Spotlight on Avian Pathology: Fowlpox virus

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Abstract

Fowlpox virus is the type species of an extensive and poorly-defined group of viruses isolated from more than 200 species of birds, together comprising the avipoxvirus genus of the poxvirus family. Long known as a significant poultry pathogen, vaccines developed in the early and middle years of the 20th century led to its effective eradication as a problem to commercial production in temperate climes in developed western countries (such that vaccination there is now far less common). Transmitted mechanically by biting insects, it remains problematic, causing significant losses to all forms of production (from back-yard, through extensive to intensive commercial flocks), in tropical climes where control of biting insects is difficult. In these regions, vaccination (via intra-dermal or subcutaneous, and increasingly *in ovo*, routes) remains necessary. Although there is no evidence that more than a single serotype exists, there are poorly-described reports of outbreaks in vaccinated flocks. Whether this is due to inadequate vaccination or penetrance of novel variants remains unclear. Some such outbreaks have been associated with strains carrying endogenous, infectious proviral copies of the retrovirus, reticulo-endotheliosis virus (REV), which might represent a pathotypic (if not newly emerging) variant in the field. Until more is known about the phylogenetic structure of the avipoxvirus genus (by more widespread genome sequencing of isolates from different species of birds) it remains difficult to ascertain the risk of novel avipoxviruses emerging from wild birds (and/or by recombination/mutation) to infect farmed poultry.

KEYWORDS:

Fowlpox virus; Poxvirus; Pathology; Control; Vaccination; Emergence

Disease impact

Fowlpox has long been recognised as a widespread, enzootic disease of domestic chickens (and other gallinaceous birds) by virtue of its distinctive dry, crusty, skin lesions, seen mainly on un-feathered areas of the comb and wattle, the face and the legs (Skinner & Laidlaw, 2009; Skinner *et al.*, 2005). It appears to be spread by direct contact (including pecking and scratching), by inhalation or ingestion of dust or aerosols, or mechanically by biting insects. Problematic outbreaks of fowlpox are rare and limited in temperate climes (so vaccination is less common) but they are more prevalent in tropical and sub-tropical climes where control of biting insects becomes more problematic and where fowlpox remains a significant problem for small-scale and backyard flocks, as well as for intensive, commercial farming (so that vaccination becomes a pre-requisite). More extensive technical reports are available, from regulators (such as the OIE; Tripathy, 2016) and poultry producers (Anon.), describing details of diagnosis and control.

Pathology

The disease caused by fowlpox virus (the ICTV approved abbreviation is FWPV) is primarily found in cutaneous and diphtheritic forms (Tripathy & Reed, 2013). The cutaneous form of the disease is mild, typically characterised by nodular cutaneous lesions on unfeathered areas of the skin and more atypically as feather folliculitis in the feathered skin (Nakamura *et al.*, 2006). The characteristic cutaneous nodules are histologically typified by marked hyperplasia of the epidermis (acanthosis) caused by the swelling and the increased number of cells in the *stratum spinosum* (C. E. Woodruff, 1930). The distribution of dermal lesions is probably linked to the mechanical transmission of the viruses by biting insects. Such lesions are rarely fatal

but can reduce performance in feeding (hunting/foraging) and predator evasion. They can become extremely extensive and persistent, which is relatively unusual for acute pox infections. Inhalation/ingestion of droplets/dust can lead to more severe infection of the oropharyngeal cavity, as so called “diphtheritic infections” (colloquially known as “wet pox”), characterised by fibronecrotic, proliferative lesions on the mucous membranes of the respiratory and digestive tracts. These lesions pose problems for diagnosis, resembling those of other respiratory infections (especially infectious laryngotracheitis) and cause up to 15% mortality in chicken flocks by occlusion of the larynx or secondary bacterial infections. Histologically, infected tissues exhibit varying degrees of ballooning of keratinocytes, with large, eosinophilic intra-cytoplasmic inclusions (Bollinger bodies) containing small, “elementary (Borrel) bodies” (virus particles), as revealed by the Gimenez method (Tripathy & Hanson, 1976).

The virus

Because the lesions were obvious and the agent could be easily propagated in embryonated eggs, then in suspensions of embryo-derived cells and finally in chick embryo fibroblast monolayer cultures (Goodpasture & Woodruff, 1930; A. M. Woodruff & Goodpasture, 1931; C. E. Woodruff & Goodpasture, 1929, 1930), fowlpox virus was one of the earliest viruses studied experimentally. As described above, its virions can just be seen by light microscopy and they form obvious inclusions upon staining. Electron microscopy reveals the characteristic bi-concave poxviral morphology of the virus particles (the Borrel bodies) and confirms their concentration in Bollinger bodies, which are poxviral A-type inclusion bodies (Eaves & Flewett, 1955; Purcell *et al.*, 1972).

Control by vaccination

The ready identification of fowlpox, its recognition as a pox disease (like the infamous smallpox, for which a vaccine had existed since the time of Edward Jenner), and its easy propagation meant that vaccines against fowlpox were among the earliest poultry vaccines introduced (the first US licence was in 1918). Numerous more-or-less attenuated, live vaccine strains were developed during the 1920s, some of which almost certainly formed the basis for the more than 70 modern commercial vaccines, the derivations of which are, therefore, not normally well-documented. They do however tend to fall into two types: those of chicken embryo origin (CEO) and those of tissue culture origin (TCO). In general, TCO are more attenuated than CEO, probably due to more extensive passage history in culture. Consequently, whereas TCO vaccines can be used in day-old chicks, the residual pathogenicity of CEO vaccines means that they cannot be used until the birds are several weeks of age. However, the more attenuated nature of TCO vaccines means that they do not provide long-lasting protection so that layers and breeders would need boosting with CEO vaccine at 6 weeks (or with the antigenically-related, cross-protective pigeonpox CEO vaccine at 4 weeks). Live, attenuated fowlpox vaccines need to be delivered percutaneously, there has been no success with drinking water or aerosol delivery, so application of TCO to day-old chicks is more practical, especially when semi-automated injectors are used.

With increasing interest in the use of recombinant fowlpox viruses as vectors to immunise against heterologous pathogens, *in ovo* delivery has been investigated (Sharma *et al.*, 2002). Commercial recombinant fowlpox virus vectored vaccines are

now available, for instance in the Trovac™ (Merial) and Vectormune™ (CEVA) ranges, against avian encephalomyelitis, avian influenza, infectious laryngotracheitis, Mycoplasma gallisepticum and Newcastle disease (TROVAC®: AIV H5, NDV; Vectormune®: FP MG, FP LT/AE, FP N). Many other recombinants are being or have been developed, *e.g.* the H5 and N1 recombinant developed by the Harbin Institute in China (Qiao *et al.*, 2009), but it is important that appropriately attenuated vaccine vector backbones are used, especially for for *in ovo* vaccination, to avoid complications such as those reported by Willams (2010).

REV, virulence and vaccine escape

An intriguing aspect of fowlpox virology is that pathogenic, field strains of fowlpox virus frequently carry an integrated, active copy of the reticuloendotheliosis virus (REV) provirus. The initial observation related to a commercial CEO vaccine (FPV-S) that proved to be contaminated with REV, was withdrawn and could not be plaque-purified free of the contaminant. PCR analysis later showed that it carried an infectious proviral copy integrated in its genome (Hertig *et al.*, 1997). We now know that REV is most closely related to mammalian retroviruses from monotremes; there has been speculation on its possible iatrogenic transfer to fowlpox virus during alleged inadvertent co-cultivation in New York in the 1940s (Niewiadomska & Gifford, 2013). However, REV-positive fowlpox carry the provirus at the same locus (passaged laboratory and commercial vaccine strains often appear to have lost most of the provirus, sometimes leaving just a single long terminal repeat sequences). This indicates a single, extremely rare, ancestral insertion event (Moore *et al.*, 2000). That may be more consistent with a natural event over evolutionary time, pre-dating the artificial propagation of fowlpox virus, but this is unlikely to be ever established

147 definitively. Nevertheless, there is anecdotal evidence that REV-containing field
148 viruses are more problematic, whether through increased virulence, resistance to
149 vaccine-induced immunity (which might equally be caused by emergence of
150 unrecognised antigenic variants) or by generally increased virus fitness.
151
152 **Phylogeny and the risk of emerging virus outbreaks**
153 Fowlpox virus is the type species of the *Avipoxvirus* genus, members of which have
154 been isolated from 280 species of birds (Bolte *et al.*, 1999). We still know relatively
155 little about their phylogenetics because of the size of their genomes (up to 300 kbp,
156 with only a handful sequenced) and because of the sequence diversity within what is
157 still classified as just a single genus. We also know little about their relationships with
158 their varied hosts, but 3 deep clades are loosely associated with broad classes of birds:
159 (A) "fowlpox-like viruses" being mainly isolated from galliforms, (B) "canarypox-
160 like viruses" from passerines and (C) psittacine viruses (Gyuranecz *et al.*, 2013), with
161 recent assignment of viruses from aquatic birds (Carulei *et al.*, 2017). The depth of
162 the clades is remarkable; the genetic distances between them are equivalent to those
163 seen between different genera of mammalian poxviruses. It has proved difficult
164 therefore to derive pan-avipoxvirus PCR probes to elucidate accurate details of
165 host/virus relationships. Most of the clade A viruses appear fairly host-specific but the
166 clade B viruses seem able to infect a wide range of species (though the picture is
167 complicated because many infections are observed in zoos, aviaries and wildlife
168 parks, veterinary clinics or quarantine facilities, where atypical species-species
169 transmissions can more readily occur). Others probably represent prey-to-predator
170 transmissions. As with many zoonotic infections, it is likely that avipoxviruses cause
171 mild or inapparent infections in their native host but present as more severe in atypical

hosts. It is almost certain that canarypox virus is relatively benign in its as-yet-undefined natural host (possibly native songbirds of temperate climes), in contrast to the severe infection it causes in non-native canaries. For all these reasons, we are therefore always vulnerable to emergence of a novel avipoxvirus that might pose a threat to poultry, so need to be vigilant. For instance, a virus that emerged in Virginia in 2003 seemed to be able to infect an unusually broad range of species (Adams *et al.*, 2005). It is clear, therefore, that we need to know more about these enigmatic viruses. Perhaps long-read, next-generation sequencing technologies will offer opportunities to understand the extent of genome variation and possibly its relationship to host range.

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REFERENCES

Adams, C.J., Feldman, S.H. & Sleeman, J.M. (2005). Phylogenetic analysis of avian poxviruses among free-ranging birds of Virginia. *Avian Diseases,* 49, 601-605.

Anon. Fowl Pox Prevention. In *Hy-Line Red Book, an Online Management Guide*.

Bolte, A.L., Meurer, J. & Kaleta, E.F. (1999). Avian host spectrum of avipoxviruses. *Avian Pathology,* 28, 415-432.

Carulei, O., Douglass, N. & Williamson, A.L. (2017). Comparative analysis of avian poxvirus genomes, including a novel poxvirus from lesser flamingos (Phoenicopterus minor), highlights the lack of conservation of the central region. *BMC Genomics,* 18, 947.

Eaves, G. & Flewett, T.H. (1955). The structure of fowl-pox inclusions (Bollinger bodies). *Journal of Hygiene,* 53, 102-105.

Goodpasture, E.W. & Woodruff, A.M. (1930). The Nature of Fowl-Pox Virus as Indicated by Its Reaction to Treatment with Potassium Hydroxide and Other Chemicals. *American Journal of Pathology,* 6, 699-712 693.

Gyuranecz, M., Foster, J.T., Dan, A., Ip, H.S., Egstad, K.F., Parker, P.G., Higashiguchi, J.M., Skinner, M.A., Hofle, U., Kreizinger, Z., Dorrestein, G.M., Solt, S., Sos, E., Kim, Y.J., Uhart, M., Pereda, A., Gonzalez-Hein, G., Hidalgo, H., Blanco, J.M. & Erdelyi, K. (2013). Worldwide phylogenetic relationship of avian poxviruses. *Journal of Virology,* 87, 4938-4951.

Hertig, C., Coupar, B.E., Gould, A.R. & Boyle, D.B. (1997). Field and vaccine strains of fowlpox virus carry integrated sequences from the avian retrovirus, reticuloendotheliosis virus. *Virology,* 235, 367-376.

Moore, K.M., Davis, J.R., Sato, T. & Yasuda, A. (2000). Reticuloendotheliosis virus (REV) long terminal repeats incorporated in the genomes of commercial fowl poxvirus vaccines and pigeon poxviruses without indication of the presence of infectious REV. *Avian Diseases,* 44, 827-841.

Nakamura, K., Waseda, K., Yamamoto, Y., Yamada, M., Nakazawa, M., Hata, E., Terazaki, T., Enya, A., Imada, T. & Imai, K. (2006). Pathology of cutaneous fowlpox with amyloidosis in layer hens inoculated with fowlpox vaccine. *Avian Diseases,* 50, 152-156.

Niewiadomska, A.M. & Gifford, R.J. (2013). The extraordinary evolutionary history of the reticuloendotheliosis viruses. *PLoS Biology,* 11, e1001642.

Purcell, D.A., Clarke, J.K., McFerran, J.B. & Hughes, D.A. (1972). The morphogenesis of pigeonpox virus. *Journal of General Virology,* 15, 79-83.

Qiao, C., Jiang, Y., Tian, G., Wang, X., Li, C., Xin, X., Chen, H. & Yu, K. (2009). Recombinant fowlpox virus vector-based vaccine completely protects chickens from H5N1 avian influenza virus. *Antiviral Research,* 81, 234-238.

Sharma, J.M., Zhang, Y., Jensen, D., Rautenschlein, S. & Yeh, H.Y. (2002). Field trial in commercial broilers with a multivalent in ovo vaccine comprising a mixture of live viral vaccines against Marek's disease, infectious bursal disease, Newcastle disease, and fowl pox. *Avian Diseases,* 46, 613-622.

Skinner, M.A. & Laidlaw, S.M. (2009). Advances in fowlpox vaccination. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources,* 4, 1-10.

Skinner, M.A., Laidlaw, S.M., Eldaghayes, I., Kaiser, P. & Cottingham, M.G. (2005). Fowlpox virus as a recombinant vaccine vector for use in mammals and poultry. *Expert Review of Vaccines,* 4, 63-76.

241 Tripathy, D.N. (2016). Fowlpox. In *OIE Terrestrial Manual 2016*.
242 Tripathy, D.N. & Hanson, L.E. (1976). A smear technique for staining elementary
243 bodies of fowlpox. *Avian Diseases,* 20, 609-610.
244 Tripathy, D.N. & Reed, W.M. (2013). Pox. In D.E. Swayne, J.R. Glisson, L.R.
245 McDougald, L.K. Nolan, D.L. Suarez & V. Nair (Eds.), *Diseases of Poultry*
246 13th ed. USA: Wiley-Blackwell.
247 Williams, S.M., Smith, J.A., Garcia, M., Brinson, D., Kiupel, M. & Hofacre, C.
248 (2010). Severe histiolymphocytic and heterophilic bronchopneumonia as a
249 reaction to in ovo fowlpox vaccination in broiler chicks. *Veterinary Pathology,*
250 47, 177-180.
251 Woodruff, A.M. & Goodpasture, E.W. (1931). The Susceptibility of the Chorio-
252 Allantoic Membrane of Chick Embryos to Infection with the Fowl-Pox Virus.
253 *American Journal of Pathology,* 7, 209-222 205.
254 Woodruff, C.E. (1930). A Comparison of the Lesions of Fowl-Pox and Vaccinia in
255 the Chick with Especial Reference to the Virus Bodies. *American Journal of*
256 *Pathology,* 6, 169-174 163.
257 Woodruff, C.E. & Goodpasture, E.W. (1929). The Infectivity of Isolated Inclusion
258 Bodies of Fowl-Pox. *American Journal of Pathology,* 5, 1-10 17.
259 Woodruff, C.E. & Goodpasture, E.W. (1930). The Relation of the Virus of Fowl-Pox
260 to the Specific Cellular Inclusions of the Disease. *American Journal of*
261 *Pathology,* 6, 713-720 713.
262