

1 ***Spotlight on Avian Pathology: Fowlpox virus***

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22 **Abstract**

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

43

44 **KEYWORDS:**

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

46

47 **Disease impact**

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

61

62 **Pathology**

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

72 but can reduce performance in feeding (hunting/foraging) and predator evasion. They
73 can become extremely extensive and persistent, which is relatively unusual for acute
74 pox infections. Inhalation/ingestion of droplets/dust can lead to more severe infection
75 of the oropharyngeal cavity, as so called “diphtheritic infections” (colloquially known
76 as “wet pox”), characterised by fibronecrotic, proliferative lesions on the mucous
77 membranes of the respiratory and digestive tracts. These lesions pose problems for
78 diagnosis, resembling those of other respiratory infections (especially infectious
79 laryngotracheitis) and cause up to 15% mortality in chicken flocks by occlusion of the
80 larynx or secondary bacterial infections. Histologically, infected tissues exhibit
81 varying degrees of ballooning of keratinocytes, with large, eosinophilic intra-
82 cytoplasmic inclusions (Bollinger bodies) containing small, “elementary (Borrel)
83 bodies” (virus particles), as revealed by the Gimenez method (Tripathy & Hanson,
84 1976).

85

86 **The virus**

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

97

98 **Control by vaccination**

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

118

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

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

130

131 **REV, virulence and vaccine escape**

132 An intriguing aspect of fowlpox virology is that pathogenic, field strains of fowlpox
133 virus frequently carry an integrated, active copy of the reticuloendotheliosis virus
134 (REV) provirus. The initial observation related to a commercial CEO vaccine (FPV-
135 S) that proved to be contaminated with REV, was withdrawn and could not be plaque-
136 purified free of the contaminant. PCR analysis later showed that it carried an
137 infectious proviral copy integrated in its genome (Hertig *et al.*, 1997). We now know
138 that REV is most closely related to mammalian retroviruses from monotremes; there
139 has been speculation on its possible iatrogenic transfer to fowlpox virus during
140 alleged inadvertent co-cultivation in New York in the 1940s (Niewiadomska &
141 Gifford, 2013). However, REV-positive fowlpox carry the provirus at the same locus
142 (passaged laboratory and commercial vaccine strains often appear to have lost most of
143 the provirus, sometimes leaving just a single long terminal repeat sequences). This
144 indicates a single, extremely rare, ancestral insertion event (Moore *et al.*, 2000). That
145 may be more consistent with a natural event over evolutionary time, pre-dating the
146 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

172 hosts. It is almost certain that canarypox virus is relatively benign in its as-yet-
173 undefined natural host (possibly native songbirds of temperate climates), in contrast to
174 the severe infection it causes in non-native canaries. For all these reasons, we are
175 therefore always vulnerable to emergence of a novel avipoxvirus that might pose a
176 threat to poultry, so need to be vigilant. For instance, a virus that emerged in Virginia
177 in 2003 seemed to be able to infect an unusually broad range of species (Adams *et al.*,
178 2005). It is clear, therefore, that we need to know more about these enigmatic viruses.
179 Perhaps long-read, next-generation sequencing technologies will offer opportunities
180 to understand the extent of genome variation and possibly its relationship to host
181 range.
182

183 **Acknowledgements**

184

185 We wish to acknowledge the support of the BBSRC (sLoLa grant BB/K002465/1:

186 “*DRREVIP - Developing Rapid Responses to Emerging Virus Infections of Poultry*”).

187

188 **Disclosure Statement**

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190 The authors disclose they have no competing financial interests.

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