Impacts of poultry vaccination on viruses of wild bird

Running title: Impacts of poultry vaccines on wild bird viruses

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Abstract

Spillover of viruses from farmed poultry into wild birds is a relatively new area of study at the livestock-wildlife interface. These transmission events can threaten the health of wild birds. There is growing evidence of transmission of vaccine viruses from poultry to wild birds, including attenuated vaccine strains of Newcastle disease virus and infectious bronchitis virus, and also spread of virulent viruses that may have evolved under the pressure of vaccine use, such as Marek’s disease virus. Viral contaminants of poultry vaccines, including reticuloendotheliosis virus, may also be transmitted to wild birds and result in disease. New, vectored vaccines are less likely to directly spread to wild birds but this risk may rise as a result of recombination.
Introduction

Global poultry production is increasing rapidly, with chicken meat forecast to become the largest meat sector worldwide from 2020 [1]. The potential for viruses in wild birds, which are often considered reservoirs of infection, to ‘spillover’ into poultry and cause disease has long been recognised. Thus biosecurity programs in poultry operations have been developed to help prevent the direct or indirect transmission of pathogens from wild birds to poultry [2]. Virus transmission in the other direction (from poultry into wild birds) lacks the immediate economic consequences that viral incursions into commercial poultry can have and has not been as thoroughly considered. Consequently, fewer biosecurity measures are in place to prevent such transmission events. This is despite the massive scale of modern commercial poultry production which can enable large-scale contamination of the environment with infectious material through practices such as disposal or re-use of poultry litter [3] or through airborne dispersal of infectious agents from infected flocks [4]. Furthermore, an increased interest in free-range and organic poultry production allows increased direct contact between wild birds and farmed poultry [2].

A developing awareness of the concept of ‘one health’, which recognises connections between animal, environmental and human health, including at the livestock-wildlife interface [5,6] has served to highlight the risks that poultry viruses may pose to the environment and to wild birds. The potential for spillover exists when the natural host range of a virus includes a domestic poultry species and a wild bird species. As viruses in wild birds are much less highly studied than those in poultry, the natural host ranges of many avian viruses are not well understood. Similarly, the degree of host susceptibility to disease caused by different viruses is often not known. However, some poultry viruses are known to have broad host ranges (e.g. Newcastle disease virus, NDV) and are thus a potential risk to a wide range of wild bird
species [7]. Other poultry viruses have narrow host ranges (e.g. some avian herpesviruses). These may only be a risk to small number of wild bird species that are closely related to farmed poultry species, such as other Galliform or Anseriform birds [8,9]. Without appropriate surveillance programmes in wild bird populations, spillover events from poultry into wild birds are unlikely to be detected.

Vaccines, along with biosecurity programmes, are critical to the control of viral diseases in poultry. Table 1 summarises vaccines used for this purpose. These vaccines typically reduce clinical signs of disease but do not prevent virus infection [2]. Vaccination can have a profound influence on virus populations within commercial poultry flocks, and, by extension, viruses that may spillover into wild bird populations. Poultry vaccines may have an impact on wild bird viruses through the transmission of attenuated vaccines from poultry to wild birds; the transmission of virulent viruses that have evolved in response to the use of poultry vaccines; or through undetected viral contaminants within commercial vaccines being transmitted to wild birds. New, vectored poultry vaccines also have the potential to have an impact on wild bird viruses. These four mechanisms through which poultry vaccines may affect wild bird viruses (Figure 1) will be explored in further detail below, with a focus on recent contributions to this field and consideration of the potential risks to wild birds.

**Attenuated vaccine viruses transmitted from poultry to wild birds**

Spillover of vaccine viruses from poultry into wild birds can only occur with live vaccines. Attenuated vaccines are commonly used in poultry (Table 1) as they are relatively inexpensive to produce and administer, particularly when they facilitate mass administration by drinking water, aerosol or spray. These vaccines also often induce more effective immunity than inactivated vaccines [2]. Prior to their registration, the safety characteristics of attenuated...
poultry vaccines are examined comprehensively in the intended target species [10] but not in wild bird species that may also be susceptible to infection.

Despite limited surveillance and characterisation of viruses in wild bird species there is evidence that poultry vaccine viruses have been transmitted to wild birds. Viruses with a high degree of genetic identity to the attenuated LaSota (genotype II) and PHY-LMV42 (genotype I) NDV vaccine strains have recently been detected in a range of different species of free-living wild birds in Mexico [11] and Luxembourg [12]. Similarly, previous studies have shown that infectious bronchitis viruses (IBVs) in wild Anseriform and Galliform birds in England have a high level of nucleotide sequence identity with the H120 vaccine strain of IBV used in poultry [13]. These findings add another dimension to our understanding of the epidemiology of these viruses but the risks to wild birds are not well understood. Other than direct impacts that the attenuated vaccine viruses may have on wild birds there is also the potential for the attenuated vaccine viruses to evolve to higher levels of virulence in wild birds, including through the process of recombination. Phylogenetic evidence of recombination is growing and has been reported for both IBV and NDV, including recombination between vaccine and wildtype viruses [14,15].

**The influence of vaccines on the evolution of poultry viruses**

Vaccines can influence the evolution of viruses within poultry flocks. Selection for viruses that ‘escape’ vaccine-induced immunity can occur when there is incomplete cross-protection between viral serotypes or antigenic types. This has been reported for a number of poultry viruses, particularly avian influenza virus (AIV) [16,17], but also other viruses, including IBV [15] and NDV [18]. The viruses that persist in poultry despite (or due to) the use of vaccines
can then be transmitted to wild birds, with the viruses detected in wild birds reflecting those that are in local farmed poultry. Recently, isolates of NDV with a high level of identity to virulent viruses in local farmed poultry have been detected in wild birds, including virulent genotype XVIIIb NDV in a wild passerine in West Africa and virulent genotype Vb NDV in captive wild birds in Mexico [11,19,20]. However, unlike vaccine viruses, the direction of transmission of virulent viruses can be more difficult to discern. Movement of AIV between farmed poultry and wild birds has been intensively studied, including transmission of highly pathogenic avian influenza viruses (HPAIV) from poultry into wild birds [21-23]. Vaccination of poultry may decrease the environmental burden of H5N1 HPAIV in endemic areas and thus limit transmission to wild birds [22]. However the potential for vaccination to drive antigenic drift of field viruses away from vaccine viruses should also be considered [16,17]. The careful deliberation required for judicious use of AIV vaccines serves to highlight many of the complexities surrounding vaccine use in poultry.

Importantly, poultry vaccines can also influence virus evolution in the absence of incomplete cross-protection between virus serotypes or antigenic types. Recent studies on Marek’s disease virus serotype 1 (MDV-1) in chickens have shown that ‘imperfect’ vaccines (‘leaky’ vaccines that keep the host alive but allow replication and transmission of challenge virus) can enhance the emergence of virulent viruses that spread to unvaccinated chickens to cause severe disease [24]. This may help to explain the evolution of MDV-1 to higher levels of virulence in the decades following the introduction of MDV-1 vaccines. The natural host range of MDV-1 is narrow, but MDV-1 infection, including infection with virulent MDV-1, has been reported in a high proportion of wild migratory and sedentary geese and ducks in Japan and Russia, showing that spillover to wild birds is possible [25,26]. As most poultry vaccines are ‘imperfect’, the potential exists for similar processes to occur with other poultry viruses.
Viral contaminants of poultry vaccines

Contamination of attenuated vaccines with viruses used to generate inactivated viruses in the same facility, or with viruses circulating in flocks used for production of embryonated eggs for vaccine production, has the potential to contribute to dissemination of virulent viruses into wild birds through poultry vaccines. In addition, inadequate inactivation of virulent viruses used to produce inactivated vaccines may also result in dissemination of viruses that pose a risk to wild birds. Although quality assurance measures in vaccine production should identify adventitious agents in vaccines, and ensure adequate inactivation, there have been a number of instances of inadvertent dissemination of viruses in poultry vaccines. Contaminating agents in viral vaccines of poultry have included chicken anaemia virus, infectious bursal disease virus (IBDV), avian leucosis viruses and reticuloendotheliosis virus (REV) [27••-29]. The initial dissemination of REV, which appears to have originated as a mammalian virus transmitted into birds during experimental passage of a model Plasmodium species in ducks [27••], has its origins in contamination of both fowlpox virus (FPV) and MDV vaccines, with extensive dissemination particularly linked to integration of the REV genome into the genome of FPV vaccine strains. Thus infection and disease associated with REV in wild birds, including wild turkeys [30,31], can be presumed to have been derived from contamination in vaccines.

The risk of contamination is greatest for undiscovered viral pathogens, as testing of vaccines for adventitious agents is focused on detection of known causes of disease in commercial poultry [28]. Future control of these risks may be improved by introduction of less targeted approaches, such as application of high throughput sequencing, to detect adventitious agents in vaccines.
New generation (vectored) vaccines

A recent dramatic expansion in the use of vectored poultry vaccines may have impacts on viruses in wild birds. Most vectored poultry vaccines use double-stranded DNA viruses as vectors. Herpesvirus of turkey (HVT) and FPV are used commercially as vaccine vectors to express genes from other poultry pathogens, including AIV, IBDV, NDV and infectious laryngotracheitis virus (ILTV) (Table 1). Fowl adenovirus (FAdV) and a number of avian herpesviruses (ILTV, MDV-1 and duck enteritis virus) have also been used experimentally as vaccine vectors [32-36]. The risk of vectored poultry vaccines being transmitted to wild birds may appear to be low because of the limited shedding of most vectored vaccines, but this risk could rise as a result of recombination. The importance of recombination as a mechanism of genome evolution in many DNA viruses is increasingly being recognised, including in virus families that are favoured as vaccine vectors, such as herpesviruses and adenoviruses [37-39]. Potential outcomes of recombination include a shift in host range, increased virulence, enhanced viral replication and enhanced virus transmission [40-42].

Analysis of the full genome of field isolates of ILTV has revealed widespread natural recombination, including recombination between attenuated vaccine strains to generate virulent viruses [41,43]. Evidence of natural recombination has also been observed between different avian poxviruses, including apparent recombination events between viruses isolated from different species of birds [44]. There are few field isolates of HVT that have been fully sequenced, making analyses of these viral genomes for evidence of recombination difficult at this time. However, a larger number of avian adenovirus isolates have been fully sequenced and their genomes are available for analyses. Recombination analyses using these sequences show extensive recombination networks (Figure 2), including recombination between adenoviruses from different bird species. These findings highlight the potential for
recombination to occur between viruses that are favoured for vectored poultry vaccines and other viruses that may be present in vaccinated poultry.

Conclusion

Vaccines are crucial for achieving high standards of animal production, health and welfare in farmed poultry, but many of the impacts of poultry vaccines on wild bird viruses are incompletely characterised. This is not unexpected given the difficulties involved in studying viruses in wild birds and the historical focus on wild birds as a potential source of infection for farmed poultry. As the scale of poultry production grows it will become increasingly important to better characterise these impacts and minimise risks to wild birds. Within poultry industries this may be assisted by continued improvements in biosecurity procedures, including an enhanced focus on preventing spillover events into wild birds, and also continued improvements in poultry vaccines and vaccination programs to avoid the selection or evolution of virulent viruses, and to avoid inadvertent dissemination of vaccine contaminants. Within wild bird populations an improved approach to viral surveillance and characterisation is indicated. Importantly, advances in genome sequencing and sequence analysis are already being used to progress our understanding of wild bird viruses and poultry vaccines, including phylogenetic relationships between viruses and the importance of recombination. New advances in these technologies are likely to further assist with these studies in the future.

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Figure legends

Figure 1: Overview of the different mechanisms through which poultry vaccines may affect viruses of wild birds. Viruses in farmed poultry (including farmed chickens, ducks, geese and turkeys) can spillover into the environment and into wild bird populations. This can include transmission of vaccine viruses (attenuated or vectored vaccines) or transmission of virulent viruses that evolved in poultry under the influence of vaccination. Viral contaminants of poultry vaccines can also be transmitted to wild birds.

Figure 2. Analysis of aviadenovirus genomes reveals evidence of widespread recombination. Global genome pairwise sequence alignments were prepared with mVista LAGAN [45] and multi-reticulate analyses performed using SplitsTree4 [46] with an uncorrected P characters transformation model, excluding all gap sites. The multi-reticulate networks indicate recombination events. A recombination pairwise homoplasy index (PHI) test was performed within SplitsTree4 and found to be significant (P < 0.01). Additional analyses in RDP4 [47] were performed as described previously [48] and confirmed the SplitsTree4 results, with 20 predicted recombination events detected by three or more of the methods used (RDP, GENECONV, Chimaera, SiScan, MaxChi and Bootscan). The abbreviations and GenBank accessions are: fowl aviadenovirus 1 (FAdV 1: NC_001720), fowl aviadenovirus 4 (FAdV 4: ON1: NC_015323, KR5: HE608152, MXSHP95: KP295475, JS13: KM096544 and HB1510: KU587519), fowl aviadenovirus 5 (FAdV 5: NC_021221), fowl aviadenovirus 8 (FAdV 8: NC_014969), fowl aviadenovirus 9 (FAdV 9: NC_000899), fowl aviadenovirus 11 (FAdV 11: KM096545 and KM096546), turkey aviadenovirus 1 (TAdV 1: NC_014564), turkey aviadenovirus 4 (TAdV 4: NC_022612), turkey aviadenovirus 5 (TAdV 5: NC_022613), pigeon aviadenovirus 1 (PAdV 1: NC_024474), goose aviadenovirus 4 (GAdV 4: NC_017979), and duck aviadenovirus 2 (DAdV 2: NC_024486 and KR135164).
References (● of special interest, ●● of outstanding interest)


The epidemiology and phylogeny of NDV isolates in Mexico are examined to show that vaccine strains and virulent strains may be escaping from poultry into the environment.


Phylogentic analyses of poultry IBV isolates in China reveals evidence of widespread IBV recombination and IBV evolution under the immune selection pressure of vaccines.


Phylogentic analyses and virus infection studies reveal that immunisation pressure from H9N2 influenza virus vaccines used in poultry selected for G57 genotype viruses that become dominant in vaccinated chickens in China and ultimately gave rise to the H7N9 viruses that caused outbreaks of human disease.


A review of the genetic diversity of NDV isolates from farmed poultry and wild birds demonstrates the wide host range of NDV and presents phylogentic evidence of frequent transmission across the domestic bird-wild bird interface.


MDV-1 infection studies in chickens showed that ‘imperfect’ vaccines can drive the evolution and spread of virulent viruses.


This study provides evidence that the origin of avian reticuloendotheliosis viruses was a mammalian virus transmitted into birds during experimental passage of a
model *Plasmodium* species in ducks that was then widely disseminated through contamination of poultry vaccines.


oncogene and co-expressing AIV-H9N2 HA and NA genes under control of exogenous promoters. *J Biotechnol* 2014, **181**:45-54.


43. Lee SW, Devlin JM, Markham JF, Noormohammadi AH, Browning GF, Ficorilli NP, Hartley CA, Markham PF: Phylogenetic and molecular epidemiological studies


Phylogenetic analyses demonstrate the wide host range of avian poxviruses and transmission of poxviruses between different species of birds. Multiple recombination events are also described.


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