Institution:
UNIVERSITY OF LIVERPOOL

Unit of Assessment:
UOA6 - Agriculture, Veterinary and Food Science

Title of case study:
Avian Metapneumovirus Research Leading to Improved Disease Control in Global Poultry

1. Summary of the impact

Since its discovery in the 1980s, avian metapneumovirus (AMPV) has spread in poultry populations worldwide with major adverse health and food security implications for commercial chickens and turkeys. Research at the University of Liverpool (UoL) led to the registration of a live vaccine in 1994 which has played a global role in AMPV control, thereby safeguarding the supply of poultry meat and eggs. Recent research and development at the UoL has identified key control measures, relating to vaccine application, vaccine selection, efficacy and safety, which have had a significant impact on poultry health and consequently, poultry producers and consumers. In particular, demonstration that live AMPV vaccines can revert to virulence, that vaccine type applied influences field protection and that continuous use of a single vaccine can influence circulating field strains, has resulted in UoL leading policy making with regard to current AMPV vaccine protocols.

2. Underpinning research

In the late 1980s, AMPV was a newly emerging respiratory disease of turkeys, initially named turkey rhinotracheitis (TRT) that caused respiratory disease, swollen head syndrome (SHS), reduced egg laying and reduced food conversion rates. It had high morbidities and mortalities in turkeys which also affected laying and broiler chickens. A vaccine industry research strategist recently estimated that 6 billion turkeys and chickens worldwide could benefit from effective AMPV vaccination.

Starting in the late 1980s, the UoL group of Dr RC Jones with Dr R Williams and Dr CJ Naylor modified a virulent AMPV field isolate to yield a protective live vaccine candidate. While protective and attenuated, this unfortunately caused some disease after serial passage in turkeys in experimental conditions, but following the UoL's use of cloning to remove virulent AMPV subpopulation, derivative clone K became a registered vaccine (Poulvac TRT) with European mutual international recognition for use in turkeys in 1997. The same product was licensed for chickens as Poulvac SHS (1998). The current licence holder is Zeotis. The vaccine is also registered in other major poultry producing regions including Latin America and Asia.

The subsequent work, starting in 2001, associated with the current impact case was led by senior lecturer Naylor of the Department of Infection Biology, UoL and frequently involved international academic and commercial collaboration in Italy and Germany. Key to much of this was the development of high level molecular AMPV expertise which included development of the first AMPV reverse genetics system [1]. Classical studies by the UoL explored the use of Liverpool's clone K and other AMPV vaccines both experimentally and on farms and demonstrated its acceptable efficacy in the face of maternal antibody, full protection in the absence of any induced anti-AMPV antibody (1997). AMPV specific molecular expertise facilitated molecular field studies, full genome sequencing studies and the construction of modified viruses to test hypotheses. Following the UoL's detection of AMPV subtype B in the UK for the first time using RT-PCR (published 1987), the UoL investigated the importance of matching within AMPV subtypes. Using in-house developed full genome sequencing protocols, the group discovered that field virus could evolve in the face of prevailing vaccine pressure [2] (2010) and that those changes involved the attachment protein [3], thus allowing current RT-PCR sequencing studies to target sections of this gene when investigating field strains likely to avoid vaccine induced protection. The UoL group previously proved that field disease was being caused by reversion to virulence of both clone K [4], then demonstrated in 2011 that similar clone K derived reverted virus could spread in the environment [5] and for the first time in 2012 that a commonly used commercial subtype B vaccine could also revert on farms (manuscript in preparation). Using reverse genetics, a molecular cause of the virulence increase in clone K was established [6].

3. References to the research

1. **Brown PA**, Edworthy N, Ling R, Jones RC, Savage C and **Naylor CJ**. Development of a reverse genetics system for Avian pneumovirus demonstrates that the small hydrophibic (SH) and attachmentproteins (G) genes are not essential for viability. J Gen Virol 2004 85 :346-54. URL http://www.ncbi.nlm.nih.gov/pubmed/15483235 Citations: 36 Impact Factor: 3.127

2. Catelli E, Lupini C, Cecchinato M, Ricchizzi E, **Brown P**, **Naylor CJ**. Field avian metapneumovirus evolution avoiding vaccine induced immunity. Vaccine 2010 28(4):916-21. URL http://www.ncbi.nlm.nih.gov/pubmed/19931381 Citations: 14 Impact Factor: 3.492

3. Cecchinato M, Catelli E, Lupini C, Ricchizzi E, **Clubbe J**, Battilani M, **Naylor CJ**. Avian metapneumovirus attachment protein involvement in probable virus evolution concurrent with mass live vaccine introduction. Veterinary Microbiology 2010 6:24-34. URL http://www.ncbi.nlm.nih.gov/pubmed/20447777 Citations: 7 Impact Factor: 3.127

4. Catelli E, Cecchinato M, **Savage CE**, **Jones RC, Naylor CJ**. Demonstration of loss of attenuation and extended field persistence of a live avian metapneumovirus vaccine. Vaccine 2006 24:6476-82.URL http://www.ncbi.nlm.nih.gov/pubmed/16901592 Citations: 32 Impact Factor: 3.492

5. Lupini C, Cecchinato M, Ricchizzi E, **Naylor CJ**, Catelli E. A turkey rhinotracheitis outbreak caused by the environmental spread of a vaccine-derived avian metapneumovirus. Avian Pathology 2011 14: 525-530URL http://www.ncbi.nlm.nih.gov/pubmed/21854180 Citations: 2 Impact Factor: 1.729

6. **Brown PA**, Lupini C, Catelli E, **Clubbe J**, Ricchizzi E, **Naylor CJ**. A single polymerase (L) mutation in avian metapneumovirus increased virulence and partially maintained virus viability at an elevated temperature. J Gen Virol 2011 92:346-54.URL http://www.ncbi.nlm.nih.gov/pubmed/21048037 Citations: 7 Impact Factor: 3.127

Key Grants

1988 – 2006. **Duphar** (now Zoetis), Weesp, The Netherlands. Studies on avian metapneumovirus including development of a live vaccine, £1.5m, PI RC Jones, UoL .

2000 – 2009. **Lohmann Animal Health**, Cuxhaven, Germany. The development of vaccines against avian metapneumovirus and associated studies, £1m, PIs **CJ Naylor** and RC Jones.

2006 – 2009. **BBSRC** ref BB/D012171/1. The application of reverse genetics to the study of pathogenicity in avian pneumovirus, £200k, PI **CJ Naylor**.

4. Details of the impact

Impacts derive from a long term concerted molecular and classical research programme led by the UoL which has directly led to improved protection of poultry against serious disease, thus improving the efficiency of meat and egg production. The beneficiaries are the protected animals in terms of animal welfare, poultry farmers globally in terms of economic efficiency and the general public in terms of economic high quality protein availability from meat and eggs, i.e. food security.

Economic and health impacts

The Liverpool vaccine has been important in global AMPV control. It is one of only two subtype A live vaccines used globally. Its profile is summarised in the DEFRA 2012 summary sheet [7].The continued use of the vaccine is enhanced by the Liverpool team's on-going research into its applications and usage, thus helping ensure the product's safety and efficacious use. It is only the

effective application AMPV vaccine in hatcheries and on farms that prevents the severe disease and losses seen prior to vaccine introduction. UoL researchers have been key to maintaining this major impact on disease protection in a major food species. Eight million doses of the vaccine were sold in Europe, Africa and the Middle East in the last financial year, much comprising UK turkeys (production approx 17 million per year) and to a lesser extent in France. This is a 20% sales increase over the previous year, an indication of both the importance of the disease and the performance of the vaccine. The figure does not include Asia/Pacific and South America (particularly Brazil) which are the biggest markets for the vaccine.

The Liverpool team's on-going research has allowed for the development of improved guidelines allowing the poultry industry to protect essential food production and maximise profit. [9,10,12,13]

Vaccine developments arising from the UoL research that have had an impact on the industry primarily by increasing effectiveness of the use of the vaccine since 2008 have included:

1. Demonstration of reversion to virulence on farms of the Zoetis clone K vaccine in 2006 led to the UoL demonstrating in 2011 that such reverted virus could circulate in the environment and in 2012 to the UoL's demonstration that a Merial subtype B vaccine could also revert to virulence on farms. The Liverpool group now advises at national (British Veterinary Poultry Association) and international (World Veterinary Poultry Association) poultry veterinary meetings that all AMPV vaccines should be administered more effectively so as to avoid unvaccinated birds becoming infected with potentially reverting shed vaccine virus. Changes of farming practice resulted, especially in Europe. More careful vaccine application became the standard, so as to avoid residual unvaccinated birds being infected with vaccine shed from littermates. Furthermore, multi-age-site-farms have fallen out of usage because of the greater level of susceptibility of young birds to any vaccine virus passaged in the environment. As a result, the current MSD product advice relating to their AMPV Nobilis TRT live vaccine, states *"The vaccine virus spreads and shows some reversion to virulence on bird to bird passage. For these reasons its use is not recommended on multi-age sites. Correct administration is important …"* [8,9,10,12,13].

2. The UoL discovered that maternal antibody did not block vaccination. This led to adoption of one-day-old hatchery vaccination where previously, administration had been later on the farm. This is formalised in the DEFRA summary sheet for the Liverpool developed clone K vaccine (Zoetis, Poulvac TRT) [7 (page 1),9].

3. The question of matching AMPV vaccine subtype (A or B) to the prevalent field subtype has been debated since the discovery of subtypes in 1993. Some parties, especially vaccine manufacturers, downplayed the issue, even though introduction of subtype B vaccine to the UK in 1995 to combat the first UK incidences of subtype B, led to a large improvement in disease control (based on research by Naylor while at IAH Compton). More influentially, from 2008 to 2010 Liverpool/Bologna studies (published 2010) conclusively proved that even small differences within a subtype had large effects on induced protection [2]. Currently AMPV vaccines of the correct subtype are favoured where the dominating field subtype is known [11].

4. The UoL showed in 2010 that the continuous use of a single AMPV vaccine (Merial Aviffa/Rhinovax) in Italy had led to evolution of field viruses in circulation which avoided previously protective immunity. This showed that exclusive use of a single vaccine type should be avoided and work has been presented to Italian veterinarians advising avoiding this practice who now avoid this practice where possible [9].

5. For some time it had been noticed that following vaccination the AMPV antibody might be absent, even though field survey evidence suggested that the birds were protected. Without the UoL's discovery that an AMPV induced antibody responses was irrelevant to protection and frequently absent, the use of AMPV vaccines might have much reduced, thus reducing disease control [13].

6. *In ovo* vaccination proved effective in producing an earlier onset of immunity. At the present time commercial restrictions have limited this to approach to small scale use [9].

5. Sources to corroborate the impact

Each source listed below provides evidence for the corresponding numbered claim made in section 4 (details of the impact).

7. DEFRA (2013). Poulvac TRT Vaccine – Summary of Product Characteristics. Listed in by the Veterinary Medicines Directorate in their product database (http://www.vmd.defra.gov.uk/ProductInformationDatabase/Default.aspx). Poulvac TRT vaccine entry at http://www.vmd.defra.gov.uk/ProductInformationDatabase/SPC_Documents/SPC_167437.doc)

8. MSD Animal Health. (2008). Nobilis TRT Live Data Sheet http://www.msd-animal-health.co.uk/Products_Public/Nobilis_TRT_Live/090_Product_Datasheet.aspx

The following individuals can confirm impact statements claimed above.

9. Contact: Poultry Director Europe, Africa and the Middle East. Pfizer Animal Health.

10. Contact: Merck Animal Health, Milton Keynes, UK and Boxmeer, The Netherlands.

11. Contact: Merial Animal Health (formerly); Wyatt Poultry Health services.

12. Contact: Agricola Tre Valli, Veronesi Group.

13. Contact: Ceva-Phylaxia