



An OFFLU Agenda for Influenza Research Priorities in Animal Species

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INTRODUCTION

A large body of animal influenza data has been generated in recent years, presenting a real opportunity to increase our understanding of how to identify risk and better control the adverse effects of influenza in animals and at the human animal interface. Many questions still remain to be answered. Structured and coordinated research towards the same goals and objectives will greatly facilitate this.

At the OFFLU technical meeting in November 2010, swine, avian, and equine experts convened to develop a list of current animal influenza research priorities, which has now been endorsed by OFFLU experts.

The 'OFFLU Research Agenda' is a first for the animal health sector and will help to steer animal influenza research in the right direction, providing maximum benefits for public and animal health. The Research Agenda can also be used to attract funds from donors, who can have more confidence that their money is being well spent.



OFFLU Research Priorities on Avian Influenza: Poultry

Control and Education

1. Conduct studies to determine if some village chicken breeds or individual chickens contain genes that confer resistance to avian influenza viruses, and if such genes, when incorporated in genetic background through traditional breeding techniques with other village chickens breeds or into commercial chickens, will increase avian influenza resistance. Conduct a review of progress in research in this area.
2. Develop training modules and ensure adequate resources for proper disease investigations and the implementation of appropriate preventive measures in places where infection is endemic.
3. Develop better field management and veterinarian training to improve understanding of biosecurity, and the correct responses and measures to take in the face of outbreaks such as carcass disposal, containment, litter treatment, emergency planning, etc.
4. Develop improved methods to control avian influenza in village poultry such as 'biosecure' husbandry methods, slaughtering, disposal of carcasses, and improved and easier methods of vaccination.
5. Undertake research and investigations to underpin development of appropriate long term plans for virus elimination in countries or part of countries.
6. Determine the virus-specific, host specific and environmental factors and animal management/husbandry-specific factors associated with zoonotic infections such as infectivity and transmissibility from birds to humans.
7. Determine exposure, infection and clinical disease rates of zoonotic infection through structured studies in both different poultry husbandry settings and humans.
8. Determine virus survival characteristics, persistence and underlying factors in a range of settings relevant to poultry production.
9. Develop innovative tools to improve assessment of the impact of disease control programs (especially vaccination).

Diagnostics

1. Develop improved, specific serological tests for H5 and H7 antibody detection on a common diagnostic platform, and transfer such developments to commercial applications.

2. Develop a rapid Newcastle disease virus field test (or adapt an existing test), such as a solid phase ELISA, with commercialization of such a test as an adjunct to existing avian influenza nucleoprotein or matrix rapid tests that would assist in more accurate field diagnosis of causes of sudden high mortality.
3. Develop a single test that will differentiate between antibodies produced in response to vaccination and those produced in response to natural infection; i.e. a single serological DIVA test.
4. Develop a sensitive, rapid test to detect both H5 and H7 avian influenza viruses in the field from clinical specimens for presumptive diagnosis of H5 and H7 notifiable AI for immediate regulatory action such as quarantine. This could be achieved through solid phase ELISA or portable RT-PCR tests.

Epidemiology

1. Determine the role of domestic ducks in the perpetuation and evolution of H5N1 HPAI, comparing husbandry systems used in different countries, i.e. grazing ducks verses village ducks; investigate viral persistence under conditions of vaccination verses non-vaccination; establish infection dynamics in ducks i.e. how they get infected, transmission from environmental sources, role of maternal antibodies and any effect from previous exposure to other AI viruses.
2. Develop standards for data collection and placement on GIS maps/GPS databases for locations where isolates from wild birds and poultry are collected to enhance traditional and molecular epidemiologic investigations, including negative results.
3. Develop quantitative tools for the evaluation of surveillance systems in both animal and human health.

Immunology and Immune responses

1. Develop a better understanding of the differences in adaptive and innate immune responses in different avian species and the relationship to susceptibility to AIV infection and responses to vaccination.
2. Develop a better understanding of duration of immunity in ducks and the possibility of re-infection of partially-immune ducks or stressed immune ducks if re-exposed to the virus.
3. Develop immunological tools to study correlates of humoral, mucosal, cellular and innate immunity.

Pathogenesis

1. Determine if H5N1 field viruses have evolved in the field that are of altered virulence from previous H5N1 HPAI viruses and if so, what the mechanism for such change was. This should include investigation of HPAI viruses from healthy birds in the field to determine if a reduction in virulence has occurred.
2. Review/clarify the role of mutations at receptor binding sites on replication and pathogenesis, especially which mutations are important in changing host specificity.
3. Determine host-virus factors that influence infection outcome in different poultry hosts, including resistance.

Transmission


1. Identify risk factors in poultry production that favour transmission and spread of avian influenza to poultry and mammalian species including humans.
2. Develop a standardized avian transmission model to better assess the inter-species transmission potential of AI viruses, especially from wild birds to poultry.
3. Identify virus-host correlates of virus transmissibility both within and between host species.

Vaccines and Vaccination

1. Develop improved vaccines for waterfowl that will give better immune responses with minimal number of booster vaccinations, such as use of improved adjuvants for killed vaccines and development of waterfowl specific viral vaccine vectors (e.g. duck viral enteritis virus).
2. Develop more effective mass immunizing methods for avian influenza vaccines such as water, feed, spray and *in ovo* application with focus on low cost, high efficiency in immunizing short-lived meat chickens (broilers).
3. Develop and use biotechnology to address antigenic drift issues and increase antigen content of inactivated AI vaccines through use of virus vectors with current avian influenza hemagglutinin gene inserts along with more development and usage of reverse genetic influenza virus A seed strains in inactivated vaccines.
4. Develop viable vaccination protocols and combinations of vaccines for use in the field, for example, the proper use of virus vectored vaccines in the field where breeders or production birds have variable immunity against the vector.
5. Develop vaccines and vaccination protocols that give longer, enhanced immunity with few vaccinations, and that are cost effective and easy to apply by the farmer.

6. Develop and standardize methods to assist manufacturers in assessing the antigen content of inactivated vaccines, and set minimum antigen standards for avian influenza inactivated vaccines.
7. Develop protocols for registration and licensure of prime-boost applications of live recombinant and inactivated avian influenza vaccines.
8. Develop protocols to rapidly update existing licensed vaccines to relevant natural and reverse genetic generated seed strains.
9. Establish standards for serological testing protocols, antisera production and antigen use for antigenic cartography that will allow comparisons between laboratories and compiling data for selection of avian influenza vaccine seed strains for challenge testing.
10. Determine the amino acid changes critical for antigenic drift and reduced vaccine protection to lead to better prediction of protection based on nucleotide sequencing.
11. Perform risk analyses on genetically modified organisms (GMOs) used for vaccines. These should include determining the potential to reassort or recombine with avian influenza viruses, potential for reversion to virulence of the vector, and the ability to infect and transmit in non-target species.
12. Review the safety and potential use of live avian influenza vaccines such as heat sensitive mutants or other reduced virulent viruses.
13. Conduct further research on DNA vaccines to improve promoters and reduce the quantity of nucleic acid and number of immunizations to give a protective response. The application of DNA vaccine that is able to provide qualified protection with acceptable cost should be encouraged.

Virus characteristics and evolution

1. Develop and maintain a database of full genome sequences for AI viruses that can be used to evaluate reassortment.
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OFFLU Research Priorities on Avian Influenza: Wild Birds

Control and Education

1. Develop a better understanding of ecological factors and risk pathways for interaction between wild birds and poultry. Provide information for improved management of prevention strategies.
2. Develop a better understanding of extensive farming systems of domestic ducks and farmed wild waterfowl species and their interactions with free ranging waterfowl with implications for virus control including interaction with other reservoir and potential bridging hosts (including wild birds).

Ecology and Epidemiology

1. Determine the role of wild bird species in the perpetuation and evolution of H5N1 HPAI.
2. Determine the role of environmental deposits (if any) in the perpetuation of H5N1 HPAI (relate to 7, 10).
3. Improve knowledge of the effect of infection with LPAI/HPAI in different populations on biological behaviours including migration.
4. Identify behavioural traits that impact on the dynamics of infection, with inputs from multidisciplinary expertise including wildlife ecologists, ornithologists, epidemiologists, virologists. Develop an improved knowledge of the demographics of wild bird populations and movements to enable better targeting of surveillance programmes.
5. Provide a more complete definition and understanding of wild bird AIV “reservoirs” and “spillover hosts” for both LPAI and HPAI.
6. Define factors, both host and viral, that influence potential maintenance of H5N1 HPAI in wild bird populations, including addressing the issue of why do some clades apparently appear more frequently in wild birds than do others.
7. Investigate the impact of prior immunity to heterologous or homologous virus subtype upon infection dynamics with HPAI
8. Identify the exact mechanisms whereby birds become infected in the wild with HPAI viruses with reference to different ecological systems. Do feeding, seasonal habitat use, migration, moult, and social or other biological traits impact virus exposure? Collate existing databases.

9. Develop standards for data collection and placement on GIS maps/GPS databases for locations where isolates from verified populations of wild birds and poultry are collected to enhance traditional and molecular epidemiologic investigations.
10. Conduct structured epidemiological investigations in response to outbreaks in poultry to robustly evaluate potential involvement of wild birds for a) virus source b) involvement in local spread.
11. Investigate whether enhanced environmental persistence of some AI viruses impacts on likelihood of infection of and adaption to wild birds?
12. Investigate the basis of host range restriction within different species populations focusing on virus subtypes of increased veterinary public health relevance.
13. Generate quantitative descriptive or predictive models to describe the (global) epidemiology of AI in wild birds.
14. Link phylogeny with migration to better understand spread and movement of viruses within and across flyways, countries, and continents.

Diagnostics and surveillance

1. Develop validated tools for assessing the immune response to influenza virus in wild birds and a specific serological test to discriminate between antibodies to H5 HPAI or LPAI.
2. Investigate use of alternative sample types for virological surveillance such as feather follicles which are less invasive and potentially as sensitive but which can also be applied in the field.
3. Develop more efficient surveillance approaches based on an improved understanding of AIV epidemiology and diagnostic approaches.

Immunology and Immune responses

1. Define the immune response in different wild bird species and the inter-relationships both between viruses of the same subtype and between different subtypes.
2. Develop a better understanding of the differences in immune responses in different wild bird species as they relate to their susceptibility to AIV infection.
3. Investigate whether the antigenicity of AI viruses differs in wild birds compared to other host groups when using tools such as cartography.
4. Develop an understanding of whether natural history cycles (spring migration, chick rearing, post-breeding moult, autumn migration) affect immune capacity or susceptibility to virus exposure or shedding.

Pathogenesis

1. Identify virus-host correlates of pathogenesis in wild bird species to explain strain specific differences upon infection course, virus persistence and pathogenicity including duration, route, and extent of viral shedding.

Transmission

1. Establish the mode of transmission and mechanisms of persistence of LPAI compared with HPAI, identifying virus-hosts factors that influence virus transmissibility.
2. Investigate whether transmission dynamics are affected by age, host species, behaviour, and immune status especially including H5N1 HPAI.

Virus characteristics and evolution

1. Enhance the depth and breadth of baseline data based upon full genome sequences of AI viruses in wild bird populations available in the public domain.



OFFLU Research Priorities on Swine Influenza

Diagnostics

1. Investigate serologic cross-reactivity among various swine influenza virus (SIV) lineages in hemagglutination-inhibition (HI) and virus-neutralization (VN) tests, as well as between human and swine influenza viruses, to facilitate better interpretation of serologic data in swine and in humans, recognizing that it is highly likely that exposure to multiple virus strains/lineages may make a correct interpretation of serologic data impossible.
2. Identify subtype- and lineage-specific B-cell epitopes. Investigate the feasibility of discriminative serologic assays based on such epitopes.
3. Develop primers for different types of PCR (generic as well as more specific for major SIV lineages).

Epidemiology and control

1. Conduct representative virological and serological surveys at regular intervals in various regions of the world.
2. Conduct studies into the population dynamics of SIVs, e.g. to address issues of how common is virus persistence at the farm level and the major routes of virus spread between farms.
3. Conduct closer monitoring and virological investigations of people in contact with pigs, especially those who present with influenza-like illness.
4. Investigate the possible role of humans in influenza virus infection of pigs and virus introduction into farms.

Immunology and immune responses

1. Conduct detailed studies of the humoral and cellular immune responses of pigs to influenza virus infections over time and to their viral targets.
2. Identify cross-reactive and cross-protective antibody- and T-cell epitopes in SIVs
3. Study the extent of heterovariant and heterosubtypic protection between influenza viruses in pigs, and the immune mechanisms involved.
4. Study the extent of cross-protection among influenza viruses of swine, humans and birds, and the immune mechanisms involved.

Pathogenesis and transmission


1. Investigate the molecular basis for tissue and cell tropism of influenza viruses in pigs, including the potential neurotropism of SIVs and intrinsic susceptibility of extra-respiratory tissues e.g. the epithelial cells of the intestinal tract.
2. Evaluate the molecular and host factors that determine adaptation of influenza viruses from birds or humans to pigs, and transmission of such viruses among pigs. More critical analysis of the significance of sialic acid receptors. Identify co-receptors and other barriers for replication.
3. Investigate the viral and host factors determining the virulence of influenza viruses for pigs.
4. Study the innate immune response to influenza viruses in pigs. Role of various cytokines in disease and pathology, as well as the adaptive immune response. Mechanisms of induction of cytokines.
5. Study the viral and host factors that contribute to the successful transmission of SIVs to other species, identifying markers of swine influenza viruses with human pandemic potential.
6. Conduct *in vivo* and *ex vivo* (organ culture) studies to gain detailed insights into the process of genetic reassortment of influenza viruses in the pig.
7. Investigate the interactions between SIV and other respiratory viruses or bacteria.

Vaccines and vaccination

1. Conduct comparative studies of the immune response and protection after infection with live virus versus vaccination with various types of vaccine.
2. Study the importance of vaccine strain (antigenic match) versus antigen dose and type of adjuvant for the efficacy of killed SIV vaccines.
3. Evaluate novel influenza vaccination strategies with the potential to induce a broader and more durable immune response, or to avoid interference from maternal immunity.
4. Investigate prime-boost immunization strategies with DNA or vector vaccines followed by killed virus vaccine.

Virus characteristics and evolution

1. Investigate virus survival in the environment in different climatic conditions.
2. Study the genetic evolution of SIVs. Viruses collected in the 10 years prior to the 2009 pandemic, should be sequenced as far as possible to improve the understanding of the emergence of the pandemic H1N1 virus.

3. Undertake thorough characterization of SIV isolates from humans, birds or other species, to identify potential factors involved in interspecies transmission of SIVs.
 4. Study the antigenic evolution of SIVs as compared with human influenza viruses, developing an understanding of the mechanisms of antigenic drift to assess why influenza viruses appear to show more rapid antigenic drift in humans than in swine.
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OFFLU Research Priorities on Equine Influenza

Virus characteristics and evolution

A formal global Equine Influenza Surveillance Programme was initiated by the OIE Biological Standards Commission in 1995. The OIE reference laboratories and other laboratories collect data on outbreaks of EI and strain characterisation which is reviewed annually by an Expert Surveillance Panel (ESP). This panel makes recommendations on the need to update vaccines which are published in the OIE Bulletin. The current disparity in the level of surveillance and virus collection in different countries results in potentially biased information about the relative prevalence of different viruses.

There is a need for:

1. Increased global surveillance/adequately funded active surveillance programmes.*
2. Development of standard procedures to characterize viruses that correctly determines their impact on vaccine efficacy.*
3. The development of a comprehensive range of specific antisera (ferret, horse and other species as appropriate) for antigenic characterization of viruses.*
4. Whole equine influenza virus genome sequencing.*
5. Identification of genetic sequences influencing antigenicity, virulence, transmissibility and attenuation.*

Diagnostics

Sensitive diagnostic techniques exist but there is a need for:

1. International Harmonisation/Standardisation of RT-PCR and other tests used for the international movement of horses.*
2. Development of sensitive “horse-side” kits (both PCR and ELISA based) for the detection of equine influenza. (The kits for the diagnosis of human influenza that are currently used to detect infected horses in quarantine and other facilities are considerably less sensitive than laboratory based RT-PCR.)
3. Development of assays that differentiate between antibodies produced in response to vaccination and those produced in response to natural infection (DIVA).

* The most important research priorities

Vaccines and vaccination

Whole virus inactivated, subunit, canary pox recombinant and modified live vaccines are used in different countries. Current vaccination regimes are roughly based on human influenza vaccination regimes.

There is a need to:

1. Systematically identify and record vaccination breakdown. *
2. Identify factors that affect the response to vaccination e.g. genetic factors, age, gender, maternal antibodies, training programmes. *
3. Identify frequency of vaccination and regimes that lead to a durable response without windows of susceptibility or excessive vaccination. Standardise intervals recommended between doses and harmonise vaccination requirements internationally. *
4. Conduct independent comparative vaccine studies.
5. Improve vaccine efficacy by developing new vaccines, adjuvants and antigen presentation systems that mimic natural infection and induce durable virological protection.
6. Develop vaccines that are easily updated with epidemiologically relevant strains using for example, reverse genetics.
7. Development of vaccines engineered to express a marker so that they can be used with a DIVA test (see above).
8. Develop and harmonise fast-track protocols for the registration and licensure of existing vaccines that have been updated with epidemiologically relevant viruses.
9. Encourage harmonisation of quality assurance protocols so that equine influenza vaccines are of a more consistent standard throughout the world.

Immunology and Immune responses

1. Characterise the nature and kinetics of the immunological response to vaccination in horses and to natural infection with a view to elucidating why vaccinated horses become infected and shed virus. *
2. Identify correlates of protection additional to antibodies against the Haemagglutinin (HA). This is particularly relevant for assessment of second generation vaccines that protect in the absence of detectable vaccinal antibodies against HA.
3. Assess the contribution of the immune response to pathogenesis.

* The most important research priorities

Transmission

The current equine influenza viruses are considered to be of avian ancestry and avian H5N1 has been associated with respiratory disease in donkeys. Interspecies transmission of equine influenza to dogs and pigs has been demonstrated.

Research priorities include:

1. Investigation of interspecies transmission of equine influenza virus by active surveillance.*
2. Elucidation of interactions of equine influenza virus proteins with host cell receptors of different species.*
3. Identification of host restriction factors as innate barriers to block transmission between different species.
4. Identification of mutations that can facilitate the replication of avian influenza viruses in horses and other equidae.*

Epidemiology

Future control strategies would be improved by:

1. The development of standard protocols for epidemiological investigations of influenza in equidae and the global sharing of the results of such investigations.
2. Determination of the impact of varied equestrian activities on virus transmission and disease expression.
3. Investigation of influenza outbreaks and transmission of virus between horses with a view to developing models for risk assessment.
4. A methodical approach to the identification of reservoirs for equine influenza viruses.

Pathogenesis

Research priorities include:

1. Identification of host determinants that influence disease expression.
2. Analysis of the transcriptional landscape of horses infected with influenza.
3. Identification of virus determinants that influence disease severity.
4. Identification of unusual manifestations of equine influenza virus infection (e.g. neurological disease) and the establishment of a causal link.

* The most important research priorities

5. Evaluation of the pathophysiological effects of influenza on respiratory function in the equine athlete.
6. Investigation of synergism between equine influenza virus and certain bacterial pathogens.

Control

Control measures differ between endemic countries and those that suffer only an occasional incursion and aim to remain influenza free.

It is important to:

1. Identify high risk populations/situations and increase awareness of the benefit of vaccination and the implementation of biosecurity measures. Review *OIE Animal Health Code*.
2. Evaluate different management and treatment regimes in the field (cohort studies) in order to develop evidence - based protocols for disease control.
3. Evaluate the therapeutic effect of antiviral drugs in controlled trials and apply cost-benefit analyses periodically.

