

**Subjek : Avian Influenza
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Kate A. Henning, Joerg Henning, John Morton, Ngo Thanh Long, Nguyen Truc Ha, Joanne Meers, Farm- and flock-level risk factors associated with Highly Pathogenic Avian Influenza outbreaks on small holder duck and chicken farms in the Mekong Delta of Viet Nam, *Preventive Veterinary Medicine*, Volume 91, Issues 2-4, 1 October 2009, Pages 179-188, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2009.05.027.

(<http://www.sciencedirect.com/science/article/B6TBK-4WP4BDF-1/2/0b0adb732fba722f0c2654843ff10d56>)

Abstract:

After 11 consecutive months of control, the Mekong Delta in Viet Nam experienced a wave of Highly Pathogenic Avian Influenza (HPAI) H5N1 outbreaks on small holder poultry farms from December 2006 to January 2007. We conducted a retrospective matched case-control study to investigate farm- and flock-level risk factors for outbreak occurrence during this period. Twenty-two case farms were selected from those where clinical signs consistent with HPAI H5N1 had been present and HPAI H5N1 had been confirmed with a positive real-time PCR test from samples obtained from affected birds. For every case farm enrolled, two control farms were selected matched on time of outbreak occurrence, farm location and species. Veterinarians conducted interviews with farmers, to collect information on household demographics, farm characteristics, husbandry practices, trading practices, poultry health, vaccination and biosecurity. Exact stratified logistic regression models were used to assess putative risk factors associated with a flock having or not having a HPAI outbreak. Nested analyses were also performed, restricted to subsets of farms using scavenging, confinement or supplementary feeding practices. Risk of an outbreak of HPAI H5N1 was increased in flocks that had received no vaccination (odds ratio (OR) = 20.2; 95% confidence interval (CI): 1.0, +infinity) or only one vaccination (OR = 85.2; 95% CI: 6.5, +infinity) of flocks compared to two vaccinations, and in flocks on farms that had family and friends visiting (OR = 8.2; 95% CI: 1.0, +infinity) and geese present (OR = 11.5; 95% CI: 1.1, +infinity). The subset analysis using only flocks that scavenged showed that sharing of scavenging areas with flocks from other farms was associated with increased risk of an outbreak (OR = 10.9; 95% CI: 1.4, 492.9). We conclude that none or only one vaccination, visitors to farms, the presence of geese on farms and sharing of scavenging areas with ducks from other farms increase the risk of HPAI H5N1 outbreaks in poultry flocks in Viet Nam.

Keywords: Poultry; Avian influenza; Risk factors; Viet Nam

Archie C.A. Clements, Dirk U. Pfeiffer, Emerging viral zoonoses: Frameworks for spatial and spatiotemporal risk assessment and resource planning, *The Veterinary Journal*, Volume 182, Issue 1, October 2009, Pages 21-30, ISSN 1090-0233, DOI: 10.1016/j.tvjl.2008.05.010.

(<http://www.sciencedirect.com/science/article/B6WXN-4T8337S-1/2/8dd2cb0abd00b5467a4f5c7daf643215>)

Abstract:

Spatial epidemiological tools are increasingly being applied to emerging viral zoonoses (EVZ), partly because of improving analytical methods and technologies for data capture and management, and partly because the demand is growing for more objective ways of allocating limited resources in the face of the emerging threat posed by these diseases. This review documents applications of geographical information systems (GIS), remote sensing (RS) and spatially-explicit statistical and mathematical models to epidemiological studies of EVZ.

Landscape epidemiology uses statistical associations between environmental variables and diseases to study and predict their spatial distributions. Phylogeography augments epidemiological

knowledge by studying the evolution of viral genetics through space and time. Cluster detection and early warning systems assist surveillance and can permit timely interventions. Advanced statistical models can accommodate spatial dependence present in epidemiological datasets and can permit assessment of uncertainties in disease data and predictions. Mathematical models are particularly useful for testing and comparing alternative control strategies, whereas spatial decision-support systems integrate a variety of spatial epidemiological tools to facilitate widespread dissemination and interpretation of disease data. Improved spatial data collection systems and greater practical application of spatial epidemiological tools should be applied in real-world scenarios.

Keywords: Spatial analysis; Geographical information systems; Rift Valley fever; West Nile virus; Highly pathogenic avian influenza; Rabies; Risk analysis

Eunok Jung, Shingo Iwami, Yasuhiro Takeuchi, Tae-Chang Jo, Optimal control strategy for prevention of avian influenza pandemic, *Journal of Theoretical Biology*, Volume 260, Issue 2, 21 September 2009, Pages 220-229, ISSN 0022-5193, DOI: 10.1016/j.jtbi.2009.05.031.

(<http://www.sciencedirect.com/science/article/B6WMD-4WGK4R5-4/2/935fdd5d08951b12c180457d609a088e>)

Abstract:

The spread of H5N1 virus to Europe and continued human infection in Southeast Asia have heightened pandemic concern. Although, fortunately, sustained human-to-human transmissions have not been reported yet, it is said that a pandemic virus which can be easily transmitted among humans certainly emerges in the future. In this study, we extended the previous studies for the prevention of the pandemic influenza to evaluate the time-dependent optimal prevention policies, which are associated with elimination policy and quarantine policy, considering its execution cost. Actually, the execution cost affects the optimal strategy of prevention policies and the prevention of the disease spread. We found that the quarantine policy is very important rather than the elimination policy during the disease spread, even if the unit execution cost of the quarantine policy is more expensive than that of the elimination policy. And also, the change of the unit execution cost does affect the total cumulative cost of the optimal prevention policies but does not affect the relative frequency of each cumulative execution cost. Furthermore, interestingly, we revealed that an optimal strategy to reduce the number of total infected humans might increase a chance of invadability of the mutant influenza.

Keywords: Epidemic model; Avian influenza; Optimal control theory; Elimination policy; Quarantine policy; Invadability

Benjamin Roche, Camille Lebarbenchon, Michel Gauthier-Clerc, Chung-Ming Chang, Frederic Thomas, Francois Renaud, Sylvie van der Werf, Jean-Francois Guegan, Water-borne transmission drives avian influenza dynamics in wild birds: The case of the 2005-2006 epidemics in the Camargue area, *Infection, Genetics and Evolution*, Volume 9, Issue 5, September 2009, Pages 800-805, ISSN 1567-1348, DOI: 10.1016/j.meegid.2009.04.009.

(<http://www.sciencedirect.com/science/article/B6W8B-4W3HX83-1/2/ea1ce6181466a3c2fe748de536c5d7a4>)

Abstract:

Transmission and persistence of avian influenza viruses (AIV) among wildlife remains an unresolved issue because it depends both on the ecology of the host (e.g. population density, migration) and on the environment (e.g. AIV persistence in water). We have developed a mathematical model that accounts for both AIV epidemics and bird community dynamics. The model is parameterized using bird counts and AIV prevalence data. Results suggest that the transmission patterns driving the dynamics of infection at our study site (Camargue, South of France) involved both a density-dependent and a water-borne transmission processes. Water-borne transmission is, however, the main determinant of the disease dynamics and observed

prevalence level. This pattern of transmission highlights the importance of the persistence of viral particles in water in AIV dynamics in wild birds.

Keywords: Influenza A; Water-borne transmission; Mathematical modeling

Kamol Suwannakarn, Alongkorn Amonsin, Jiroj Sasipreeyajan, Pravina Kitikoon, Rachod Tantilertcharoen, Sujira Parchariyanon, Arunee Chaisingh, Bandit Nuansrichay, Thaweesak Songserm, Apiradee Theamboonlers, Yong Poovorawan, Molecular evolution of H5N1 in Thailand between 2004 and 2008, *Infection, Genetics and Evolution*, Volume 9, Issue 5, September 2009, Pages 896-902, ISSN 1567-1348, DOI: 10.1016/j.meegid.2009.06.004.

(<http://www.sciencedirect.com/science/article/B6W8B-4WJ3DV8-3/2/9f5faacbf8dc92b3531d7a55de79a98d>)

Abstract:

Highly pathogenic avian influenza (HPAI) H5N1 viruses have seriously affected the Asian poultry industry since their occurrence in 2004. Thailand has been one of those countries exposed to HPAI H5N1 outbreaks. This project was designed to compare the molecular evolution of HPAI H5N1 in Thailand between 2004 and 2008. Viruses with clade 1 hemagglutinin (HA) were first observed in early 2004 and persisted until 2008. Viruses with clade 2.3.4 HA were first observed in the northeastern region of Thailand between 2006 and 2007. Phylogenetic analysis among Thai isolates indicated that clade 1 viruses in Thailand consist of three distinct lineages: CUK2-like, PC168-like, and PC170-like viruses. The CUK2-like virus represents the predominant lineage and has been circulating throughout the course of the 4-year outbreaks. Analysis of recently isolated viruses has shown that the genetic distance was slightly different from viruses of the early outbreak and that CUK2-like viruses comprise the native strain. Between 2005 and 2007, PC168-like and PC170-like viruses were first observed in several areas around central and lower northern Thailand. In 2008, viruses reassorted from these two lineages, PC168-like and PC170-like viruses, were initially isolated in the lower northern provinces of Thailand and subsequently spread to the upper central part of Thailand. On the other hand, CUK2-like viruses were still detected around the lower northern and the upper central part of Thailand. Furthermore, upon emergence of the reassorted viruses, the PC168-like and PC170-like lineages could not be detected, suggesting that the only predominant strains still circulating in Thailand were CUK2-like and reassorted viruses. The substitution rate among clade 1 viruses in Thailand was lower. The virus being limited to the same area might explain the lower nucleotide substitution rate. This study has demonstrated that nationwide attempts to monitor the virus may help curb access and propagation of new HPAI viral genes.

Keywords: H5N1; Influenza; Thailand; Molecular evolution

C.P. Jewell, T. Kypraios, R.M. Christley, G.O. Roberts, A novel approach to real-time risk prediction for emerging infectious diseases: A case study in Avian Influenza H5N1, *Preventive Veterinary Medicine*, Volume 91, Issue 1, Special Issue: GisVet 2007, 1 September 2009, Pages 19-28, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2009.05.019.

(<http://www.sciencedirect.com/science/article/B6TBK-4WJ2CBB-1/2/66e9bce866a210bea2478c960f9f7e13>)

Abstract:

Mathematical simulation modelling of epidemic processes has recently become a popular tool in guiding policy decisions for potential disease outbreaks. Such models all rely on various parameters in order to specify quantities such as transmission and detection rates. However, the values of these parameters are peculiar to an individual outbreak, and estimating them in advance of an epidemic has been the major difficulty in the predictive credibility of such approaches.

The obstruction to classical approaches in estimating model parameters has been that of missing data: (i) an infected individual is only detected after the onset of clinical signs, we never observe the time of infection directly; (ii) if we wish to make inference on an epidemic while it is in progress

(in order to predict how it might unfold in the future), we must take into account the fact that there may be individuals who are infected but not yet detected.

In this paper we apply a reversible-jump Markov chain Monte Carlo algorithm to a combined spatial and contact network model constructed in a Bayesian context to provide a real-time risk prediction during an epidemic. Using the example of a potential Avian H5N1 epidemic in the UK poultry industry, we demonstrate how such a technique can be used to give real-time predictions of quantities such as the probability of individual poultry holdings becoming infected, the risk that individual holdings pose to the population if they become infected, and the number and whereabouts of infected, but not yet detected, holdings. Since the methodology generalises easily to many epidemic situations, we anticipate its use as a real-time decision-support tool for targetting disease control to critical transmission processes, and for monitoring the efficacy of current control policy.

Keywords: Epidemic; Inference; Prediction; Bayesian; Reversible jump MCMC; Risk

Donata Kalthoff, Anja Globig, Martin Beer, (Highly pathogenic) Avian Influenza as a zoonotic agent, *Veterinary Microbiology*, In Press, Accepted Manuscript, Available online 26 August 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.08.022.

(<http://www.sciencedirect.com/science/article/B6TD6-4X378C8-1/2/ada689a3019ccf26319614a35694aeb5>)

Abstract: Summary

Zoonotic agents challenging the world every year afresh are influenza A viruses. In the past, human pandemics caused by influenza A viruses had been occurring periodically. Wild aquatic birds are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses. Whereas avian influenza viruses in their natural avian reservoir are generally of low pathogenicity (LPAIV), some have gained virulence by mutation after transmission and adaptation to susceptible gallinaceous poultry. Those so-called highly pathogenic avian influenza viruses (HPAIV) then cause mass die-offs in susceptible birds and lead to tremendous economical losses when poultry is affected. Besides a number of avian influenza virus subtypes that have sporadically infected mammals, the HPAIV H5N1 Asia shows strong zoonotic characteristics and it was transmitted from birds to different mammalian species including humans. Theoretically, pandemic viruses might derive directly from avian influenza viruses or arise after genetic re-assortment between viruses of avian and mammalian origin. So far, HPAIV H5N1 already meets two conditions for a pandemic virus: as a new subtype it has been hitherto unseen in the human population and it has infected at least 387 people, and caused severe illness and high lethality in 245 humans to date (Sep 08). The acquisition of efficient human-to-human transmission would complete the emergence of a new pandemic virus. Therefore, fighting H5N1 at its source is the prerequisite to reduce pandemic risks posed by this virus. Other influenza viruses regarded as pandemic candidates derive from subtypes H2, H7, and H9 all of which have infected humans in the past. Here, we will give a comprehensive overview on avian influenza viruses in concern to their zoonotic potential.

Keywords: influenza A virus; highly pathogenic avian influenza viruses; zoonoses

Luca Busani, Maria Grazia Valsecchi, Emanuela Rossi, Marica Toson, Nicola Ferre, Manuela Dalla Pozza, Stefano Marangon, Risk factors for highly pathogenic H7N1 avian influenza virus infection in poultry during the 1999-2000 epidemic in Italy, *The Veterinary Journal*, Volume 181, Issue 2, August 2009, Pages 171-177, ISSN 1090-0233, DOI: 10.1016/j.tvjl.2008.02.013.

(<http://www.sciencedirect.com/science/article/B6WXN-4T4WM3Y-2/2/d5d2563ba5a131096503475d8c5e59aa>)

Abstract:

In 1999-2000, Italian poultry production was disrupted by an H7N1 virus subtype epidemic of highly pathogenic avian influenza (HPAI). The objectives of the present study were to identify risk

factors for infection on poultry farms located in regions that had the highest number of outbreaks (Veneto and Lombardia) and the impact of pre-emptive culling as a complementary measure for eradicating infection. A Cox regression model that included spatial factors, such as the G index, was used. The results confirmed the relationship between risk of infection and poultry species, production type and size of farms. The effectiveness of pre-emptive culling was confirmed. An increased risk of infection was observed for poultry farms located near an infected farm and those at altitudes less than 150 m above sea level. The measures for the control and eradication of AI virus infection need to consider species differences in susceptibility, the types of production and the density of poultry farms in the affected areas.

Keywords: Avian influenza; Poultry; Epidemiology; Survival analysis; Risk factors

Shingo Iwami, Yasuhiro Takeuchi, Xianning Liu, Shinji Nakaoka, A geographical spread of vaccine-resistance in avian influenza epidemics, *Journal of Theoretical Biology*, Volume 259, Issue 2, 21 July 2009, Pages 219-228, ISSN 0022-5193, DOI: 10.1016/j.jtbi.2009.03.040.

(<http://www.sciencedirect.com/science/article/B6WMD-4W1BVDP-3/2/571cc59966dfefb43256289f0896bede>)

Abstract:

Vaccination can be a useful tool for control of avian influenza outbreaks in poultry, but its use is reconsidered in most of the countries worldwide because of its negative effects on the disease control. One of the most important negative effects is the potential for emergence of vaccine-resistant viruses. Actually, in the vaccination program in China and Mexico, several vaccine-resistant strains were confirmed. Vaccine-resistant strains usually cause a loss of the protection effectiveness of vaccination. Therefore, a vaccination program that engenders the emergence of the resistant strain might promote the spread of the resistant strain and undermine the control of the infectious disease, even if the vaccination protects against the transmission of a vaccine-sensitive strain. We designed and analyzed a deterministic patch-structured model in heterogeneous areas (with or without vaccination) illustrating transmission of vaccine-sensitive and vaccine-resistant strains during a vaccination program. We found that the vaccination program can eradicate the vaccine-sensitive strain but lead to a prevalence of vaccine-resistant strain. Further, interestingly, the replacement of viral strain could occur in another area without vaccination through a migration of non-infectious individuals due to an illegal trade of poultry. It is also a novel result that only a complete eradication of both strains in vaccination area can achieve the complete eradication in another areas. Thus we can obtain deeper understanding of an effect of vaccination for better development of vaccination strategies to control avian influenza spread.

Keywords: Epidemic model; Patch-structured model; Avian influenza; Vaccination program; Geographical spread; Illegal poultry trade

Suwarak Wanaratana, Rachod Tantilertcharoen, Jiroj Sasipreeyajan, Somsak Pakpinyo, The inactivation of avian influenza virus subtype H5N1 isolated from chickens in Thailand by chemical and physical treatments, *Veterinary Microbiology*, In Press, Corrected Proof, Available online 10 July 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.07.008.

(<http://www.sciencedirect.com/science/article/B6TD6-4WR66FD-9/2/b969a58ef06b73362c3770a87695f5fc>)

Abstract:

The objectives of this study were to determine the survival of avian influenza virus (AIV) subtype H5N1 under various physical and chemical treatments, including disinfectants, temperature and pH. The highly pathogenic AIVs subtype H5N1 were isolated from internal organs of suspected chickens and were characterized by the inoculation into chicken embryonated eggs (CEEs), hemagglutination (HA) test, hemagglutination inhibition (HI) test, reverse transcriptase polymerase chain reaction (RT-PCR) and nucleotide sequencing of hemagglutinin (H) and neuraminidase (N) genes. Three H5N1 isolates, at the concentration of 10⁹ 50% embryo lethal dose (ELD₅₀)/ml,

were used for the determination of the survival of the virus under different chemical and physical treatments. The chemical treatments were performed by incubating the viruses with various types of disinfectants including glutaraldehyde (Glu), hydrogen peroxide, quaternary ammonium compounds (QAC), Glu + QAC, iodine, chlorine, formalin and phenol, at 25 and 37 [degree sign]C, for 0, 5, 7, and 14 days. The physical treatments included incubation of the viruses at 55, 60, 65, 70 and 75 [degree sign]C for 10, 15, 30, 45 and 60 min or pH 3, 5, 7, 9 and 12. The results revealed that AIV H5N1 reference viruses, 2004.1, CUK-2/04 and 2004.2, showed low or no resistance against Glu + QAC, chlorine and phenol at both tested temperatures. Incubations at 70 [degree sign]C for 60 min or at least 75 [degree sign]C for at least 45 min could effectively inactivate all of the isolates, whereas all ranges of pH could not inactivate any of them. In this study, CUK-2/04 was more resistant to the disinfectants, temperatures, and pH compared to the other isolates.

Keywords: H5N1; Inactivation; Disinfectants; Temperature; pH

Rui Wu, Hongbo Zhang, Keli Yang, Wangwang Liang, Zhongliang Xiong, Zewen Liu, Xuehai Yang, Huabin Shao, Xinmin Zheng, Mingxin Chen, Diping Xu, Multiple amino acid substitutions are involved in the adaptation of H9N2 avian influenza virus to mice, *Veterinary Microbiology*, Volume 138, Issues 1-2, 2 July 2009, Pages 85-91, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.03.010. (<http://www.sciencedirect.com/science/article/B6TD6-4VTPY8-B/2/31b1f0e01e83be2bd9fd30696dc7eb53>)

Abstract:

To explore adaptation of avian influenza virus to mice we previously performed serial lung-to-lung passages of the influenza A/Chicken/Jiangsu/7/2002 (H9N2) strain, resulting in the isolation of a variant influenza strain lethal for mice. We now report that virulence correlates with improved growth characteristics on mammalian cells and extended tissue tropism *in vivo*. Sequencing of the complete genomes of the wild-type and mouse-adapted viruses revealed 25 amino acid substitutions. Some were found to reiterate known substitutions in human and swine H9N2 influenza isolates. Functions affected include nuclear localization signals and sites of protein and RNA interaction, while others are known determinants of pathogenicity and host specificity such as the viral polymerase PB2 E627K substitution. These observations suggest that enhanced growth characteristics and modified cell tropism may contribute to increased virulence in mice. We conclude that multiple amino acid substitutions are likely to be involved in the adaptation of H9N2 avian influenza virus to mice.

Keywords: Avian influenza virus; H9N2 subtype; Mouse; Adaptation; Pathogenicity

Carol J. Cardona, Zheng Xing, Christian E. Sandrock, Cristina E. Davis, Avian influenza in birds and mammals, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 255-273, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.001.

(<http://www.sciencedirect.com/science/article/B6T5H-4SHFSM3-1/2/c8b96214bd2d8c39a9e941cb8a727721>)

Abstract:

The disease syndromes caused by avian influenza viruses are highly variable depending on the host species infected, its susceptibility and response to infection and the virulence of the infecting viral strain. Although avian influenza viruses have a broad host range in general, it is rare for an individual strain or subtype to infect more than one species. The H5N1 highly pathogenic avian influenza virus (HPAIV) lineages of viruses that descended from A/goose/Guangdong/96 (H5N1 HPAIV) are unusual in the diversity of species they have infected worldwide. Although the species affected by H5N1 HPAI in the field and those that have been experimentally studied are diverse, their associated disease syndromes are remarkably similar across species. In some species, multi-organ failure and death are rapid and no signs of the disease are observed. Most

prominently in this category are chickens and other avian species of the order Galliformes. In other species, neurologic signs develop resulting in the death of the host. This is what has been reported in domestic cats (Carnivora), geese (Anseriformes), ratites (Struthioniformes), pigeons inoculated with high doses (Columbiformes) and ducks infected with H5N1 HPAIV isolated since 2002 (Anseriformes). In some other species, the disease is more prolonged and although multi-organ failure and death are the eventual outcomes, the signs of disease are more extensive. Predominantly, these species include humans (Primates) and the laboratory models of human disease, the ferret (Carnivora), mouse (Rodentia) and cynomolgous macaques (Primates). Finally, some species are more resistant to infection with H5N1 HPAIV and show few or no signs of disease. These species include pigeons in some studies (Columbiformes), ducks inoculated with pre-2002 isolates (Anseriformes), and pigs (Artiodactyla).

Keywords: Avian influenza; H5N1 highly pathogenic avian influenza; Disease; Lesions; Birds; Mammals; Grippe aviaire; Grippe aviaire H5N1 hautement pathogene; Maladie; Lesions; Oiseaux; Mammiferes

Walter M. Boyce, Christian Sandrock, Chris Kreuder-Johnson, Terra Kelly, Carol Cardona, Avian influenza viruses in wild birds: A moving target, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 275-286, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.002.

(<http://www.sciencedirect.com/science/article/B6T5H-4SK4X9J-1/2/e2bf0af0dcf6121de9ef62a2a061fddb>)

Abstract:

The long-standing evolutionary and ecological relationships between wild birds and influenza A viruses has created a broad pool of viral genetic diversity and a reservoir of potentially transmissible viruses. An understanding of these relationships can help us identify and modify critical control points to reduce transmission of avian influenza viruses into animal and human populations.

Keywords: Avian influenza virus; Wild bird; Ecology; Interspecies transmission; Virus aviaire de grippe; Oiseau sauvage; Ecologie; Transmission d'Interspecies

Marguerite Pappaioanou, Highly pathogenic H5N1 avian influenza virus: Cause of the next pandemic?, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 287-300, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.003.

(<http://www.sciencedirect.com/science/article/B6T5H-4VX7GRW-2/2/74b19bde26e47022ca7d8297f06aa03f>)

Abstract:

Since 1997, when human infections with a highly pathogenic (HP) avian influenza A virus (AIV) subtype H5N1 - previously infecting only birds - were identified in a Hong Kong outbreak, global attention has focused on the potential for this virus to cause the next pandemic. From December 2003, an unprecedented H5N1 epizootic in poultry and migrating wild birds has spread across Asia and into Europe, the Middle East, and Africa. Humans in close contact with sick poultry and on rare occasion with other infected humans, have become infected. As of early March 2007, 12 countries have reported 167 deaths among 277 laboratory-confirmed human infections to WHO. WHO has declared the world to be in Phase 3 of a Pandemic Alert Period. This paper reviews the evolution of HP AIV H5N1, molecular changes that enable AIVs to infect and replicate in human cells and spread efficiently from person-to-person, and strategies to prevent the emergence of a pandemic virus.

Keywords: Influenza; Avian influenza; Pandemic; H5N1

Chang-Won Lee, Yehia M. Saif, Avian influenza virus, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 301-310, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.007.

(<http://www.sciencedirect.com/science/article/B6T5H-4SK4X9J-2/2/f9a099bc1518418e868f5c55c2346f1f>)

Abstract:

Avian influenza viruses do not typically replicate efficiently in humans, indicating direct transmission of avian influenza virus to humans is unlikely. However, since 1997, several cases of human infections with different subtypes (H5N1, H7N7, and H9N2) of avian influenza viruses have been identified and raised the pandemic potential of avian influenza virus in humans. Although circumstantial evidence of human to human transmission exists, the novel avian-origin influenza viruses isolated from humans lack the ability to transmit efficiently from person-to-person. However, the on-going human infection with avian-origin H5N1 viruses increases the likelihood of the generation of human-adapted avian influenza virus with pandemic potential. Thus, a better understanding of the biological and genetic basis of host restriction of influenza viruses is a critical factor in determining whether the introduction of a novel influenza virus into the human population will result in a pandemic. In this article, we review current knowledge of type A influenza virus in which all avian influenza viruses are categorized.

Keywords: Avian influenza virus; H5N1; Transmission; Virus de la grippe aviaire; H5N1; Transmission

Blanca Lupiani, Sanjay M. Reddy, The history of avian influenza, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 311-323, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.004.

(<http://www.sciencedirect.com/science/article/B6T5H-4SNGRKV-1/2/bd67bb37c3c1cb5a8c6c85f3bef9de39>)

Abstract:

The first description of avian influenza (AI) dates back to 1878 in northern Italy, when Perroncito [Perroncito E. *Epizoozia tifoide nei gallinacei. Annali Accad Agri Torino* 1878;21:87-126] described a contagious disease of poultry associated with high mortality. The disease, termed 'fowl plague', was initially confused with the acute septicemic form of fowl cholera. However, in 1880, soon after its first description, Rivolta and Delprato [as reported by Stubs EL. *Fowl pest*, In: Biester HE, Devries L, editors. *Diseases of poultry*. 1st ed. Ames, IO: Iowa State College Press; 1943. p. 493-502] showed it to be different from fowl cholera, based on clinical and pathological properties, and called it *Typhus exudatious gallinarum*. In 1901, Centanni and Savunzzi [Centanni E, Savunzzi E, *La peste aviaria I & II, Comunicazione fatta all'accademia delle scienze mediche e naturali de Ferrara*, 1901] determined that fowl plague was caused by a filterable virus; however, it was not until 1955 that the classical fowl plague virus was shown to be a type A influenza virus based on the presence of type A influenza virus type-specific ribonucleoprotein [Schafer W. *Vergleichender sero-immunologische Untersuchungen über die Viren der Influenza und klassischen Geflügelpest. Z Naturf* 1955;10b:81-91]. The term fowl plague was substituted by the more appropriate term highly pathogenic avian influenza (HPAI) at the First International Symposium on Avian Influenza [Proceedings of the First International Symposium on Avian Influenza. Beltsville, MD. 1981, *Avian Dis* 47 (Special Issue) 2003.] and will be used throughout this review when referring to any previously described fowl plague virus.

Keywords: Virus de la grippe aviaire; Peste aviaire; Hautement pathogene; Faiblement pathogene; Pandemie de grippe; Fowl plague; Avian influenza; History; Highly pathogenic; Low pathogenic; Waterfowl

Karen S. Yee, Tim E. Carpenter, Carol J. Cardona, Epidemiology of H5N1 avian influenza, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 325-340, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.005.

(<http://www.sciencedirect.com/science/article/B6T5H-4SD1KNP-1/2/ed4758258e9993f95311f101c157867e>)

Abstract:

High pathogenic (HP) H5N1 avian influenza (AI) infection has been reported in domestic poultry, wildlife, and human populations since 1996. Risk of infection is associated with direct contact with infected birds. The mode of H5N1 spread from Asia to Europe, Africa and the Far East is unclear; risk factors such as legal and illegal domestic poultry and exotic bird trade, and migratory bird movements have been documented. Measures used to control disease such as culling, stamping out, cleaning and disinfection, and vaccination have not been successful in eradicating H5N1 in Asia, but have been effective in Europe.

Keywords: Orthomyxoviridae; Highly Pathogenic Avian Influenza; Poultry diseases; Pandemic surveillance; Wild aquatic birds; Distribution; Spread; Volaille; Maladies aviaires; Gibier migratoire; Grippe aviaire hautement pathogene; Orthomyxoviridae; Surveillance de la pandémie; Epidemiologie

Bruce Charlton, Beate Crossley, Sharon Hietala, Conventional and future diagnostics for avian influenza, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 341-350, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.009.

(<http://www.sciencedirect.com/science/article/B6T5H-4SD1KNP-2/2/e58d343a801f0468d2a8d4000f8f7e27>)

Abstract:

The significant and continued transboundary spread of Asian avian influenza H5N1 since 2003, paired with documented transmission from avian species to humans and other mammals, has focused global attention on avian influenza virus detection and diagnostic strategies. While the historic and conventional laboratory methods used for isolation and identification of the virus and for detection of specific antibodies continued to be widely applied, new and emerging technologies are rapidly being adapted to support avian influenza virus surveillance and diagnosis worldwide. Molecular tools in particular are advancing toward lab-on-chip and fully integrated technologies that are capable of same day detection, pathotyping, and phylogenetic characterization of influenza A viruses obtained from clinical specimens. The future of avian influenza diagnostics, rather than moving toward a single approach, is wisely adopting a strategy that takes advantage of the range of conventional and advancing technologies to be used in 'fit-for-purpose' testing.

Keywords: Avian influenza; Detection; Diagnosis; 'Fit-for-purpose'; Grippe aviaire; Detection; Diagnostic; << sur mesure >>

David E. Swayne, Avian influenza vaccines and therapies for poultry, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 4, Avian Influenza, July 2009, Pages 351-363, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.01.006.

(<http://www.sciencedirect.com/science/article/B6T5H-4SCTSPD-1/2/020ef2ca93aa7527081fa5e4643e5853>)

Abstract:

Vaccines have been used in avian influenza (AI) control programs to prevent, manage or eradicate AI from poultry and other birds. The best protection is produced from the humoral response against the hemagglutinin (HA) protein. A variety of vaccines have been developed and tested under experimental conditions with a few receiving licensure and field use following demonstration of purity, safety, efficacy and potency. Current licensed vaccines are predominately inactivated whole AI vaccines, typically produced from low pathogenicity (LP) AI virus strains, or occasionally from high pathogenicity AI virus strains. Recently, reverse genetic procedures have been

developed that allow construction of vaccine strains using a genetically altered HA gene (changing HP HA proteolytic cleavage site to LP) and a backbone of internal gene segments for safe, high growth production. Other licensed AI vaccines include recombinant fowl poxvirus vector with an AI H5 insert and a recombinant Newcastle disease virus vector with an AI H5 gene insert. The latter vaccine can be mass administered via aerosol application.

Keywords: Avian influenza; Vaccine; Protection; Efficacy; Potency; grippe aviaire; vaccin; protection; efficacite; puissance

Etienne Thiry, Diane Addie, Sandor Belak, Corine Boucraut-Baralon, Herman Egberink, Tadeusz Frymus, Tim Gruffydd-Jones, Katrin Hartmann, Margaret J. Hosie, Albert Lloret, Hans Lutz, Fulvio Marsilio, Maria Grazia Pennisi, Alan D. Radford, Uwe Truyen, Marian C. Horzinek, H5N1 avian influenza in cats. ABCD guidelines on prevention and management, *Journal of Feline Medicine & Surgery*, Volume 11, Issue 7, July 2009, Pages 615-618, ISSN 1098-612X, DOI: 10.1016/j.jfms.2009.05.011.

(<http://www.sciencedirect.com/science/article/B6WJC-4WCWP0G-G/2/23d1a0053bdfed62d45152409117676d>)

Abstract: Overview

Avian influenza is a disease of birds, caused by a type A influenza virus. The subtype H5N1 avian influenza occurs primarily in birds and infection varies from mild disease with little or no mortality to a highly fatal, rapidly spreading epidemic (highly pathogenic avian influenza). It is extremely rare for cats to be infected and there are only very few confirmed reports of the disease in cats in Europe. Infection

Cats can be infected via the respiratory and oral routes (eg, by eating infected birds). The key precondition for infection is that the cat lives in an area where H5N1 virus infection has been confirmed in birds. Additionally, the cat should have had outdoor access to an environment where waterfowl is present, or contact with poultry or uncooked poultry meat, or close contact with an H5N1-infected, sick cat during the first week of infection. Clinical suspicion

Clinical signs in cats may include fever, lethargy, dyspnoea, conjunctivitis and rapid death. Neurological signs (circling, ataxia) have also been recorded. Diagnosis

The veterinary authorities should be notified. Oropharyngeal, nasal and/or rectal swabs or faecal samples of suspected cases should be submitted for PCR and/or virus isolation. Post-mortem samples of lung and mediastinal lymph nodes should be obtained. Particular care should be taken when handling the cat and/or samples. Disease management

The virus is sensitive to all standard medical disinfectants. Cats with suspected H5N1 infection should be kept in strict isolation. Owners should be advised to confine the cat to a separate room prior to bringing it to the veterinary clinic. Vaccination and disease prevention

No H5N1 vaccines are commercially available for cats. In the event of confirmed cases of H5N1 avian influenza in birds in the area, owners should keep their cats indoors until further information is available, and follow official regulations.

Chulseung Lee, Daesub Song, Bokyu Kang, Dongsuk Kang, Jungeun Yoo, Kwonil Jung, Gunsuk Na, Kichang Lee, Bongkyun Park, Jinsik Oh, A serological survey of avian origin canine H3N2 influenza virus in dogs in Korea, *Veterinary Microbiology*, Volume 137, Issues 3-4, 12 June 2009, Pages 359-362, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.01.019.

(<http://www.sciencedirect.com/science/article/B6TD6-4VDS8DH-2/2/1edcfdc4a80fa580688a98323afde8df>)

Abstract:

Canine H3N2 influenza viruses of avian origin were recently isolated and found to induce disease in dogs. Results of serologic analysis indicate that avian origin canine influenza virus can spread rapidly through local dog populations, which indicates its potential for becoming established in dogs throughout Korea.

Keywords: Canine influenza virus; H3N2; Serologic analysis; Dog; Avian

Yimeng Wang, Chunqiao Shan, Shuangxi Ming, Yan Liu, Yuchun Du, Guotuo Jiang, Immunoadjuvant effects of bacterial genomic DNA and CpG oligodeoxynucleotides on avian influenza virus subtype H5N1 inactivated oil emulsion vaccine in chicken, *Research in Veterinary Science*, Volume 86, Issue 3, June 2009, Pages 399-405, ISSN 0034-5288, DOI: 10.1016/j.rvsc.2008.09.006.

(<http://www.sciencedirect.com/science/article/B6WWR-4TTDYXN-1/2/35174b1c7ced08df8d02c8fdb6c3ae8>)

Abstract:

This study investigated the immunoadjuvant effects of three types of bacterial genomic DNA and CpG oligonucleotides (CpG ODN) on the avian influenza virus (AIV) subtype H5N1 inactivated oil emulsion vaccine under two immunization strategies. The genomic DNA extracted from *Escherichia coli* O2, *Staphylococcus aureus*, *Streptococcus faecalis* FQ68, and synthetic CpG ODN were used as adjuvants, and their effects on the AIV oil emulsion vaccine were examined in chickens. The results indicated that when administered separately from the vaccine, adjuvants induced lower haemagglutination inhibition (HI) titres and serum IgG titres but resulted in higher concentrations of IFN- γ and IL-10. In contrast, when combined with the oil emulsion vaccine prior to inoculation, CpG ODN induced higher HI, IgG titres and IFN- γ concentration but resulted in lower IL-10 concentration. These data suggest that, depending on the immunization approaches, adjuvants may exert distinct immune effects in chickens receiving AIV H5N1 oil emulsion vaccine: the prior incorporation of CpG ODN into the vaccine may augment both the humoral and Th1 type immune responses, while separate inoculation of adjuvants has not shown better adjuvanticity.

Keywords: CpG ODN; Bacterial genomic DNA; Avian influenza virus subtype H5N1 inactivated oil emulsion vaccine; Adjuvant; Immunization approaches; Immune responses

Qingping Luo, Hongliang Huang, Wei Zou, Hanbing Dan, Xuebo Guo, Anding Zhang, Zhengjun Yu, Huanchun Chen, Meilin Jin, An indirect sandwich ELISA for the detection of avian influenza H5 subtype viruses using anti-hemagglutinin protein monoclonal antibody, *Veterinary Microbiology*, Volume 137, Issues 1-2, 28 May 2009, Pages 24-30, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.12.009.

(<http://www.sciencedirect.com/science/article/B6TD6-4V4KPTB-2/2/9d87cdf80646cfe38c9c04b8e67494ac>)

Abstract:

A sandwich ELISA test using AIV H5 subtype specific monoclonal antibody (clone 2H4) to an epitope of hemagglutinin protein has been developed. The monoclonal antibody was used to capture the antigen from clinical samples (swabs and tissues). Captured antigens from clinical samples were detected using polyclonal sera, purified AIV H5N1 particles were titrated in the sandwich ELISA and the limit of detection was determined to be approximately 1.0 ng of influenza viral protein in virus preparations. Fifteen AIV strains of H1-H15 subtypes and some other pathogens were tested by this system, and the test is specific to H5 subtype viruses as it failed to detect other AIV subtype viruses and other pathogens. Varieties of clinical samples originating from laboratory experiments (n = 382) and from fields (n = 288) were employed to test the efficacy of DAS-ELISA test. The test compared very well with the traditional method for detection of influenza virus: virus isolation (VI) in embryonated chicken eggs. In comparison to virus isolation the sensitivity and specificity of sandwich ELISA were found to be 98.6% and 97.6% respectively. In addition, the DAS-ELISA was used to test samples of experimentally infected birds and clinical samples obtained from central China in 2005. The assay proved to be sensitive and specific for the rapid detection of AIV H5 subtype virus from the tissues and swabs in infected animals.

Keywords: Avian infectious H5 subtype viruses; AIV; DAS-ELISA; Monoclonal antibody

N. Sedlmaier, K. Hoppenheidt, H. Krist, S. Lehmann, H. Lang, M. Buttner, Generation of avian influenza virus (AIV) contaminated fecal fine particulate matter (PM2.5): Genome and infectivity detection and calculation of immission, *Veterinary Microbiology*, In Press, Corrected Proof, Available online 20 May 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.05.005.

(<http://www.sciencedirect.com/science/article/B6TD6-4WBC1R3-1/2/d3683be347b1aa9b25d28b53d7fbcf50>)

Abstract:

As a model for aerosol transmission, chicken feces was spiked with avian influenza virus (AIV) subtype H10N7 and used to generate a fine particulate matter aerosol. For this an innovative aerosol chamber was developed, that collected PM2.5 on quartz microfiber filters. With AIV contaminated PM2.5 dust-coated filters different incubation times ranging from 0 to 4 days and storage mainly at +4 and +20 [degree sign]C and at different relative humidity (RH) were performed. Embryonic death in inoculated hen's eggs with filter elute was the AIV infectivity read out. To determine viral genome presence quantitative real time RT-PCR was applied.

The filter elutes contained AIV genome as well as viable virus whereby +20 [degree sign]C indicated a borderline temperature for infectious virus stability. In addition, high relative humidity was critical for AIV viability in PM2.5. The results allowed a dispersion calculation of infectious AIV in aerosols assuming a worst case scenario for an AIV outbreak in poultry farms. Thus exposure to AIV associated with PM2.5 is possible near to infected farms and may be a serious risk for fatal influenza disease in both man and animals. Airborne transmission should be effectively preventable by dispersion of water combined with disinfection into the inside air as well as the exhaust air stream of AIV infected farms.

Keywords: Avian influenza virus; Chicken feces; Fine particulate matter (PM2.5); Airborne transmission; Quartz microfiber filters

Bernd Hoffmann, Martin Beer, Scott M. Reid, Peter Mertens, Chris A.L. Oura, Piet A. van Rijn, Marek J. Slomka, Jill Banks, Ian H. Brown, Dennis J. Alexander, Donald P. King, A review of RT-PCR technologies used in veterinary virology and disease control: Sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health, *Veterinary Microbiology*, In Press, Corrected Proof, Available online 6 May 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.04.034.

(<http://www.sciencedirect.com/science/article/B6TD6-4W7B0DY-1/2/b6967a20cb4ec01c7696a72e96ee6979>)

Abstract:

Real-time, reverse transcription polymerase chain reaction (rRT-PCR) has become one of the most widely used methods in the field of molecular diagnostics and research. The potential of this format to provide sensitive, specific and swift detection and quantification of viral RNAs has made it an indispensable tool for state-of-the-art diagnostics of important human and animal viral pathogens. Integration of these assays into automated liquid handling platforms for nucleic acid extraction increases the rate and standardisation of sample throughput and decreases the potential for cross-contamination. The reliability of these assays can be further enhanced by using internal controls to validate test results. Based on these advantageous characteristics, numerous robust rRT-PCRs systems have been developed and validated for important epizootic diseases of livestock. Here, we review the rRT-PCR assays that have been developed for the detection of five RNA viruses that cause diseases that are notifiable to the World Organisation for Animal Health (OIE), namely: foot-and-mouth disease, classical swine fever, bluetongue disease, avian influenza and Newcastle disease. The performance of these tests for viral diagnostics and disease control and prospects for improved strategies in the future are discussed.

Keywords: Polymerase chain reaction; Real-time PCR; FMDV; AIV; NDV; CSFV; BTM

Sylvia S. Reemers, Marian J. Groot Koerkamp, Frank C. Holstege, Willem van Eden, Lonneke Vervelde, Cellular host transcriptional responses to influenza A virus in chicken tracheal organ cultures differ from responses in in vivo infected trachea, *Veterinary Immunology and Immunopathology*, In Press, Corrected Proof, Available online 4 May 2009, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2009.04.021.

(<http://www.sciencedirect.com/science/article/B6TD5-4W6Y7WV-2/2/8778418bc7c02a2d5bb6c1c8eb84e9f9>)

Abstract:

In this study a viral infection of a tissue culture model system was compared to an in vivo infection, which is of importance to gauge the utility of the model system. The aim was to characterize early immune responses induced by avian influenza virus using tracheal organ cultures (TOC) as a model system. First, the in vitro system was optimized to ensure that the host transcription responses were only influenced by virus infection and not by differences in viral load. Upper and lower trachea both could be used in the cultures because the virus load was the same. Cilia motility was not affected in non-infected TOC and only slightly in infected TOC at 24 h post-inoculation. Gene expression profiles of early immune responses were analyzed in in vitro infected TOC, and were compared to the responses found in in vivo infected trachea. The gene expression profile in infected TOC suggested the up regulation of innate anti-viral responses that were triggered by attachment, entry and uptake of virus leading to several signalling cascades including NF- κ B regulation. Genes associated with IFN mediated responses were mainly type I IFN related. Overlapping gene expression profiles between non-infected and infected TOC suggested that tissue damage during excision induced wound healing responses that masked early host responses to the virus. These responses were confirmed by real-time quantitative RT-PCR showing up regulation of IL-1 β and IL-6. Microarray analysis showed that gene expression profiles of infected and non-infected TOC had a large overlap. This overlap contained many immune-related genes associated with inflammatory responses, apoptosis and immune system process and development. Infected TOC and in vivo infected trachea shared few significantly differentially expressed genes. The gene expression profile of infected TOC contained fewer genes which were expressed at reduced amplitude of change. Genes that were common between TOC and trachea were associated with early immune responses likely triggered by virus attachment and entry. Most of the genes were associated with IFN-mediated responses, mainly type I IFN related. Our study implicates that although the TOC model is suitable for culturing of virus and lectin or virus binding studies, it is not suitable for measuring early immune responses upon viral infection at host transcriptional level.

Keywords: Avian influenza virus; Organ culture; Host-pathogen interaction; Genomics; Innate immunity

H.M. Yassine, M. Khatri, Y.J. Zhang, C.W. Lee, B.A. Byrum, J. O'Quin, K.A. Smith, Y.M. Saif, Characterization of triple reassortant H1N1 influenza A viruses from swine in Ohio, *Veterinary Microbiology*, In Press, Corrected Proof, Available online 4 May 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.04.028.

(<http://www.sciencedirect.com/science/article/B6TD6-4W6YJ7P-1/2/e0ce57dda2fbdd81bd172efbdeba37e4>)

Abstract:

An H1N1 influenza A virus, A/swine/Ohio/24366/07, was isolated from pigs in an Ohio county fair. Twenty-six people who came in contact with the infected pigs developed respiratory disease and two of these people were laboratory confirmed as H1N1 by the Centers for Disease Control and Prevention (CDC). The A/swine/Ohio/24366/07 virus we isolated from swine was shown at the CDC to have 100% identical genome sequence to the human virus associated with the county fair. This prompted us to characterize three swine and two human origin H1N1 influenza A viruses isolated at different time points in the State of Ohio. The three swine viruses were shown to be

triple reassortant viruses harboring genes of human (PB1), swine (HA, NA, NP, M, and NS), and avian (PB2 and PA) lineage viruses. Although viruses evaluated in this study were isolated during a short time interval (3 years), genetic drift was observed within the HA and NA genes, including changes at the receptor binding and antigenic sites of HA1 protein. Nevertheless, all viruses exhibited antigenic similarity as evaluated with hemagglutination inhibition and virus neutralizing tests. Internal genes were similar to other reassortant viruses of various subtypes currently circulating in the United States. Interestingly, two of the swine viruses including the 2007 isolate replicated well in human airway epithelial cells, however, another virus isolated in 2006 showed very little replication.

Keywords: Influenza A viruses; H1N1; Triple reassortants

Shimon Perk, Natalia Golender, Caroline Banet-Noach, Ester Shihmanter, Shimon Pokamunsky, Michael Pirak, Yevgenii Tendler, Michael Lipkind, Alexander Panshin, Phylogenetic analysis of hemagglutinin, neuraminidase, and nucleoprotein genes of H9N2 avian influenza viruses isolated in Israel during the 2000-2005 epizootic, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 32, Issue 3, May 2009, Pages 221-238, ISSN 0147-9571, DOI: 10.1016/j.cimid.2007.06.008.

(<http://www.sciencedirect.com/science/article/B6T5H-4RRXJ92-1/2/b3b7b371a243afc8bceab9fd77b807cf>)

Abstract:

The first two isolates of H9N2 influenza virus in Israel were collected from turkey and chicken hosts in May 2000. The actual epizootic of the H9N2 virus started in December 2001, after a 1.5-year period of silence, and still continues. A total of more than 500 isolations from turkeys and chickens were registered during the outbreaks. The present study has revealed some genetic peculiarities among the local isolates, namely: all the isolates belong to the same G1-like phylogenetic lineage, within which they form a single group, which, in turn, is divided into three subgroups in the cases of the HA and NP genes, and two subgroups in the case of the NA gene. The results present a basis for suggesting the existence of two parallel evolutionary trends originating from the same local 'prototype' isolate.

Keywords: Influenza virus; Hemagglutinin; Neuraminidase; Nucleoprotein; Phylogenetic analysis; Virus de la grippe aviaire; Gene de l'hemagglutinine; Neuraminidase et nucleoproteine; Analyse phylogénique

H.-K. Froschle, U. Gonzales-Barron, K. McDonnell, S. Ward, Investigation of the potential use of e-tracking and tracing of poultry using linear and 2D barcodes, *Computers and Electronics in Agriculture*, Volume 66, Issue 2, May 2009, Pages 126-132, ISSN 0168-1699, DOI: 10.1016/j.compag.2009.01.002.

(<http://www.sciencedirect.com/science/article/B6T5M-4VH8XYN-2/2/192cb879e096c8ce59219af1e857d53a>)

Abstract:

Contemporary Precision Livestock Technology in poultry production is very limited and does not meet European standards for traceability and Best Available Technology (BAT), as laid down in EN ISO 2205:2007 standards (2007) and the European Directive 2008/1/EC (2008). A worldwide occurrence of Avian Influenza additionally calls for a fraud-proof tagging device and source verification system for poultry and poultry products in order to complete partially existing documentary trails.

During a preliminary laboratory trial, a procedure for the application of miniature linear and two-dimensional Data Matrix (DM) barcodes onto poultry beaks and legs through inkjet printing was set up and assessed. Results regarding the proportion of readability (p%), the standard error in readability (SE) and general statistics on the reading time were calculated. Tests for independence based on Chi-square and Pearson's were performed on the categorical data, to estimate the

differences between proportions of readability of reading groups. The resulting data was used to define the optimal position of barcodes as well as the optimal reading mode of the barcode scanner to be used for further trials. As this experiment provided an estimate of readability of barcodes imprinted on chicken beaks and legs, it is intended to serve as a basis for sample size calculation for an ongoing live trial.

Keywords: Traceability; Animal identification; Barcode; Poultry

Phan Q. Minh, Roger S. Morris, Birgit Schauer, Mark Stevenson, Jackie Benschop, Hoang V. Nam, Ron Jackson, Spatio-temporal epidemiology of highly pathogenic avian influenza outbreaks in the two deltas of Vietnam during 2003-2007, *Preventive Veterinary Medicine*, Volume 89, Issues 1-2, 1 May 2009, Pages 16-24, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2009.01.004.

(<http://www.sciencedirect.com/science/article/B6TBK-4VNCBVK-1/2/8fab1a4f50d25bd017d7d9c311b59667>)

Abstract:

Outbreaks of highly pathogenic avian influenza A subtype H5N1 have occurred in Vietnam as a series of epidemic waves since December 2003. We describe the spatial and temporal patterns of the HPAI H5N1 epidemics in the Red River Delta in the north (785 outbreaks in 606 communes) and the Mekong River Delta in the south of Vietnam (1313 outbreaks in 837 communes), where the epidemics were concentrated. Throughout the study period the percentage of outbreaks affecting ducks increased steadily to a peak of 78% during the 2006/2007 epidemic in both deltas. Five of the seven epidemic waves occurred in the period of active poultry population buildup immediately prior to the Vietnamese New Year (Tết festival). Recorded outbreaks were clustered in space and time within both deltas, consistent with infection transmission occurring via a combination of local and long-distance spread. Our analyses demonstrate that the epidemiology of HPAI in Vietnam has changed over the 4-year study period, with outbreaks now occurring in the warmer months of the year and ducks featuring more prominently as affected species. To determine the relative importance of local and long-distance spread on infection transmission, precise details of outbreak location, date of onset of clinical signs, and size and composition of the poultry population at risk need to be recorded during future outbreak responses.

Keywords: Avian influenza; Spatio-temporal analysis; Poultry; Disease control; Vietnam

Dawn Su-Yin Yeo, Sock-Hoon Ng, Chin-Wen Liaw, Ley-Moy Ng, Eugene Jing-Hui Wee, Elizabeth Ai-Sim Lim, Shirely Lay-Kheng Seah, Wai-Kwan Wong, Chee-Wee Lim, Richard J. Sugrue, Boon-Huan Tan, Molecular characterization of low pathogenic avian influenza viruses, isolated from food products imported into Singapore, *Veterinary Microbiology*, In Press, Corrected Proof, Available online 23 April 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.04.025.

(<http://www.sciencedirect.com/science/article/B6TD6-4W4JDPJ-1/2/a7998090fbb4d988a1e619c0a4171bfe>)

Abstract:

We have completed the genetic characterization of all eight gene segments for four low pathogenic avian influenza (LPAI) viruses. The objective of this study was to detect the presence of novel signatures that may serve as early warning indicators of the conversion of LPAI viruses to high pathogenic avian influenza (HPAI) viruses. This study included three H5N2 and one H5N3 viruses that were isolated from live poultry imported into Singapore as part of the national avian influenza virus (AIV) surveillance program. Based on the molecular criterion of the World Organisation for Animal Health (OIE), sequence analysis with the translated amino acid (aa) sequence of the hemagglutinin (HA) gene revealed the absence of multibasic aa at the HA cleavage site, identifying all four virus isolates as LPAI. Detailed phylogenetic tree analyses using the HA and neuraminidase (NA) genes clustered these isolates in the Eurasian H5 lineage, but away from the HPAI H5 subtypes. This analysis further revealed that the internal genes clustered to different avian and swine subtypes, suggesting that the four isolates may possibly share their

ancestry with these different influenza subtypes. Our results suggest that the four LPAI isolates in this study contained mainly avian signatures, and the phylogenetic tree for the internal genes further suggests the potential for reassortment with other different circulating avian subtypes. This is the first comprehensive report on the genetic characterization of LPAI H5N2/3 viruses isolated in South-East Asia.

Keywords: Low pathogenic avian influenza virus; H5N2; H5N3; Imported poultry; Singapore

Zsofia Szeleczy, Adam Dan, Krisztina Ursu, Eva Ivanics, Istvan Kiss, Karoly Erdelyi, Sandor Belak, Claude P. Muller, Ian H. Brown, Adam Balint, Four different sublineages of highly pathogenic avian influenza H5N1 introduced in Hungary in 2006-2007, *Veterinary Microbiology*, In Press, Corrected Proof, Available online 19 April 2009, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2009.04.017.

(<http://www.sciencedirect.com/science/article/B6TD6-4W3PT5Y-7/2/44468caf7011e6562dfb545bfb4672be>)

Abstract:

Highly pathogenic avian influenza (HPAI) H5N1 viruses were introduced to Hungary during 2006-2007 in three separate waves. This study aimed at determining the full-length genomic coding regions of the index strains from these epizootics in order to: (i) understand the phylogenetic relationship to other European H5N1 isolates, (ii) elucidate the possible connection between the different outbreaks and (iii) determine the putative origin and way of introduction of the different virus variants. Molecular analysis of the HA gene of Hungarian HPAI isolates obtained from wild birds during the first introduction revealed two groups designated Hungarian1 (HUN1) and Hungarian2 (HUN2) within sublineage 2.2B and clade 2.2.1, respectively. Sequencing the whole coding region of the two index viruses A/mute swan/Hungary/3472/2006 and A/mute swan/4571/Hungary/2006 suggests the role of wild birds in the introduction of HUN1 and HUN2 viruses: the most similar isolates to HUN1 and HUN2 group were found in wild avian species in Croatia and Slovakia, respectively. The second introduction of HPAI H5N1 led to the largest epizootic in domestic waterfowl in Europe. The index strain of the epizootic A/goose/Hungary/14756/2006 clustered to sublineage 2.2.A1 forming the Hungarian3 (HUN3) group. A common ancestry of HUN3 isolates with Bavarian strains is suggested as the most likely scenario of origin. Hungarian4 (HUN4) viruses isolated from the third introduction clustered with isolate A/turkey/United Kingdom/750/2007 forming a sublineage 2.2.A2. The origin and way of introduction of HUN4 viruses is still obscure, thus further genetic, phylogenetic, ecological and epidemiological data are required in order to elucidate it.

Keywords: Avian influenza; H5N1; Highly pathogenic; Hungary; Phylogenetic characterisation

Justin D. Brown, Ginger Goekjian, Rebecca Poulson, Steve Valeika, David E. Stallknecht, Avian influenza virus in water: Infectivity is dependent on pH, salinity and temperature, *Veterinary Microbiology*, Volume 136, Issues 1-2, 14 April 2009, Pages 20-26, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.10.027.

(<http://www.sciencedirect.com/science/article/B6TD6-4TVSFKD-1/2/4d7b4598cb26ab4b4fd5ea480d220a64>)

Abstract:

Wild birds in the Orders Anseriformes and Charadriiformes are the natural reservoir for avian influenza (AI) viruses. Transmission within these aquatic bird populations occurs through an indirect fecal-oral route involving contaminated water on shared aquatic habitats. In order to better understand the influence that aquatic environments exert on AI transmission and maintenance in the wild-bird reservoir system, we determined the duration of persistence for 12 wild-bird origin AI viruses under natural ranges of pH, salinity, and temperature. Viral persistence was measured using a laboratory-based distilled water model system. The AI viruses varied in their response to each of the examined variables, but, generally, the viruses were most stable at a slightly basic pH

(7.4-8.2), low temperatures (<17 [degree sign]C), and fresh to brackish salinities (0-20,000 parts per million (ppm)). Alternatively, the AI viruses had a much shorter duration of persistence in acidic conditions (pH < 6.6), warmer temperatures (>32 [degree sign]C), and high salinity (>25,000 ppm). The results of this research suggest that the pH, temperature, and salinity in natural aquatic habitats can influence the ability of AI viruses to remain infective within these environments. Furthermore, these results provide insight into chemical and physical properties of water that could enhance or restrict AI virus transmission on an aquatic bird habitat.

Keywords: pH; Salinity; Temperature; Avian influenza virus; Water; Environment

V. Bavinck, A. Bouma, M. van Boven, M.E.H. Bos, E. Stassen, J.A. Stegeman, The role of backyard poultry flocks in the epidemic of highly pathogenic avian influenza virus (H7N7) in the Netherlands in 2003, *Preventive Veterinary Medicine*, Volume 88, Issue 4, 1 April 2009, Pages 247-254, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.10.007.

(<http://www.sciencedirect.com/science/article/B6TBK-4VGDNK6-4/2/fa3f5854d85d27bdcfef782fe46a05ab>)

Abstract:

In recent years, outbreaks of highly pathogenic avian influenza (HPAI) viruses have caused the death of millions of poultry and of more than 200 humans worldwide. A proper understanding of the transmission dynamics and risk factors for epidemic spread of these viruses is key to devising effective control strategies. The aim of this study was to quantify the epidemiological contributions of backyard flocks using data from the H7N7 HPAI epidemic in the Netherlands in 2003. A dataset was constructed in which flocks in the affected area were classified as susceptible (S), infected but not yet infectious (E), infectious (I), and removed (R). The analyses were based on a two-type SEIR epidemic model, with the two types representing commercial poultry farms and backyard poultry flocks. The analyses were aimed at estimation of the susceptibility (g) and infectiousness (f) of backyard flocks relative to commercial farms. The results show that backyard flocks were considerably less susceptible to infection than commercial farms (g), while estimates of the relative infectiousness of backyard flocks varied widely (f). Our results indicate that, from an epidemiological perspective, backyard flocks played a marginal role in the outbreak of highly pathogenic avian influenza in the Netherlands in 2003.

Keywords: Avian influenza; Backyard poultry; Transmission; Reproduction number; SEIR model

Marian E.H. Bos, Mirjam Nielen, Guus Koch, Annemarie Bouma, Mart C.M. De Jong, Arjan Stegeman, Back-calculation method shows that within-flock transmission of highly pathogenic avian influenza (H7N7) virus in the Netherlands is not influenced by housing risk factors, *Preventive Veterinary Medicine*, Volume 88, Issue 4, 1 April 2009, Pages 278-285, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.12.003.

(<http://www.sciencedirect.com/science/article/B6TBK-4VGDNK6-1/2/ba5fe9f1b9db7f84d9e63aff65b59242>)

Abstract:

To optimize control of an avian influenza outbreak knowledge of within-flock transmission is needed. This study used field data to estimate the transmission rate parameter ($[\beta]$) and the influence of risk factors on within-flock transmission of highly pathogenic avian influenza (HPAI) H7N7 virus in the 2003 epidemic in The Netherlands. The estimation is based on back-calculation of daily mortality data to fit a susceptible-infectious-dead format, and these data were analysed with a generalized linear model. This back-calculation method took into account the uncertainty of the length of the latent period, the survival of an infection by some birds and the influence of farm characteristics. After analysing the fit of the different databases created by back-calculation, it could be concluded that an absence of the latency period provided the best fit. The transmission rate parameter ($[\beta]$) from these field data was estimated at 4.50 per infectious chicken per day (95% CI: 2.68-7.57), which was lower than what was reported from experimental data. In contrast

to general belief, none of the studied risk factors (housing system, flock size, species, age of the birds in weeks and date of depopulation) had significant influence on the estimated [beta].

Keywords: Avian influenza; H7N7; Transmission; Risk factors; Field data

S.R. Fereidouni, E. Starick, C. Grund, A. Globig, T.C. Mettenleiter, M. Beer, T. Harder, Rapid molecular subtyping by reverse transcription polymerase chain reaction of the neuraminidase gene of avian influenza A viruses, *Veterinary Microbiology*, Volume 135, Issues 3-4, 30 March 2009, Pages 253-260, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.09.077.

(<http://www.sciencedirect.com/science/article/B6TD6-4TMJ3Y2-2/2/68991578985f24081ce82368533d0ad0>)

Abstract:

Accurate identification of hemagglutinin (HA) and neuraminidase (NA) subtypes of influenza A viruses is an integral part of monitoring programs targeting avian influenza viruses (AIV). Use of highly sensitive molecular screening methods such as pan influenza-specific real-time RT-PCR (rRT-PCR) yields an increasing number of samples which are positive for AIV RNA but negative by virus isolation and, therefore, require molecular, instead of serological, subtyping. We developed specific RT-PCR assays for all known nine AIV NA subtypes. Validation using 43 reference isolates from different animal species revealed good performance characteristics regarding sensitivity and specificity. On basis of serial tenfold dilution series of reference isolates a benchmark value of Ct 32 in an M gene-specific rRT-PCR became evident below which all nine NA subtypes were readily detectable by the subtype-specific RT-PCRs. For subtypes N1, N2, N4 and N6 detection was extended to dilutions with Ct values of up to 35. Diagnostic applicability of the whole set of conventional NA-specific RT-PCRs was evaluated by analysis of 119 different diagnostic samples from wild birds which proved to be positive for AIV by M gene-specific rRT-PCR. Diagnostic sensitivity and specificity was confirmed by sequencing NA amplicons from 41 field isolates generated from this set and by NA inhibition assays. A universal molecular HA/NA subtyping algorithm for rRT-PCR positive avian influenza virus monitoring samples is proposed which may complement classical serological subtyping of influenza A virus isolates.

Keywords: Influenza A virus; Neuraminidase; Subtyping; RT-PCR

Winfried G.J. Degen, Jacqueline Smith, Bartjan Simmelink, Elizabeth J. Glass, Dave W. Burt, Virgil E.J.C. Schijns, Molecular immunophenotyping of lungs in naive and vaccinated chickens early after pulmonary avian influenza A (H9N2) virus infection, *Veterinary Immunology and Immunopathology*, Volume 128, Issues 1-3, Special Issue: The 8th International Veterinary Immunology Symposium (8th IVIS), 15 March 2009, Page 325, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2008.10.245.

(<http://www.sciencedirect.com/science/article/B6TD5-4VPG66R-8M/2/0d6c13ae30af8b6e87fc3eca1b047011>)

Keywords: Adjuvant; Avian influenza H9N2; Microarray; Immunophenotyping

Matthias Giese, Timm C. Harder, Jens P. Teifke, Thomas C. Mettenleiter, Thomas W. Vahlenkamp, The role of T cells in avian influenza H5N1 infected cats, *Veterinary Immunology and Immunopathology*, Volume 128, Issues 1-3, Special Issue: The 8th International Veterinary Immunology Symposium (8th IVIS), 15 March 2009, Page 343, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2008.10.282.

(<http://www.sciencedirect.com/science/article/B6TD5-4VPG66R-9Y/2/20b904595cd6e74de26f8e3ca212279a>)

Keywords: T cells; Influenza virus; Viraemia

Shingo Iwami, Yasuhiro Takeuchi, Xianning Liu, Avian flu pandemic: Can we prevent it?, *Journal of Theoretical Biology*, Volume 257, Issue 1, 7 March 2009, Pages 181-190, ISSN 0022-5193, DOI: 10.1016/j.jtbi.2008.11.011.

(<http://www.sciencedirect.com/science/article/B6WMD-4V166J5-1/2/b0790b5b68a515ec061ad87267f23ecc>)

Abstract:

Outbreaks of highly pathogenic H5N1 avian influenza in Southeast Asia, Europe and Africa have led to devastating consequences for poultry, and have resulted in numerous infections in humans. Although these infections from the animal reservoir continue to accumulate, the virus does not seem to spread extensively among humans. However, for example, a process of genetic reassortment could occur in a human who is co-infected with avian influenza A virus and a human strain of influenza A virus. The resulting new virus might then be able to easily infect humans and spread from human to human. Therefore, many experts expect the occurrence of a pandemic due to a mutant virus which can be easily transmitted among humans. Thus, currently, a major public health concern is the next influenza pandemic; yet it remains unclear how to control such a crisis. In this paper, we investigate relations between the evolution of virulence and an effectiveness of pandemic control measures after the emergence of mutant avian influenza; one is an elimination policy of infected birds with avian influenza and the other is a quarantine policy of infected humans with mutant avian influenza. We found that each of these prevention policies can be ineffective (i.e., increase human morbidity or mortality). Further, interestingly, the same intervention might, under the same conditions, increase human morbidity and decrease human mortality, or vice versa. Our practical findings are that the quarantine policy can effectively reduce both human morbidity and mortality but the elimination policy increases either human morbidity or mortality in a worst case situation.

Keywords: Epidemic model; Avian influenza; Mutation; Elimination policy; Quarantine policy

Andrew W. Hill, Robert P. Guralnick, Meredith J.C. Wilson, Farhat Habib, Daniel Janies, Evolution of drug resistance in multiple distinct lineages of H5N1 avian influenza, *Infection, Genetics and Evolution*, Volume 9, Issue 2, March 2009, Pages 169-178, ISSN 1567-1348, DOI: 10.1016/j.meegid.2008.10.006.

(<http://www.sciencedirect.com/science/article/B6W8B-4TT9GK9-1/2/6965b7602d0b2be5b6322a3170d44185>)

Abstract:

Some predict that influenza A H5N1 will be the cause of a pandemic among humans. In preparation for such an event, many governments and organizations have stockpiled antiviral drugs such as oseltamivir (Tamiflu(R)). However, it is known that multiple lineages of H5N1 are already resistant to another class of drugs, adamantane derivatives, and a few lineages are resistant to oseltamivir. What is less well understood is the evolutionary history of the mutations that confer drug resistance in the H5N1 population. In order to address this gap, we conducted phylogenetic analyses of 676 genomic sequences of H5N1 and used the resulting hypotheses as a basis for asking 3 molecular evolutionary questions: (1) Have drug-resistant genotypes arisen in distinct lineages of H5N1 through point mutation or through reassortment? (2) Is there evidence for positive selection on the codons that lead to drug resistance? (3) Is there evidence for covariation between positions in the genome that confer resistance to drugs and other positions, unrelated to drug resistance, that may be under selection for other phenotypes? We also examine how drug-resistant lineages proliferate across the landscape by projecting or phylogenetic analysis onto a virtual globe. Our results for H5N1 show that in most cases drug resistance has arisen by independent point mutations rather than reassortment or covariation. Furthermore, we found that some codons that mediate resistance to adamantane derivatives are under positive selection, but did not find positive selection on codons that mediate resistance to oseltamivir. Together, our phylogenetic methods, molecular evolutionary analyses, and geographic visualization provide a

framework for analysis of globally distributed genomic data that can be used to monitor the evolution of drug resistance.

Keywords: Influenza; H5N1; Viral evolution; Adamantane; Oseltamivir; Drug resistance; Antiviral; Tamiflu(R); Neuraminidase; M2 protein; Mutation

Alexandre Caron, Nicolas Gaidet, Michel de Garine-Wichatitsky, Serge Morand, Elissa Z. Cameron, Evolutionary biology, community ecology and avian influenza research, *Infection, Genetics and Evolution*, Volume 9, Issue 2, March 2009, Pages 298-303, ISSN 1567-1348, DOI: 10.1016/j.meegid.2008.12.001.

(<http://www.sciencedirect.com/science/article/B6W8B-4V42J93-1/2/3ead16c9424af5a97f307ced0e276df2>)

Abstract:

The epidemiology of H5N1 HPAI is still unclear despite the efforts of the research community. Studies bringing new insights add more variability in the host-pathogen system and uncertainty in the prediction of local risks. Global analyses of the pathways of wild birds in parallel with virus outbreaks have brought limited conclusions once the raw information was extracted from relevant maps. In this article, we propose an integration of epidemiology, evolutionary biology and community ecology on a local level in a research framework. This multidisciplinary approach aims at understanding the pathogen transmission processes at the interface between different bird groups whether wild or domesticated. We believe that this ecological data brought together with the epidemiological and molecular data is a key element to explore the mechanism of the AIV ecology in their hosts.

Keywords: Avian influenza; Epidemiology; Community ecology; Evolutionary biology

Perk Shimon, Natalia Golender, Ekaterina Lapin, Shimon Pokamunski, Elyakum Berman, Yevgeny Tendler, Michel Bellaiche, Alexander Panshin, Irit Davidson, Genetic characterization of H5N1 influenza virus that caused new outbreak in Israel at the beginning of 2008, *Comparative Immunology, Microbiology and Infectious Diseases*, In Press, Corrected Proof, Available online 3 February 2009, ISSN 0147-9571, DOI: 10.1016/j.cimid.2008.12.002.

(<http://www.sciencedirect.com/science/article/B6T5H-4VHS7P2-1/2/f1843946a45d9495df37c8a29e4fb119>)

Abstract:

Our aim was to characterize the A/ck/Israeli/1055/2008 (H5N1) avian influenza virus that was isolated at the beginning of 2008, and to establish the phylogenetic relationship of this isolate to other H5N1 viruses that were recently isolated in adjacent countries. In light of a study of complete nucleotide sequences of all the genes we found that the isolate (year 2008) was closely related to the H5N1 viruses isolated in Egypt, Israel and Gaza in 2006. The Israeli isolate had the hemagglutinin-connecting peptide with a polybasic amino acid insertion. The most host-restriction sites of the 2008 isolate were typical of avian hosts, with one exception: K627 at the PB2 protein. As compared with previous local H5N1 isolates, a high mutation rate was found at the HA gene, which antigenic sites were under positive selection pressure.

Keywords: Avian influenza virus; H5N1 subtype; Hemagglutinin gene; Phylogenetic analysis; Virus de la grippe aviaire de type H5N1; Gene l'hemagglutinine; Analyse phylogénique

Cristobal Verdugo, Carol J. Cardona, Tim E. Carpenter, Simulation of an early warning system using sentinel birds to detect a change of a low pathogenic avian influenza virus (LPAIV) to high pathogenic avian influenza virus (HPAIV), *Preventive Veterinary Medicine*, Volume 88, Issue 2, Special Section: Schwabe Symposium 2007 - Field Disease Investigation and Population Health - A Symposium Honoring the Legacy of Dr. Clive C. Gay, Professor Emeritus from Washington State University, 1 February 2009, Pages 109-119, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.08.007.

(<http://www.sciencedirect.com/science/article/B6TBK-4TTNC88-1/2/5a76d1a17c66e32d28d97059cfac6be2>)

Abstract:

The placement of sentinel birds in a commercial poultry flock infected with low pathogenic avian influenza virus (LPAIV) may be an effective way of detecting subsequent change in the isolate to a high pathogenic avian influenza virus (HPAIV). Data collected from the 2002 Chilean HPAIV outbreak, along with information from a literature review of laboratory studies involving A/chicken/Chile/176822/02 (H7N3/LP) and A/chicken/Chile/184240-1/02 (H7N3/HP) viruses, were used to construct a computer simulation model. Mortality rates of the original LPAIV-infected population and the sentinel population were compared to detect the presence of HPAIV. A total of 12 increased mortality threshold scenarios were examined, using one-day absolute (2, 3, or 4 birds) or relative (0.5, 1.0, or 1.5%) mortality thresholds, and two-day absolute (1, 2, or 3 birds) or relative (0.25, 0.50, or 1.00%) mortality thresholds, to indicate the change from LPAIV to HPAIV in the sentinel and original populations, respectively. Results showed that following a one-day approach, threshold mortalities occurred on average at 7.35, 7.82, and 8.17 (0.5, 1.0, or 1.5%) and 6.21, 6.38, and 6.45 (2, 3, or 4 birds) days after the first infectious case for the original and sentinel populations, respectively. The two-day approach delayed the occurrence of threshold mortalities, on average, to 7.64, 8.05, and 8.62 (0.25, 0.50, or 1.00%) and 6.86, 6.78, and 7.23 (1, 2, or 3 birds) days after the first infectious case for the original and sentinel populations, respectively. Although, significant ($p < 0.10$) differences were observed among different combinations of detection times for the original and sentinel populations, the use of sentinel birds has a maximum mean advantage, over monitoring mortality exclusively in the original population, of 1.96 and 1.84 days for one- and two-day threshold mortalities, respectively. Additionally, the early warning system based on a sentinel vs. original population presented a decrease of the probabilities of a false alarm, from 0.04-0.45 to <0.01 -0.10%. These findings may be used by decision makers to evaluate the risk of not depopulating a flock infected with a H5 or H7 LPAIV strain and the benefit of using sentinel birds as an early warning system of a change to HPAIV.

Keywords: Avian influenza; Sentinel birds; Early warning system; Broiler chickens; Chile; Simulation model

Alexander Nagy, Veronika Vostinakova, Zuzana Pindova, Jitka Hornickova, Lenka Cernikova, Kamil Sedlak, Miroslav Mojzis, Zuzana Dirbakova, Jirina Machova, Molecular and phylogenetic analysis of the H5N1 avian influenza virus caused the first highly pathogenic avian influenza outbreak in poultry in the Czech Republic in 2007, *Veterinary Microbiology*, Volume 133, Issue 3, 13 January 2009, Pages 257-263, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.07.013.

(<http://www.sciencedirect.com/science/article/B6TD6-4T3M67R-1/2/d23c6cf715a67f2b9de9e2e85ab51c72>)

Abstract:

On 19th July 2007 re-occurrence of the H5N1 highly pathogenic avian influenza (HPAI) virus was noticed in Europe. The index strain of this novel H5N1 lineage was identified in the Czech Republic where it caused historically the first HPAI outbreak in commercial poultry. In the present study we performed molecular and phylogenetic analysis of the index strain of the re-emerging H5N1 virus lineage along with the Czech and the Slovak H5N1 strains collected in 2006 and established the evolutionary relationships to additional viruses circulated in Europe in 2005-2006. Our analysis revealed that the Czech and the Slovak H5N1 viruses collected during 2006 were separated into two sub-clades 2.2.1 and 2.2.2, which predominated in Europe during 2005-2006. On the contrary the newly emerged H5N1 viruses belonged to a clearly distinguishable sub-clade 2.2.3. Within the sub-clade 2.2.3 the Czech H5N1 strains showed the closest relationships to the simultaneously circulated viruses from Germany, Romania and Russia (Krasnodar) in 2007 and were further clustered with the viruses from Afghanistan and Mongolia circulated in 2006. The origin of the Czech 2007 H5N1 HPAI strains was also discussed.

Keywords: H5N1; Avian influenza; Highly pathogenic avian influenza; H5N1 outbreak

P.K. Biswas, H. Barua, G.M.N. Uddin, D. Biswas, A. Ahad, N.C. Debnath, Serosurvey of five viruses in chickens on smallholdings in Bangladesh, *Preventive Veterinary Medicine*, Volume 88, Issue 1, 1 January 2009, Pages 67-71, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.06.018.

(<http://www.sciencedirect.com/science/article/B6TBK-4T8R1M5-1/2/b0d1327cf20f07fa1cdbcb9d909ef207>)

Abstract:

A serologic survey was undertaken in chickens in smallholdings in Bangladesh for avian influenza A virus (AIV), egg drop syndrome '76 virus (EDS'76V), infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and reovirus (RV) in three phases: January 2002-May 2003, September 2003-August 2004, and August 2005-March 2006. Four hundred thirty-six sera collected in the 2nd phase, 295 in the first phase, 755 in the 1st plus 2nd phases and 295 in the 1st phase were investigated for AIV, EDS'76V, IBV and RV, respectively, using enzyme linked immunosorbent assays. All 854 sera collected in the three phases were screened for NDV using hemagglutination inhibition test. In chickens 20% were seropositive to AIV, 3% to EDS'76V, 74% to IBV, 88% to NDV, and 47% to RV. The seroprevalence in flocks was 23% to AIV, 6% to EDS'76V, 79% to IBV, 89% to NDV and 56% to RV. Twenty-five percent chickens had $\geq 10 \log_2$ HI titers to NDV.

Keywords: Seroprevalences; Avian influenza virus; Newcastle disease virus; Village chickens

Dergham A. Roussan, Ghassan Y. Khawaldeh, Rami H. Al Rifai, Waheed S. Totanji, Ibrahim A. Shaheen, Avian influenza virus H9 subtype in poultry flocks in Jordan, *Preventive Veterinary Medicine*, Volume 88, Issue 1, 1 January 2009, Pages 77-81, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.06.021.

(<http://www.sciencedirect.com/science/article/B6TBK-4T5S486-1/2/d68173bfab0084d8463898aff5d6c50>)

Abstract:

Avian influenza virus (AIV) has been recognized as one of the most important pathogens in poultry. This study was designed to investigate the prevalence of AIV H9 subtype in commercial chicken flocks in Jordan by serological and molecular methods. Serum samples from 180 chicken flocks (120 broilers and 60 layers) free from respiratory symptoms, were examined by hemagglutination inhibition (HI) test for specific antibodies against AIV H9 subtype, and 83 chicken flocks (60 broilers and 23 layers) with respiratory symptoms, were examined by reverse transcription-polymerase chain reaction (RT-PCR) using universal primers for influenza A viruses, then specific primers targeting AIV H9 gene were used for the flocks that were positive by universal primers. Overall, 65 out of 120 broiler flocks (54.2%), and 47 out of 60 layer flocks (78.3%) were positive for AIV H9 subtype antibodies. Nucleic acid of influenza A viruses was detected in 31 out of 60 broiler flocks (51.7%), and 15 out of 23 layer flocks (65.2%). AIV H9 subtype was detected in all flocks that were positive for influenza A viruses. The current study confirmed the endemic nature of AIV H9 subtype in broiler and layer flocks in Jordan. It is essential that the biosecurity on poultry farms should be improved to prevent the introduction and dissemination of influenza and other viruses. Furthermore, farmers need to be educated about the signs, lesions, and the importance of this virus.

Keywords: Chicken flocks; Hemagglutination inhibition; AIV H9 subtype; Jordan; Reverse transcription-polymerase chain reaction

Takehiko Saito, Chiaki Watanabe, Nobuhiro Takemae, Arunee Chaisingh, Yuko Uchida, ChantaneeBuranathai, Hirofumi Suzuki, Masatoshi Okamoto, Tadao Imada, Sujira Parchariyanon, Nimit Traivanatam, Shigeo Yamaguchi, Pathogenicity of highly pathogenic avian influenza viruses of H5N1 subtype isolated in Thailand for different poultry species, *Veterinary*

Microbiology, Volume 133, Issues 1-2, 1 January 2009, Pages 65-74, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.06.020.

(<http://www.sciencedirect.com/science/article/B6TD6-4SX9FY5-5/2/9414a0d2a76745d3a27f51f8d6ef583a>)

Abstract:

Highly pathogenic avian influenza (HPAI) viruses of the H5N1 subtype have caused several rounds of outbreaks in Thailand. In this study, we used 3 HPAI viruses isolated in Thailand in January 2004 from chicken, quail, and duck for genetic and pathogenetic studies. Sequence analysis of the entire genomes of these isolates revealed that they were genetically similar to each other. Chickens, quails, domestic ducks, and cross-bred ducks were inoculated with these isolates to evaluate their pathogenicity to different host species. A/chicken/Yamaguchi/7/04 (H5N1), an HPAI virus isolated in Japan, was also used in the chicken and quail studies for comparison. All four isolates were shown to be highly pathogenic to chickens and quails, with 100% mortality by 106 EID₅₀ inoculants of the viruses. They caused sudden death in chickens and quails within 2-4 days after inoculation. The mean death times (MDT) of quails infected with the Thai isolates were shorter than those of chickens infected with the same isolates. Mortality against domestic and cross-bred ducks ranged from 50 to 75% by intranasal inoculation with the 106 EID₅₀ viruses. Neurological symptoms were observed in most of the inoculated domestic ducks and appeared less severe in the cross-bred ducks. The MDTs of the ducks infected with the Thai isolates were 4.8-6 days post-inoculation. Most of the surviving ducks infected with the Thai isolates had seroconverted until 14 dpi. Our study illustrated the pathobiology of the Thai isolates against different poultry species and would provide useful information for improving control strategies against HPAI. **Keywords:** HPAI; Poultry; Influenza virus; Pathogenicity

S. Nagarajan, K. Rajukumar, C. Tosh, V. Ramaswamy, K. Purohit, G. Saxena, P. Behera, B. Pattnaik, H.K. Pradhan, S.C. Dubey, Isolation and pathotyping of H9N2 avian influenza viruses in Indian poultry, *Veterinary Microbiology*, Volume 133, Issues 1-2, 1 January 2009, Pages 154-163, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.06.013.

(<http://www.sciencedirect.com/science/article/B6TD6-4STGRVG-1/2/9bca9f09c2085033d7e00b05f26c4b8e>)

Abstract:

A total of 1246 faecal and tissue samples collected/received from 119 farms located in various states of India were processed for isolation of avian influenza viruses (AIV) during 2003-2004 as part of a program to monitor AIV infection in Indian poultry population. Avian influenza virus was isolated for the first time in India from poultry farms with history of drop in egg production, respiratory illness and increased mortality in Haryana state. A total of 29 H9N2 AIV isolates were obtained from the states of Punjab, Haryana, Uttar Pradesh, Gujarat, and Orissa and Union Territory Delhi. Subtyping was done by HI, RT-PCR and neuraminidase inhibition assay. Pathotyping of six representative isolates by intravenous pathogenicity index (0.0/3.0) in 6-8 weeks old chicken, trypsin dependency in cell culture and HA cleavage site analysis (335RSSR*GLF341) confirmed that these isolates are low pathogenic. Nucleotide sequence analysis of the HA gene showed that the Indian isolates are very closely related (95.0-99.6%) and shared a homology of 92-96% with H9N2 isolates from Germany and Asian regions other than that of mainland China. Deduced amino acid sequences showed the presence of L226 (234 in H9 numbering) which indicates a preference to binding of [alpha] (2-6) sialic acid receptors. Two of the six isolates had 7 glycosylation sites in the HA1 cleaved protein and the remaining four had 5 sites. Phylogenetic analysis showed that they share a common ancestor Qa/HK/G1/97 isolate which had contributed internal genes of H5N1 virus circulating in Vietnam. Further characterization of Indian H9N2 isolates is required to understand their nature and evolution.

Keywords: Avian influenza; H9N2; Indian poultry; LPAIV

Jesse. D. Thomas, Kirsten. R. Morris, Dale. I. Godfrey, John. W. Lowenthal, Andrew. G.D. Bean, Expression, purification and characterisation of recombinant Escherichia coli derived chicken interleukin-12, *Veterinary Immunology and Immunopathology*, Volume 126, Issues 3-4, 15 December 2008, Pages 403-406, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2008.08.004.

(<http://www.sciencedirect.com/science/article/B6TD5-4T8SKWY-2/2/dd85aa5d789b3e264f68ffc90e845492>)

Abstract:

Zoonotic viruses, such as H5N1 Avian Influenza, pose major threats to both animals and humans, and with this in mind there is a need for the development of new anti-viral strategies. The cytokine interleukin-12 (IL-12) is known to play a pivotal regulatory role in the anti-viral response due to its role in the induction of the key anti-viral cytokine IFN- γ . Therefore, strategies which provide a means for the production of therapeutic quantities of IL-12 may be of major benefit. Here we describe the development of biologically active Escherichia coli (*E. coli*) derived chicken IL-12 (ChIL-12). The single chain ChIL-12 gene was cloned into the pET32b expression vector, transformed into the BL-21 *E. coli* strain and expression induced with IPTG. Over expressed protein was solubilised with zwittergent detergent and isolated utilising Nickel ion affinity chromatography. Biological activity was determined as ChIL-12 stimulated proliferation of pre-treated T-cells in vitro. This study is the first example of a biologically active *E. coli* derived IL-12 from a non-mammalian vertebrate subsequently providing a means for testing the anti-viral therapeutic potential of ChIL-12 in an in vivo model.

Keywords: Chicken; Interleukin-12; Prokaryotic expression

Ruo Qian Yan, Zhi Ming Wu, Qin Mei Fang, Zhi Ling Zhang, Jian Zhang, Xin Sheng Li, Hui Fang Hao, Chun Xia, Reconstruction of a chicken BF2 protein complex and identification of binding nonamer peptides derived from avian influenza virus hemagglutinin, *Veterinary Immunology and Immunopathology*, Volume 126, Issues 1-2, 15 November 2008, Pages 91-101, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2008.06.007.

(<http://www.sciencedirect.com/science/article/B6TD5-4SVV8DC-2/2/526fbe91fc2851f3b0133301a97bdf1f>)

Abstract:

In order to reconstruct the system for identification of short antigenic peptides, the chicken BF2 gene of Chinese Sanhuang (SH) chicken line was linked to the $[\beta]_2m$ gene via (G4S)₃, a linker encoding a 15-amino acid glycine-rich peptide, by splicing overlap extension PCR (SOE-PCR). The MBP-BF2-(G4S)₃- $[\beta]_2m$ fusion protein was expressed and purified in a pMAL-p2X/*E. coli* TB1 system. The purified MBP-BF2-(G4S)₃- $[\beta]_2m$ protein was cleaved by Factor Xa protease, and further purified by DEAE-Sepharose chromatography. The conformation of the BF2-(G4S)₃- $[\beta]_2m$ protein was determined by circular dichroism (CD). In addition, the refolded BF2-(G4S)₃- $[\beta]_2m$ protein was used to bind three predicted nonameric peptides derived from the hemagglutinins of the avian influenza virus (AIV) H5N1 and H9N2 subtypes. The BF2-(G4S)₃- $[\beta]_2m$ -associated peptides were detected by mass spectrometry. The molecular weights and amino acid sequences of the peptides were confirmed by primary and tandem mass spectrometry analysis, respectively. The results indicate that the secondary structures and predicted three-dimensional crystal structure of BF2-(G4S)₃- $[\beta]_2m$ are similar to those of the monomers of chicken BF2 and $[\beta]_2m$. The BF2-(G4S)₃- $[\beta]_2m$ protein could bind two of the three predicted nonamer peptides derived from AIV hemagglutinin. The experimental system demonstrated that the reconstructed BF2-(G4S)₃- $[\beta]_2m$ protein complex can be used to identify nonamer peptides, including T-cell epitopes in chicken.

Keywords: Chicken; BF2 protein complex; AIV; T-cell epitopes; Nonamer peptides

Luciana Sarmiento, Claudio L. Afonso, Carlos Estevez, Jamie Wasilenko, Mary Pantin-Jackwood, Differential host gene expression in cells infected with highly pathogenic H5N1 avian influenza

viruses, *Veterinary Immunology and Immunopathology*, Volume 125, Issues 3-4, 15 October 2008, Pages 291-302, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2008.05.021.

(<http://www.sciencedirect.com/science/article/B6TD5-4SNGM1C-1/2/c239f36d7cfb855192c99f95c00c350e>)

Abstract:

In order to understand the molecular mechanisms by which different strains of avian influenza viruses overcome host response in birds, we used a complete chicken genome microarray to compare early gene expression levels in chicken embryo fibroblasts (CEF) infected with two avian influenza viruses (AIV), A/CK/Hong Kong/220/97 and A/Egret/Hong Kong/757.2/02, with different replication characteristics. Gene ontology revealed that the genes with altered expression are involved in many vital functional classes including protein metabolism, translation, transcription, host defense/immune response, ubiquitination and the cell cycle. Among the immune-related genes, MEK2, MHC class I, PDCD10 and Bcl-3 were selected for further expression analysis at 24 hpi using semi-quantitative RT-PCR. Infection of CEF with A/Egret/Hong Kong/757.2/02 resulted in a marked repression of MEK2 and MHC class I gene expression levels. Infection of CEF with A/CK/Hong Kong/220/97 induced an increase of MEK2 and a decrease in PDCD10 and Bcl-3 expression levels. The expression levels of alpha interferon (IFN-[alpha]), myxovirus resistance 1 (Mx1) and interleukin-8 (IL-8) were also analyzed at 24 hpi, showing higher expression levels of all of these genes after infection with A/CK/Hong Kong/220/97 compared to A/Egret/Hong Kong/757.2/02. In addition, comparison of the NS1 sequences of the viruses revealed amino acid differences that may explain in part the differences in IFN-[alpha] expression observed. Microarray gene expression analysis has proven to be a useful tool on providing important insights into how different AIVs affect host gene expression and how AIVs may use different strategies to evade host response and replicate in host cells.

Keywords: Avian influenza virus; Gene expression; Immune response; Chicken embryo fibroblasts

Hai Yu, Rong-Hong Hua, Tian-Chao Wei, Yan-Jun Zhou, Zhi-Jun Tian, Guo-Xin Li, Tian-Qiang Liu, Guang-Zhi Tong, Isolation and genetic characterization of avian origin H9N2 influenza viruses from pigs in China, *Veterinary Microbiology*, Volume 131, Issues 1-2, 18 September 2008, Pages 82-92, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.02.024.

(<http://www.sciencedirect.com/science/article/B6TD6-4S0JN0D-2/2/cc3f76a94103d194e7c6e2d655138181>)

Abstract:

As pigs are susceptible to infection with both avian and human influenza A viruses, they have been proposed to be an intermediate host for the adaptation of avian influenza viruses to humans. In April 2006, a disease caused by highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) occurred in several pig farms and subsequently overwhelmed almost half of China with more than 2,000,000 cases of pig infection. Here we report a case in which four swine H9N2 influenza viruses were isolated from pigs infected by highly pathogenic PRRSVs in Guangxi province in China. All the eight gene segments of the four swine H9N2 viruses are highly homologous to A/Pigeon/Nanchang/2-0461/00 (H9N2) or A/Wild Duck/Nanchang/2-0480/00 (H9N2). Phylogenetic analyses of eight genes show that the swine H9N2 influenza viruses are of avian origin and may be the descendants of A/Duck/Hong Kong/Y280/97-like viruses. Molecular analysis of the HA gene indicates that our H9N2 isolates might have high-affinity binding to the [alpha]2,6-NeuAcGal receptor found in human cells. In conclusion, our finding provides further evidence about the interspecies transmission of avian influenza viruses to pigs and emphasizes the importance of reinforcing swine influenza virus (SIV) surveillance, especially after the emergence of highly pathogenic PRRSVs in pigs in China.

Keywords: Swine influenza; Avian H9N2 influenza virus; Porcine reproductive and respiratory syndrome virus; Genetic analysis; Molecular analysis

George W. Beran, Disease and destiny-mystery and mastery, Preventive Veterinary Medicine, Volume 86, Issues 3-4, Special Issue:Schwabe Symposia 2004-2006. Perspectives on Veterinary Epidemiology in Public Health, Animal Production and Preventive Medicine, 15 September 2008, Pages 198-207, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.05.001.

(<http://www.sciencedirect.com/science/article/B6TBK-4T0X2HR-1/2/57d01a06fe70bd0be5e944eb3a08f62a>)

Abstract:

When early people made their appearance, zoonotic infectious diseases were already waiting, but epidemic diseases did not appear in human history until people began to live in large numbers under conditions of close contact, mainly during the last 10,000 years. Disease has decimated urban populations, conquered armies, and disrupted society. The focus here is on (1) the plague of Athens and the Black Death; (2) smallpox, influenza, and rabies; (3) avian influenza prion diseases, and foot & mouth disease; and (4) emerging and re-emerging diseases. All have veterinary public health associations.

In Athens, Greece, in 430 BC, when the Spartans ravaged the countryside, hordes crowded into Athens so that orderly movements, space in which to live, and adequate supplies of food became impossible. Crowding of any population fosters disease transmission; chaos and disorder enhance it all the more. Out of northern Egypt came a terrible plague from across the Mediterranean Sea. The identity of the plague of Athens remains unsure, but the well-considered conclusion is Rift Valley Fever, a mosquito borne, viral zoonosis. The Black Death, also called the Plague, raged in Asia for centuries. In 1347, the Black Death was brought by a ship out of Asia to Sicily. The scenes of devastation were repeated throughout Europe, with 90% or more of the people dying in city after city.

Influenza, too, has been a cause of periodic human epidemics, but the great pandemic of influenza occurred in the last months of World War I. In the years of highest occurrence, more than half the world's population became clinically infected. If veterinary public health had been born earlier, it could have led to elucidating the epidemiology of influenza and the plagues of Athens, Europe, and Asia. In turn, smallpox had also caused continual tragedy. In 1796, Edward Jenner began to harvest pustules of cowpox from children or infected cows and inject them into susceptible children. In 1980, the World Health Organization declared that smallpox had been eliminated from the world. Rabies, though, still strikes terror.

A number of animal diseases, broadly termed emerging and re-emerging diseases, need surveillance because they have the potential to impact human health. From late in 2003 to 2007, the highly pathogenic H5N1 influenza virus in poultry infected at least 121 people and caused 62 deaths in four countries. The prion diseases, too, all have very high numbers in concentrated contacts. To control these diseases, veterinary public health is essential, with diagnosis, epidemiological surveillance, clinical manifestations, and prevention as primary measures.

Keywords: Zoonoses; Pandemics; Historic diseases

Frederick A. Murphy, Emerging zoonoses: The challenge for public health and biodefense, Preventive Veterinary Medicine, Volume 86, Issues 3-4, Special Issue:Schwabe Symposia 2004-2006. Perspectives on Veterinary Epidemiology in Public Health, Animal Production and Preventive Medicine, 15 September 2008, Pages 216-223, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.02.009.

(<http://www.sciencedirect.com/science/article/B6TBK-4S9R1TD-2/2/74bf428a8db13ce7f83cb0e6b36021c3>)

Abstract:

The concept of new and emerging diseases has captured the public interest and has revitalized the public health infectious disease research community. This interest has also resulted in competition for funding and turf wars between animal health and public health scientists and public officials and, in some cases, has delayed and hindered progress toward effective prevention,

control and biodefense. There is a dynamic list of outbreaks causing substantial morbidity and mortality in humans and often in the reservoir animal species. Some agents have the potential to grow into major epidemics. There are many determinants that influence the emergence of diseases of concern that require the use of current understanding of the nature of agent persistence and spread. Additional factors that are global must be added to plans for prevention and control. To this complex mix has been added the potential for accidental or malicious release of agents. The nature of emerging infectious agents and their impact is largely unpredictable. Models that strive to predict the dynamics of agents may be useful but can also blind us to increasing disease risks if it does not match a specific model. Field investigations of early events will be critical and should drive prevention and control actions. Many disease agents have developed strategies to overcome extremes of reservoir qualities like population size and density. Every infectious agent spreads easier when its hosts are closer together. Zoonoses must be dealt with at the interface of human and animal health by all available information. Lessons learned from the emergence of and response to agents like West Nile virus, H5N1 avian influenza, SARS and bovine spongiform encephalopathy, the cause of new-variant Creutzfeldt-Jakob disease in humans, must be used to create better plans for response and meet the challenge for public health and biodefense.

Keywords: Emerging zoonoses; Control and prevention; Preparedness and biodefense

Jae Min Song, Youn Jeong Lee, Ok Mi Jeong, Hyun Mi Kang, Hye Ryoung Kim, Jun Hun Kwon, Jae Hong Kim, Baik Lin Seong, Yong Joo Kim, Generation and evaluation of reassortant influenza vaccines made by reverse genetics for H9N2 avian influenza in Korea, *Veterinary Microbiology*, Volume 130, Issues 3-4, 25 August 2008, Pages 268-276, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.02.005.

(<http://www.sciencedirect.com/science/article/B6TD6-4RVG3K3-2/2/cea7e8fc47d9240eef8290d5e0fe8443>)

Abstract:

The prevalence and continuous evolution of H9N2 avian influenza viruses in poultry have necessitated the use of vaccines in veterinary medicine. Because of the inadequate growth properties of some strains, additional steps are needed for producing vaccine seed virus. In this study, we generated three H9N2/PR8 reassortant viruses using a total cDNA plasmid-transfection system, as an alternative strategy for developing an avian influenza vaccine for animals. We investigated the vaccine potency of the reassortant viruses compared with the existing vaccine strain which was adapted by the 20th serial passages in embryonated eggs with A/Ck/Kor/01310/01 (H9N2). The H9N2/PR8 reassortant viruses, containing the internal genes of the high-yielding PR8 strain and the surface gene of the A/Ck/Kor/01310/01 strain, could be propagated in eggs to the same extent as existing vaccine strain without additional processing. Similar to vaccine strain, the H9N2/PR8 reassortant viruses induced hemagglutination-inhibiting antibodies in chickens and prevented virus shedding and replication in multiple organs in response to homologous infection. However, due to the continuing evolution and increasing biologic diversity of H9N2 influenza in Korea, the vaccine provided only partial protection against currently isolates. Taken together, our results suggest that the H9N2/PR8 reassortant virus can be used as a seed virus for avian influenza vaccines in poultry farm. Considering the constant genetic changes in H9 strains isolated in Korea, this reverse genetic system may offer a prompt and simple way to change the vaccine seed virus and mitigate the impact of unexpected influenza outbreaks.

Keywords: Avian influenza virus; Reverse genetics; Vaccine

Richard Njouom, Jean-Thierry Aubin, Assumpta Lucienne Bella, Baschirou Moussa Demsa, Pierre Rouquet, Bouba Gake, Andre Ngangnou, Yacouba Foupouapouognigni, Sylvie Van Der Werf, Jocelyne Rocourt, Dominique Rousset, Highly pathogenic avian influenza virus subtype H5N1 in

ducks in the Northern part of Cameroon, *Veterinary Microbiology*, Volume 130, Issues 3-4, 25 August 2008, Pages 380-384, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2008.02.006.

(<http://www.sciencedirect.com/science/article/B6TD6-4RVG3K3-1/2/59b611d358a2df67bb5170c50b531e19>)

Abstract:

Highly pathogenic avian influenza (HPAI) virus was first detected in Cameroon in February 2006. Analysis of NA sequences of the virus demonstrated that it is closely related to the H5N1 isolates from Northern Nigeria, Sudan and Ivory Coast, suggesting a common virus ancestor.

Keywords: HPAI; H5N1; Cameroon

Peter N. Thompson, Marna Sinclair, Boto Ganzevoort, Risk factors for seropositivity to H5 avian influenza virus in ostrich farms in the Western Cape Province, South Africa, *Preventive Veterinary Medicine*, Volume 86, Issues 1-2, 15 August 2008, Pages 139-152, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2008.03.011.

(<http://www.sciencedirect.com/science/article/B6TBK-4SJ950B-1/2/20a79dc070f4a00f0c9967785618be44>)

Abstract:

In a 2005 serological survey, carried out in response to an outbreak of H5N2 avian influenza (AI) in ostriches in the Eastern Cape Province, 16.3% of ostrich farms in the Western Cape Province of South Africa were found to be seropositive to H5 AI virus. We subsequently carried out a questionnaire-based census survey on all available registered Western Cape ostrich farms that still existed at the end of 2005 (367 farms, of which 82 were seropositive), in order to identify risk factors associated with farm-level seropositivity. A farm was classified as seropositive for H5 AI virus if one or more birds had tested positive (haemagglutination inhibition titre >1:16) in the 2005 survey, which had been designed to detect a minimum within-group seroprevalence of 10%. For each farm, risk factor information was collected using a questionnaire administered during a face-to-face interview with each farm owner or manager. Information was obtained on the ostrich population, movements of birds, environmental factors, management practices, and frequency of contact between ostriches and various wild bird species. Multiple logistic regression models were developed for the whole Western Cape Province and also for the two largest ostrich farming regions, 'Klein Karoo' and 'Southern Cape'. Seroprevalence differed between regions, being highest in the Klein Karoo (31.6%). In all three models, increased risk of farm-level H5 AI virus seropositivity was associated with increasing numbers of ostriches, excluding chicks, present on the farm. Increased risk of seropositivity was associated with reduced frequency of cleaning of feed troughs (<1x/week vs. >1x/week), both overall (odds ratio (OR) = 4.5; 95% confidence interval (CI): 1.5, 13.3) and in the Southern Cape (OR = 53.6; 95% CI: 3.3, 864), and with failure to clean and disinfect transport vehicles, both overall (OR = 2.3; 95% CI: 1.1, 4.8) and in the Klein Karoo (OR = 2.6; 95% CI: 1.1, 6.5). Increased risk of seropositivity was also associated with increasing frequency of contact of ostriches with certain wild bird species: overall with white storks (*Ciconia ciconia*), in the Southern Cape with gulls (*Larus* spp.), and in the Klein Karoo with Egyptian geese (*Alopochen aegyptiaca*).

Keywords: Avian influenza; Ostriches; Risk factors; Epidemiology; Survey; South Africa

Julia Marschall, Bianka Schulz, Timm C. Harder Priv-Doz, Thomas W. Vahlenkamp Priv-Doz, Janine Huebner, Elke Huisinga, Katrin Hartmann, Prevalence of influenza A H5N1 virus in cats from areas with occurrence of highly pathogenic avian influenza in birds, *Journal of Feline Medicine & Surgery*, Volume 10, Issue 4, August 2008, Pages 355-358, ISSN 1098-612X, DOI: 10.1016/j.jfms.2008.03.007.

(<http://www.sciencedirect.com/science/article/B6WJC-4T1SKJH-1/2/91bca948cb049afd87c8ce2688cc387b>)

Abstract:

Natural and experimental infections have shown that cats are susceptible to highly pathogenic avian influenza A virus subtype H5N1 (HPAIV H5N1). Cats can be severely affected and die from the disease, but subclinical infections have also been reported. To learn more about the role of cats in the spread of the virus and about the risk posed to cats, the prevalence of H5N1 virus was examined in 171 cats from areas in Germany and Austria in which birds infected with HPAIV H5N1 had been found. Pharyngeal swabs were examined for H5N1 virus using real-time polymerase chain reaction, and serum samples were tested for antibodies to influenza virus. None of the cats showed evidence of infection with H5N1 virus. Prevalence of H5N1 virus was determined to be <1.8% (95% confidence interval (CI): 0.000000-0.017366); prevalence of antibodies was <2.6% (95% CI: 0.000000-0.025068).

Julia Marschall, Katrin Hartmann, Avian influenza A H5N1 infections in cats, *Journal of Feline Medicine & Surgery*, Volume 10, Issue 4, August 2008, Pages 359-365, ISSN 1098-612X, DOI: 10.1016/j.jfms.2008.03.005.

(<http://www.sciencedirect.com/science/article/B6WJC-4SYDB1R-2/2/09c16ad8d8f88eb36448d7de8817d8a4>)

Abstract:

Although cats had been considered resistant to disease from influenza virus infection, domestic cats and large felids are now known to be naturally and experimentally susceptible to infection with highly pathogenic avian influenza virus H5N1 (HPAIV H5N1). The virus causes systemic infection, lung and liver being the mainly affected organs. Infected cats show fever, depression, dyspnoea, and neurological signs, but subclinical infections have also occurred. Mostly, cats have been infected by direct contact with affected birds, especially by eating raw poultry; transmission from cat to cat may also occur. Little is known about the role of cats in the epidemiology of the virus. So far, no reassortment between avian and mammalian influenza viruses has occurred in cats, but experts fear that cats might give the virus an opportunity to adapt to mammals. This publication gives a review on avian influenza in cats with a focus on practical aspects for veterinarians.

Chi-Young Wang, Chia-Jen Hsu, Heng-Ju Chen, Julius L.C. Chulu, Hung-Jen Liu, Development of a reliable assay protocol for identification of diseases (RAPID)-bioactive amplification with probing (BAP) for detection of Newcastle disease virus, *Veterinary Microbiology*, Volume 130, Issues 1-2, 27 July 2008, Pages 28-36, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2007.12.015.

(<http://www.sciencedirect.com/science/article/B6TD6-4RH37WJ-1/2/d86c5c6b61324082d458671c0272b764>)

Abstract:

Due to appearance of new genotypes of Newcastle disease virus (NDV) with no cross-protection and with vaccine strains, some outbreaks have been reported in Taiwan that caused significant damage to the poultry industry. A reliable assay protocol, (RAPID)-bioactive amplification with probing (BAP), for detection of NDV that uses a nested PCR and magnetic bead-based probe to increase sensitivity and specificity, was developed. Primers and probes were designed based on the conserved region of the F protein-encoding gene sequences of all NDV Taiwan isolates. The optimal annealing temperature for nested reverse transcription-polymerase chain reaction (RT-PCR) to amplify the gene was 61 [degree sign]C and optimal hybridization occurred when buffer 1x SSC and 0.5% SDS were used at 50 [degree sign]C. The sensitivity of RAPID-BAP was 1 copy/[mu]l for standard plasmids and 10 copy/[mu]l for transcribed F protein-encoding gene of NDV with comparable linearity ($R^2 = 0.984$ versus $R^2 = 0.99$). This sensitivity was superior to that of other techniques currently used. The assay was also highly specific because the negative controls, including classical swine fever virus, avian influenza virus, avian reovirus, and infectious bursa disease virus could not be detected. Thirty-four field samples were tested using conventional RT-PCR, nested RT-PCR, real-time quantitative RT-PCR, and RAPID-BAP assay and the positive rates were 24%, 30%, 41%, and 53%, respectively. The developed assay allows

for rapid, correct, and sensitive detection of NDV and fulfils all of the key requirements for clinical applicability. It could reliably rule out false negative results from antibody-based assays and also facilitate a rapid diagnosis in the early phase of the disease for emergency quarantine that may help prevent large-scale outbreaks.

Keywords: Newcastle disease virus; RAPID-BAP assay; Real-time quantitative RT-PCR

Shingo Iwami, Yasuhiro Takeuchi, Andrei Korobeinikov, Xianning Liu, Prevention of avian influenza epidemic: What policy should we choose?, *Journal of Theoretical Biology*, Volume 252, Issue 4, 21 June 2008, Pages 732-741, ISSN 0022-5193, DOI: 10.1016/j.jtbi.2008.02.020.

(<http://www.sciencedirect.com/science/article/B6WMD-4RX07B3-2/2/3a747d911010b0470474f48b4c08ce5a>)

Abstract:

Human-to-human transmission of the avian influenza has been extremely rarely reported, and is considered as limited, inefficient and unsustainable. However, experts warn an occurrence of 'mutant avian influenza', which can easily spread among humans, because the avian influenza is already endemic, in particular in Asian poultry, and it is evolving in domestic and wild birds, pigs and humans. Outbreak of such mutant avian influenza in the human world may have devastating consequences, which are comparable with these for the 1918 'Spanish influenza'. In this paper we develop a mathematical model for the spread of the mutant avian influenza, and explore the effectivity of the prevention policies, namely the elimination policy which increases the effective additional death rate of the infected birds and the quarantine policy which reduces the number of infective contacts.

Keywords: Epidemic model; Avian influenza; Mutation; Elimination policy; Quarantine policy

Hong-Ying Chen, Bao-An Cui, Ping-An Xia, Xin-Sheng Li, Gong-Zheng Hu, Ming-Fan Yang, Hong-Ying Zhang, Xue-Bin Wang, Su-fang Cao, Long-Xian Zhang, Xiang-Tao Kang, Ke Tu, Cloning, in vitro expression and bioactivity of duck interleukin-18, *Veterinary Immunology and Immunopathology*, Volume 123, Issues 3-4, 15 June 2008, Pages 205-214, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2008.01.036.

(<http://www.sciencedirect.com/science/article/B6TD5-4RSRDC6-2/2/b60d5f590a4ce7b2292cc55bd32be92f>)

Abstract:

The encoding sequence for duck IL-18 was obtained, using reverse transcription-polymerase chain reaction, from mRNA harvested from Con A-stimulated Gushi (GS) duck splenic mononuclear cells. Recombinant duck IL-18 (rdulL-18) was produced in a prokaryotic expression system. In vitro bioactivity of rdulL-18 was determined in a lymphocyte proliferation assay and in vivo bioactivity of rdulL-18 was assessed by addition to a vaccine. Monoclonal antibody (mAb) and polyclonal antibodies (pAbs) specific for rdulL-18 were generated and subsequently characterized by ELISA, Western blot and neutralizing assays. Sequence analysis of GS duck IL-18 demonstrated an open reading frame (ORF) of 603 base pairs encoding for a 200 amino acid precursor protein. The duck encoding sequence shares 85.3% similarity to the chicken equivalent, at the nucleotide level. A His-dulL-18 fusion protein was recognized in Western blot by mAbs against duck and chicken IL-18 (chIL-18), but not by mAb against human IL-18. Recombinant dulL-18 induced in vitro proliferation of Con A-stimulated duck splenocytes and enhanced the immune response of ducks vaccinated with an inactivated oil emulsion vaccine against avian influenza virus. PAb and mAb 5B2 against rdulL-18 had neutralizing ability, inhibiting the biological activities of both recombinant dulL-18 and endogenous dulL-18. The results indicate that rdulL-18 has the potential to be used as an immunoadjuvant, and the mAb against rdulL-18 further facilitates basic immunobiological studies of the role of IL-18 in the avian immune system.

Keywords: Duck; Interleukin-18; In vitro expression; In vivo bioactivity

Geoff Vana, Kristi M. Westover, Origin of the 1918 Spanish influenza virus: A comparative genomic analysis, *Molecular Phylogenetics and Evolution*, Volume 47, Issue 3, June 2008, Pages 1100-1110, ISSN 1055-7903, DOI: 10.1016/j.ympev.2008.02.003.

(<http://www.sciencedirect.com/science/article/B6WNH-4RV17KV-2/2/451977a1d1a8873e73950a1747926060>)

Abstract:

To test the avian-origin hypothesis of the 1918 Spanish influenza virus we surveyed influenza sequences from a broad taxonomic distribution and collected 65 full-length genomes representing avian, human and 'classic' swine H1N1 lineages in addition to numerous other swine (H1N2, H3N1, and H3N2), human (H2N2, H3N2, and H5N1), and avian (H1N1, H4N6, H5N1, H6N1, H6N6, H6N8, H7N3, H8N4, H9N2, and H13N2) subtypes. Amino acids from all eight segments were concatenated, aligned, and used for phylogenetic analyses. In addition, the genes of the polymerase complex (PB1, PB2, and PA) were analyzed individually. All of our results showed the Brevig-Mission/1918 strain in a position basal to the rest of the clade containing human H1N1s and were consistent with a reassortment hypothesis for the origin of the 1918 virus. Our genome phylogeny further indicates a sister relationship with the 'classic' swine H1N1 lineage. The individual PB1, PB2, and PA phylogenies were consistent with reassortment/recombination hypotheses for these genes. These results demonstrate the importance of using a complete-genome approach for addressing the avian-origin hypothesis and predicting the emergence of new pandemic influenza strains.

Keywords: Avian-origin hypothesis; 1918 Spanish influenza; Genomic analysis H5N1; Reassortment

Wanpo Zhang, Huiying Li, Guofu Cheng, Sishun Hu, Zili Li, Dingren Bi, Avian influenza virus infection induces differential expression of genes in chicken kidney, *Research in Veterinary Science*, Volume 84, Issue 3, June 2008, Pages 374-381, ISSN 0034-5288, DOI: 10.1016/j.rvsc.2007.05.015.

(<http://www.sciencedirect.com/science/article/B6WWR-4PCXXN4-2/2/a0d1e5b4432d956888fbaa5565646794>)

Abstract:

The pathogenic process of highly pathogenic avian influenza virus (HPAIV) infection is poorly understood. To explore the differential expression of kidney genes as a result of HPAIV infection, two cDNA libraries were constructed from uninfected and infected kidneys by suppression subtractive hybridization (SSH). Fifteen genes including IFN-stimulated genes (ISG12), lymphocyte antigen 6 complex locus E gene (LY6E), matrix Gla protein gene (MGP), lysozyme gene, haemopoiesis related membrane protein 1 gene, KIAA1259, MGC68696, G6pc-prov protein gene (G6PC), MGC4504, alcohol dehydrogenase gene (ADH), glutathione S-transferase gene (GST), sodium-dependent high-affinity dicarboxylate transporter gene (SDCT), Synaptotagmin XV (SytXV) and two novel genes were found significantly up-regulated or dramatically suppressed. Differential expression of these genes was further identified by Northern blot. Functional analysis indicated that the regulation of their expression might contribute to the pathogenic process of HPAIV infection. In contrast, the increased expression of three IFN-stimulated genes named ISG12, LY6E, and haemopoiesis related membrane protein 1 gene might reflect host defense responses. Further study showed that ISG12 protein failed to directly interact with NS1 protein of HPAIV which expressed simultaneously in the organs where HPAIV replication occurred, by use of BacterioMatch two-hybrid system. Therefore, our findings may provide new insights into understanding the molecular mechanism underlying the pathophysiological process of HPAIV infection in chicken.

Keywords: Avian influenza virus (AIV); Chicken; Suppression subtractive hybridization (SSH); Gene; Differential expression

S.P.S. Pillai, D.L. Suarez, M. Pantin-Jackwood, C.-W. Lee, Pathogenicity and transmission studies of H5N2 parrot avian influenza virus of Mexican lineage in different poultry species, *Veterinary Microbiology*, Volume 129, Issues 1-2, 25 May 2008, Pages 48-57, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2007.11.003.

(<http://www.sciencedirect.com/science/article/B6TD6-4R40SM1-1/2/4a596915ce95176401a907f5acf8ed9c>)

Abstract:

In 2004, a low pathogenic H5N2 influenza virus (A/parrot/CA/6032/04) was identified in a psittacine bird for the first time in the United States. Sequence and phylogenetic analysis of the hemagglutinin gene grouped the parrot isolate under the Mexican lineage H5N2 viruses (subgroup B) with highest similarity to recent chicken-origin isolates from Guatemala. Antigenic analysis further confirmed the close relatedness of the parrot isolate to Mexican lineage viruses, the highest cross-reactivity being demonstrated to Guatemala isolates. In vivo studies of the parrot isolate in chickens, ducks and turkeys showed that the virus, though did not cause any clinical signs, could replicate to high titers in these birds and efficiently transmit to contact control cage mates. The possibility that the parrot harboring the virus was introduced into the United States as a result of illegal trade across the border provides additional concern for the movement of foreign animal diseases from neighboring countries. Considering the potential threat of the virus to domestic poultry, efforts should be continued to prevent the entry and spread of influenza viruses by imposing effective surveillance and monitoring measures.

Keywords: H5N2; Influenza; Parrot; Pathogenicity; Transmission

E. Starick, M. Beer, B. Hoffmann, C. Staubach, O. Werner, A. Globig, G. Strebelow, C. Grund, M. Durban, F.J. Conraths, T. Mettenleiter, T. Harder, Phylogenetic analyses of highly pathogenic avian influenza virus isolates from Germany in 2006 and 2007 suggest at least three separate introductions of H5N1 virus, *Veterinary Microbiology*, Volume 128, Issues 3-4, 30 April 2008, Pages 243-252, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2007.10.012.

(<http://www.sciencedirect.com/science/article/B6TD6-4PXM6BC-2/2/4a7ccee4a9016a612e6e934721b5a7e3>)

Abstract:

In spring 2006, highly pathogenic avian influenza virus (HPAIV) of subtype H5N1 was detected in Germany in 343 dead wild birds, as well as in a black swan (*Cygnus atratus*) kept in a zoo, three stray cats, one stone marten (*Martes foina*), and in a single turkey farm. In 2007 (June-July) the virus reoccurred in 96 wild birds at six geographically separate locations in the Southeast of Germany. In addition, a backyard mixed duck and goose holding was affected. Real-time RT-PCR [Hoffmann, B., Harder, T., Starick, E., Depner, K., Werner, O., Beer, M., 2007. Rapid and highly sensitive pathotyping of avian influenza A H5N1 virus by using real-time reverse transcription-PCR. *J. Clin. Microbiol.* 45, 600-603] and nucleotide sequencing confirmed that these H5-viruses belonged to the Qinghai lineage of HPAIV H5N1 (clade 2.2). For a more detailed analysis, the hemagglutinin and neuraminidase genes of 27 selected German H5N1 viruses isolated 2006 or 2007 and originating from different regions and animal species were sequenced and analysed phylogenetically. As a result, three closely related but distinguishable H5N1 subclades could be defined: In 2006 a 'Northern type' (subclade 2.2.2), representing virus isolates from the German federal states Mecklenburg-Western Pomerania, Schleswig-Holstein, Brandenburg, and Lower Saxony, and a 'Southern type' (subclade 2.2.1) from Baden-Wuerttemberg and Bavaria were detected. Interestingly, representatives of both types were present in Central Germany and caused the outbreak in turkeys (subclade 2.2.2) and in a case in a tufted duck (*Aythya fuligula*) (subclade 2.2.1) in Saxony. Furthermore, one isolate from the South of Germany was identified as 2.2.2 and vice versa a 2.2.1-like isolate was found in Northern Germany. H5N1 viruses isolated in 2007 belonged to a third type (subclade 2.2.3) which was not detected in 2006. Our data suggest the introduction of three distinct H5N1 variants into the wild bird population of Germany. The source of

these viruses and the exact time of introduction remain obscure. Based on the identification of closely related H5N1 viruses from Southern and Central Russia, a recent introduction via wild birds on winter escape from these regions, early in 2006 constitutes the most likely scenario for the 2006 outbreaks. The viruses detected in 2007 most likely represent another new incursion from an as yet unknown source.

Keywords: Avian influenza; H5N1; Highly pathogenic; Phylogenetic analysis; Molecular epidemiology

Lih-Chiann Wang, Chu-Hsiang Pan, Lucia Liu Severinghaus, Lu-Yuan Liu, Chi-Tsong Chen, Chang-En Pu, Dean Huang, Jihn-Tsair Lir, Shih-Chien Chin, Ming-Chu Cheng, Shu-Hwae Lee, Ching-Ho Wang, Simultaneous detection and differentiation of Newcastle disease and avian influenza viruses using oligonucleotide microarrays, *Veterinary Microbiology*, Volume 127, Issues 3-4, 18 March 2008, Pages 217-226, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2007.08.019.

([http://www.sciencedirect.com/science/article/B6TD6-4PFW697-](http://www.sciencedirect.com/science/article/B6TD6-4PFW697-2/2/535d9a6336dcd4c50bb1398d88f0c52f)

[2/2/535d9a6336dcd4c50bb1398d88f0c52f](http://www.sciencedirect.com/science/article/B6TD6-4PFW697-2/2/535d9a6336dcd4c50bb1398d88f0c52f))

Abstract:

Newcastle disease (ND) and avian influenza (AI) are two of the most important zoonotic viral diseases of birds throughout the world. These two viruses often have a great impact upon the poultry industry. Both viruses are associated with transmission from wild to domestic birds, and often display similar signs that need to be differentiated. A rapid surveillance among wild and domestic birds is important for early disease detection and intervention, and is the basis for what measures should be taken. The surveillance, thus, should be able to differentiate the diseases and provide a detailed analysis of the virus strains. Here, we described a fast, simultaneous and inexpensive approach to the detection of Newcastle disease virus (NDV) and avian influenza virus (AIV) using oligonucleotide microarrays. The NDV pathotypes and the AIV haemagglutinin subtypes H5 and H7 were determined at the same time. Different probes on a microarray targeting the same gene were implemented in order to encompass the diversified virus strains or provide multiple confirmations of the genotype. This ensures good sensitivity and specificity among divergent viruses. Twenty-four virus isolates and twenty-four various combinations of the viruses were tested in this study. All viruses were successfully detected and typed. The hybridization results on microarrays were clearly identified with the naked eyes, with no further imaging equipment needed. The results demonstrate that the detection and typing of multiple viruses can be performed simultaneously and easily using oligonucleotide microarrays. The proposed method may provide potential for rapid surveillance and differential diagnosis of these two important zoonoses in both wild and domestic birds.

Keywords: Avian influenza; Newcastle disease; Oligonucleotide microarrays

Thierry van den Berg, Benedicte Lambrecht, Sylvie Marche, Mieke Steensels, Steven Van Borm, Michel Bublot, Influenza vaccines and vaccination strategies in birds, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 31, Issues 2-3, Aspects of vaccine development, March 2008, Pages 121-165, ISSN 0147-9571, DOI: 10.1016/j.cimid.2007.07.004.

([http://www.sciencedirect.com/science/article/B6T5H-4PRHKXN-](http://www.sciencedirect.com/science/article/B6T5H-4PRHKXN-1/2/c276a27406c4f7b2e666ec051a001c72)

[1/2/c276a27406c4f7b2e666ec051a001c72](http://www.sciencedirect.com/science/article/B6T5H-4PRHKXN-1/2/c276a27406c4f7b2e666ec051a001c72))

Abstract:

Although it is well accepted that the present Asian H5N1 panzootic is predominantly an animal health problem, the human health implications and the risk of human pandemic have highlighted the need for more information and collaboration in the field of veterinary and human health. H5 and H7 avian influenza (AI) viruses have the unique property of becoming highly pathogenic (HPAI) during circulation in poultry. Therefore, the final objective of poultry vaccination against AI must be eradication of the virus and the disease. Actually, important differences exist in the control of avian and human influenza viruses. Firstly, unlike human vaccines that must be adapted to the

circulating strain to provide adequate protection, avian influenza vaccination provides broader protection against HPAI viruses. Secondly, although clinical protection is the primary goal of human vaccines, poultry vaccination must also stop transmission to achieve efficient control of the disease. This paper addresses these differences by reviewing the current and future influenza vaccines and vaccination strategies in birds.

Keywords: Influenza aviaire; Grippe; Vaccin; Vecteur; Sous-unite; Immunité; Surveillance; DIVA; Eradication; H5N1; Avian influenza; Flu; Vaccine; Vector; Subunit; Immunity; Surveillance; DIVA; Eradication; H5N1

Nicole Schauerte, Heike Kuck, Beschreibung und Durchführung der Impfkation bei Zoovogeln im Zoo am Meer Bremerhaven gegen Aviare Influenza, hervorgerufen durch das Virus H5N1, Der Zoologische Garten, Volume 77, Issue 3, 2 January 2008, Pages 172-181, ISSN 0044-5169, DOI: 10.1016/j.zoolgart.2007.10.002.

(<http://www.sciencedirect.com/science/article/B8JHX-4RSS54D-5/2/8d8e3ffa96701a324db1b891674028a1>)

Abstract: Zusammenfassung

Das Virus, das die Aviaren Influenza hervorruft, ist in der Wildtierpopulation latent immer vorhanden. Der Zoo am Meer Bremerhaven hat mit seiner Lage in Küstennahe und in der Nähe von Vogelrastplätzen ein erhöhtes Einschleppungsrisiko für den Erreger der Geflügelgrippe. Als Alternative zu den drastischen Totungsmaßnahmen im Falle eines Ausbruchs der Geflügelgrippe wurde im Einvernehmen mit der zuständigen Behörde im Rahmen „des Programms der Bundesrepublik Deutschland zur Impfung gegen die Aviare Influenza von in Zoos gehaltenen Vögeln“ im Mai 2006 die Impfkation gestartet. Dazu bedurfte es einer Impfgenehmigung der Behörde, an die zahlreiche Auflagen und Bedingungen gekoppelt waren und einen hohen logistischen Aufwand bedeuteten.

Am Ende der Impfkation bleiben einige Fragen unbeantwortet. Zum Beispiel ist bisher ungeklärt, ob bei Zoovogeln bei einer Infektion mit H5N1 der Impfschutz ausreicht und ob und in welchem zeitlichen Abstand in Zukunft weitere Impfungen erfolgen sollen. Die gesetzliche Grundlage über die Abgabe von geimpften Zoovogeln an andere Einrichtungen ist im Bundesanzeiger veröffentlicht.

The avian influenza A virus (H5N1), which causes the bird flu is latently present in populations of wild birds. The Bremerhaven Zoo is subject to an especially high infection risk owing to its close proximity to the North Sea coast and to resting places of birds. As an alternative to drastically killing all birds in an event of infection, a vaccination campaign was initiated in May 2006 as part of the 'Program of the Federal Republic of Germany for the Vaccination of Birds in Zoos against Bird Flu'. The campaign is carried out in cooperation with the responsible authorities. This required formal approval of the authorities was subject to multiple conditions, the fulfilment of which required a large logistic effort. Some issues remained unresolved after the campaign had been completed. It is unclear, for instance, whether or not the applied inoculation is sufficient to immunize infected zoo birds and in which time intervals the vaccination has to be repeated in the future. The legal basis for the handing over of inoculated zoo birds to other institutions is published in the Federal Gazette.

Keywords: Bird flu; Avian influenza A virus (H5N1); Vaccination

John N. Sofos, Challenges to meat safety in the 21st century, Meat Science, Volume 78, Issues 1-2, Symposium on Meat safety: From Abattoir to Consumer, January-February 2008, Pages 3-13, ISSN 0309-1740, DOI: 10.1016/j.meatsci.2007.07.027.

(<http://www.sciencedirect.com/science/article/B6T9G-4P96265-1/2/35ca99c35796b19ef8f45ea5d3ceb832>)

Abstract:

The safety of meat has been at the forefront of societal concerns in recent years, and indications exist that challenges to meat safety will continue in the future. Major meat safety issues and related challenges include the need to control traditional as well as 'new,' 'emerging,' or 'evolving' pathogenic microorganisms, which may be of increased virulence and low infectious doses, or of resistance to antibiotics or food related stresses. Other microbial pathogen related concerns include cross-contamination of other foods and water with enteric pathogens of animal origin, meat animal manure treatment and disposal issues, foodborne illness surveillance and food attribution activities, and potential use of food safety programs at the farm. Other issues and challenges include food additives and chemical residues, animal identification and traceability issues, the safety and quality of organic and natural products, the need for and development of improved and rapid testing and pathogen detection methodologies for laboratory and field use, regulatory and inspection harmonization issues at the national and international level, determination of responsibilities for zoonotic diseases between animal health and regulatory public health agencies, establishment of risk assessment based food safety objectives, and complete and routine implementation of HACCP at the production and processing level on the basis of food handler training and consumer education. Viral pathogens will continue to be of concern at food service, bacterial pathogens such as *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* will continue affecting the safety of raw meat and poultry, while *Listeria monocytogenes* will be of concern in ready-to-eat processed products. These challenges become more important due to changes in animal production, product processing and distribution; increased international trade; changing consumer needs and increased preference for minimally processed products; increased worldwide meat consumption; higher numbers of consumers at-risk for infection; and, increased interest, awareness and scrutiny by consumers, news media, and consumer activist groups. Issues such as bovine spongiform encephalopathy will continue to be of interest mostly as a target for eradication, while viral agents affecting food animals, such as avian influenza, will always need attention for prevention or containment.

Keywords: Meat; Safety; Pathogens; Hazards; Bacteria

Gert Jan Boender, Ronald Meester, Edo Gies, Mart C.M. De Jong, The local threshold for geographical spread of infectious diseases between farms, *Preventive Veterinary Medicine*, Volume 82, Issues 1-2, 15 November 2007, Pages 90-101, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2007.05.016.

(<http://www.sciencedirect.com/science/article/B6TBK-4P2S21X-3/2/4cb5a84120cdd5555a6e9a83b87a4a4f>)

Abstract:

We investigated the influence of the spatial pattern of farms on the geographical spread of infectious livestock diseases, such as classical swine fever, foot-and-mouth disease and avian influenza in a combined analytical-numerical approach. Our purpose of this paper is to develop a method to identify the areas in which an infection has the potential to spread in an outbreak. In our model, each infected farm can infect neighbouring farms and the probability of transmission is a function of the inter-farm distance (spatial kernel). Therefore, the density of farms in an area is a good indicator for the probability of a major outbreak. In the epidemiological nomenclature, such density corresponds to a local reproduction ratio and we studied the critical behaviour of both the local density and the local reproduction ratio. We found that a threshold can be defined above which major outbreaks can occur, and the threshold value depends on the spatial kernel. Our expression for the threshold value is derived based on scaling arguments and contains two parameters in the exponents of the equation. We estimated these parameters from numerical results for the spatial spread using one particular mathematical function for the form of the spatial kernel. Subsequently, we show that our expression for the threshold using these estimated parameters agrees very well with numerical results for a number of different other functional forms of the spatial kernel (thus suggesting that we are dealing with universal parameters). As an

illustration of the practical relevance of the presented method, we calculated the threshold value for avian influenza in the Netherlands and use it to produce a risk map for this disease.

Keywords: Veterinary epidemiology; Spatial spread; Spatial kernel; High-risk areas

Zvonimir Poljak, Catherine E. Dewey, S. Wayne Martin, Jette Christensen, Susy Carman, Robert M. Friendship, Spatial clustering of swine influenza in Ontario on the basis of herd-level disease status with different misclassification errors, *Preventive Veterinary Medicine*, Volume 81, Issue 4, 16 October 2007, Pages 236-249, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2007.04.018.

(<http://www.sciencedirect.com/science/article/B6TBK-4NT93NR-2/2/a1f6a4788760bd33ba130d6c926018f4>)

Abstract:

This approach maximizes sensitivity of serology-based monitoring systems by considering spatial clustering of herds classified as false positive by herd testing, allowing outbreaks to be detected in an early phase. The primary objective of this study was to determine whether swine herds infected with influenza viruses cluster in space, and if so, where they cluster. The secondary objective was to investigate the combining of a multivariate spatial scan statistic with herd test results to maximize the sensitivity of the surveillance system for swine influenza. We tested for spatial clustering of swine influenza using the Cuzick-Edwards test as a global test. The location of the most likely spatial clusters of cases for each subtype and strain in a sample of 65 sow and 72 finisher herds in 2001 (Ontario, Canada), and 76 sow herds in 2003 (Ontario, Canada) was determined by a spatial scan statistic in a purely spatial Bernoulli model based on single and multiple datasets.

A case herd was defined by true herd-disease status for sow or finisher herds tested for H1N1, and by apparent herd-disease status for sow herds tested for two H3N2 strains (A/Swine/Colorado/1/77 (Sw/Col/77) and A/Swine/Texas/4199-2/98 (Sw/Tex/98)). In sow herds, there was no statistically significant clustering of H1N1 influenza after adjustment for pig-farm density. Similarly, spatial clustering was not found in finisher herds. In contrast, clustering of H3N2 Sw/Col/77 (prevalence ratio = 12.5) and H3N2 Sw/Tex/98 (prevalence ratio = 15) was identified in an area close to a region with documented isolation of avian influenza isolates from pigs.

For the H1N1 subtype tested by ELISA, we used an approach that minimized overall misclassification at the herd level. This could be more applicable for detecting clusters of positive farms when herd prevalence is moderate to high than when herd prevalence is low. For the H3N2 strains we used an approach that maximized herd-level sensitivity by minimizing the herd cut-off. This is useful in situations where prevalence of the pathogen is low. The results of applying a multivariate spatial scan statistic approach, led us to generate the hypothesis that an unknown variant of influenza of avian origin was circulating in swine herds close to an area where avian strains had previously been isolated from swine. Maximizing herd sensitivity and linking it with the spatial information can be of use for monitoring of pathogens that exhibit the potential for rapid antigenic change, which, consequently, might then lead to diminished cross-reactivity of routinely used assays and lower test sensitivity for the newly emerged variants. Veterinary authorities might incorporate this approach into animal disease surveillance programs that either substantiate freedom from disease, or are aimed at detecting early incursion of a pathogen, such as influenza virus, or both.

Keywords: Influenza; Swine; Scan statistic; Sentinel surveillance; Herd test; Spatial

A. Mannelli, L. Busani, M. Toson, S. Bertolini, S. Marangon, Transmission parameters of highly pathogenic avian influenza (H7N1) among industrial poultry farms in northern Italy in 1999-2000, *Preventive Veterinary Medicine*, Volume 81, Issue 4, 16 October 2007, Pages 318-322, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2007.04.017.

(<http://www.sciencedirect.com/science/article/B6TBK-4NT93NR-1/2/def8d9a67889bd031e2ca5403f9555ef>)

Abstract:

We estimated between-farm transmission parameters of the highly pathogenic avian-influenza (HPAI) epidemic that struck the poultry industry of northern Italy (including turkeys, layer hens, broilers, gamebirds, and waterfowl) from December 1999 through April 2000. We estimated the average number of susceptible farms that were infected with HPAI virus by each infectious farm during a day ($[\beta]$) with a generalised linear model (GLM). The HPAI's reproductive ratios (R_h ; the average number of new infected farms (IFs) that were caused by an infectious farm) were calculated separately for the regions of Lombardy and Veneto, where 382 out of 413 (92.5%) of IFs were located. In both regions, R_h decreased to ~ 1 during the second month of the epidemic (showing that its containment had been initiated). Subsequently, during the last two months of the epidemic, $[\beta]$ and R_h were reduced to 0.04/day and 0.6, respectively, in Veneto and to 0.07/day and 0.8 in Lombardy. The reduction of the susceptible population through strict control measures, including pre-emptive slaughter of at-risk poultry flocks, was implemented to a greatest extent in Veneto and this might have been associated with a more rapid control of the epidemic in this region than in Lombardy.

Keywords: Avian influenza; Epidemic; Italy; Reproductive ratio; Generalised linear model

M.S. Lee, M.C. Deng, Y.J. Lin, C.Y. Chang, Happy K. Shieh, J.Z. Shiau, C.C. Huang, Characterization of an H5N1 avian influenza virus from Taiwan, *Veterinary Microbiology*, Volume 124, Issues 3-4, 6 October 2007, Pages 193-201, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2007.04.021.

(<http://www.sciencedirect.com/science/article/B6TD6-4NFH0BB-M/2/9f9566b0b26d1d05261b322feabea1c4>)

Abstract:

In 2003, an avian influenza (AI) virus of H5N1 subtype (A/Duck/China/E319-2/03; Dk/CHN/E319-2/03) was isolated from a smuggled duck in Kinmen Island of Taiwan. Phylogenetic analysis and pairwise comparison of nucleotide and amino acid sequences revealed that the virus displayed high similarity to the H5N1 viruses circulating in Asia during 2004 and 2005. The hemagglutinin (HA) protein of the virus contained multiple basic amino acid residues (-RERRRKR-) adjacent to the cleavage site between the HA1 and HA2 domains, showing the highly pathogenic (HP) characteristics. The HP phenotype was confirmed by experimental infection of chickens, which led up to 100% mortality within 24-72 h postinfection. The virus replicated equally well in the majority of organs of the infected chickens with titers ranging from $10^{7.5}$ to $10^{4.7}$ 50% embryo lethal dose (ELD₅₀) per gram of tissue. In a mouse model the virus exhibits low pathogenic characteristics with a lethal infection observed only after applying high inoculating dose ($\geq 10^{7.6}$ ELD₅₀) of the virus. The infectious virus particles were recovered only from the pulmonary system including trachea and lungs. Our study suggests that ducks infected with H5N1 AIV of HPAI pathotype showing no disease signs can carry the virus silently and that bird smuggling represent a serious risk for H5N1 HPAI transmission.

Keywords: Highly pathogenic avian influenza; H5N1; Surveillance; Interspecies transmission

Chongmas Antarasena, Rungtiva Sirimujalin, Porntip Prommuang, Naruepol Promkuntod, Praisorn Prommuang, Stuart D. Blacksell, The indirect immunofluorescence assay using cardiac tissue from chickens, quails and ducks for identification of influenza A virus during an outbreak of highly pathogenic avian influenza virus (H5N1): A rapid and simple screening tool for limited resource settings, *Research in Veterinary Science*, Volume 83, Issue 2, October 2007, Pages 279-281, ISSN 0034-5288, DOI: 10.1016/j.rvsc.2006.12.007.

(<http://www.sciencedirect.com/science/article/B6WWR-4N0GDWY-1/2/d009dd5635cc6e926c707e5ec4cf47e5>)

Abstract:

Here we describe the diagnostic utility of the indirect immunofluorescence assay (IFA) during a recent outbreak of highly pathogenic avian influenza (HPAI) subtype H5N1 virus in southern Thailand and demonstrate the usefulness of the cardiac tissue from infected chickens, quail, and ducks for diagnosis. The most reliable sample for IFA diagnosis of influenza A virus was cardiac tissue (83.0%; 44/53) which when divided by species (chicken, quail and duck cardiac tissues) gave respective positivity rates of 88% (22/25), 88.9% (16/18) and 60.0% (6/10). Cardiac tissue also gave the highest IFA intensity for the three species. We believe that the IFA method has wide applicability in developing countries or remote settings where clinically similar avian diseases with high morbidity and mortality such as Newcastle disease and fowl cholera are common and could be rapidly excluded thereby conserving valuable reference laboratory capacity for true HPAI outbreaks.

Keywords: Avian influenza; HPAI; H5N1; Immunofluorescence; Cardiac tissue

Masatoshi Okamoto, Takehiko Saito, Yu Yamamoto, Masaji Mase, Satoko Tsuduku, Kikuyasu Nakamura, Kenji Tsukamoto, Shigeo Yamaguchi, Low pathogenicity H5N2 avian influenza outbreak in Japan during the 2005-2006, *Veterinary Microbiology*, Volume 124, Issues 1-2, 20 September 2007, Pages 35-46, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2007.04.025.

(<http://www.sciencedirect.com/science/article/B6TD6-4NJ0THB-1/2/96b15cdeabe524ffc3457e71badb5422>)

Abstract:

At the end of May 2005, a low-pathogenicity avian influenza (LPAI) virus of subtype H5N2 was isolated for the first time from chickens in Japan. Through active and epidemiological surveillance, 5.78 million chickens on 41 farms were found to be affected and 16 H5N2 viruses were isolated. Antigenic analysis revealed antigenic similarity of these isolates. Phylogenetic analysis showed that they originated from a common ancestor and clustered with the H5N2 strains prevalent in Central America that have been circulating since 1994. Experimental infection of chickens with the index isolate (*A/chicken/Ibaraki/1/05*) demonstrated that this virus replicated efficiently in the respiratory tract without clinical signs, and dust-borne and/or droplet-borne transmission was considered as a possible mode of transmission. These results suggested that the H5N2 LPAI viruses isolated in Japan were highly adapted to chickens.

Keywords: Avian influenza; H5N2; Antigenicity; Phylogenetic analysis; Transmission

Camille Lebarbenchon, Sylvie van der Werf, Frederic Thomas, Jean-Thierry Aubin, Saliha Azebi, Frederique Cuvelier, Patricia Jeannin, Vanessa Roca, Chung-Ming Chang, Yves Kayser, Benjamin Roche, Jean-Francois Guegan, Francois Renaud, Michel Gauthier-Clerc, Absence of detection of highly pathogenic H5N1 in migratory waterfowl in southern France in 2005-2006, *Infection, Genetics and Evolution*, Volume 7, Issue 5, September 2007, Pages 604-608, ISSN 1567-1348, DOI: 10.1016/j.meegid.2007.05.009.

(<http://www.sciencedirect.com/science/article/B6W8B-4NSMMS-3/2/67d509cea089c1db6b9c922ede48188d>)

Abstract:

During fall 2005, the rapid and wide spread of highly pathogenic (HP) H5N1 avian influenza viruses (AIV) outside Asia alerted European health authorities. Because of abnormal and recurrent field mortality, wild migratory birds were considered to be the main dispersing agent of the virus at an intercontinental scale. European wintering wetlands, such as the Camargue (Rhône delta, France), are identified as potential hot spots for the risk of introduction and transmission of bird-borne diseases. In this study, we investigated the role of migratory waterbirds (mainly ducks) in the spread of HP H5N1 viruses. We combined molecular analysis of living and freshly killed birds with population surveillance (aerial censuses and death surveillance). We sampled 1345 birds belonging to 17 waterbird species (3 orders) in the Camargue between September 2005 and March 2006. The prevalence of AIV was 1.8%. We did not detect HP H5N1 virus. Population

censuses did not reveal any population decreases nor abnormal mortalities. We discuss, in the light of these results, the implication of wild migratory ducks in the arrival of HP H5N1 AIV in Europe.

Keywords: Avian influenza; Anseriforms; Camargue

Ilaria Capua, Avian influenza: We have the chance to make a difference, *The Veterinary Journal*, Volume 174, Issue 2, September 2007, Pages 213-214, ISSN 1090-0233, DOI: 10.1016/j.tvjl.2007.07.004.

(<http://www.sciencedirect.com/science/article/B6WXN-4PDT02D-1/2/cee3fae931f00716d3254c4758dad286>)

Dirk U. Pfeiffer, Phan Q. Minh, Vincent Martin, Michael Epprecht, Martin J. Otte, An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occurrence in Vietnam using national surveillance data, *The Veterinary Journal*, Volume 174, Issue 2, September 2007, Pages 302-309, ISSN 1090-0233, DOI: 10.1016/j.tvjl.2007.05.010.

(<http://www.sciencedirect.com/science/article/B6WXN-4P3M2CR-2/2/a7cf4e5234a636ef9407eef23ac9c8a1>)

Abstract:

The objectives of this study were to describe the spatio-temporal pattern of an epidemic of highly pathogenic avian influenza (HPAI) in Vietnam and to identify potential risk factors for the introduction and maintenance of infection within the poultry population. The results indicate that during the time period 2004-early 2006 a sequence of three epidemic waves occurred in Vietnam as distinct spatial and temporal clusters. The risk of outbreak occurrence increased with a greater percentage of rice paddy fields, increasing domestic water bird and chicken density. It increased with reducing distance to higher population density aggregations, and in the third epidemic wave with increasing percentage of aquaculture. The findings indicate that agri-livestock farming systems involving domestic water birds and rice production in river delta areas are important for the maintenance and spread of infection. While the government's control measures appear to have been effective in the South and Central parts of Vietnam, it is likely that in the North of Vietnam the vaccination campaign led to transmission of infection which was subsequently brought under control.

Keywords: Avian influenza; Epidemiology; Vietnam; Poultry; Vaccination; Disease control

R. Klopffleisch, P.U. Wolf, C. Wolf, T. Harder, E. Starick, M. Niebuhr, T.C. Mettenleiter, J.P. Teifke, Encephalitis in a Stone Marten (*Martes foina*) after Natural Infection with Highly Pathogenic Avian Influenza Virus Subtype H5N1, *Journal of Comparative Pathology*, Volume 137, Issues 2-3, August-October 2007, Pages 155-159, ISSN 0021-9975, DOI: 10.1016/j.jcpa.2007.06.001.

(<http://www.sciencedirect.com/science/article/B6WHW-4PCH4NB-1/2/1f5c14807a847a82f0dcf45645e252c7>)

Abstract: Summary

Recent outbreaks of disease in different avian species, caused by the highly pathogenic avian influenza virus (HPAIV), have involved infection by subtype H5N1 of the virus. This virus has also crossed species barriers and infected felines and humans. Here, we report the natural infection of a stone marten (*Martes foina*) from an area with numerous confirmed cases of H5N1 HPAIV infection in wild birds. Histopathological examination of tissues from this animal revealed a diffuse nonsuppurative panencephalitis with perivascular cuffing, multifocal gliosis and neuronal necrosis. Additionally, focal necrosis of pancreatic acinar cells was observed. Immunohistochemically, lesions in these organs were associated with avian influenza virus antigen in neurons, glial cells and pancreatic acinar cells. Thus, the microscopical lesions and viral antigen distribution in this stone marten differs from that recently described for cats naturally and experimentally infected with the same virus subtype. This is the first report of natural infection of a mustelid with HPAIV H5N1.

Keywords: brain; highly pathogenic avian influenza virus (HPAIV); *Martes foina*; stone marten

Jing LI, Jing-fei WANG, Chun-yan WU, Yan-tao YANG, Zeng-tao JI, Hong-bin WANG, Establishment of a Risk Assessment Framework for Analysis of the Spread of Highly Pathogenic Avian Influenza, *Agricultural Sciences in China*, Volume 6, Issue 7, July 2007, Pages 877-881, ISSN 1671-2927, DOI: 10.1016/S1671-2927(07)60125-4.

(<http://www.sciencedirect.com/science/article/B82XG-4P9F020-H/2/a8d4faf7b3375d3ebc8616bac8b84546>)

Abstract: Abstract

To evaluate the risk of highly pathogenic avian influenza (HPAI) in mainland China, a risk assessment framework was built. Risk factors were determined by analyzing the epidemic data using the brainstorming method; the analytic hierarchy process was designed to weigh risk factors, and the integrated multicriteria analysis was used to evaluate the final result. The completed framework included the risk factor system, data standards for risk factors, weights of risk factors, and integrated assessment methods. This risk assessment framework can be used to quantitatively analyze the outbreak and spread of HPAI in mainland China.

Keywords: highly pathogenic avian influenza (HPAI); risk factor; risk assessment framework; analytical hierarchy process (AHP); weights; integrated assessment

Jinyong Wang, Jie Fang, Junqing Guo, Qiaoyang Teng, Zhenyu Huang, Jianyou Gu, Huigang Shen, Jiyong Zhou, Molecular cloning and characterization of Duck CD25, *Veterinary Immunology and Immunopathology*, Volume 117, Issues 3-4, 15 June 2007, Pages 266-274, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2007.02.007.

(<http://www.sciencedirect.com/science/article/B6TD5-4N43RJH-1/2/46d126e6ef96d5f9e0413cb037512d28>)

Abstract:

The IL-2R[alpha] chain (CD25, Tac) is an essential component of high affinity IL-2Rs, playing critical role for the immune specificity of antigen-activated T-cell clonal expansion. Up to now, no duck cytokine receptor has been described. Here, the cDNA segment of a duck cytokine receptor (duCD25), encoding a 226 aa precursor protein with a 20 aa signal peptide, was isolated. Then a novel mouse monoclonal antibody (mAb) was generated using the prokaryotically expressed duCD25 protein as immunogen. Using this mAb, the endogenous duCD25 molecule was localized on the surface of duck lymphocytes, and the duck IL-2-induced lymphocyte proliferation was further inhibited. Furthermore, flow cytometry analysis showed that duCD25 positive cells were upregulated in ducks infected with avian influenza virus (H9N2). Our findings confirm that duCD25 is a receptor of duck interleukin-2, and duCD25 positive cells play a potential role in H9N2 virus infection.

Keywords: Duck CD25 molecule; In vitro and in vivo expression; Monoclonal antibody; Avian influenza virus; DuCD25 positive cell

Shu-hong SUN, Zhi-zhong CUI, Li-xin QU, Maternal Antibody Protected Chicks from Growth Retardation and Immunosuppression Induced by Early Reticuloendotheliosis Virus Infection, *Agricultural Sciences in China*, Volume 6, Issue 6, June 2007, Pages 762-768, ISSN 1671-2927, DOI: 10.1016/S1671-2927(07)60110-2.

(<http://www.sciencedirect.com/science/article/B82XG-4P48RF7-K/2/0e9a5e748982ddf14bb326ed4b14087d>)

Abstract: Abstract

To determine if the maternal antibody from breeders vaccinated with cell culture-adapted reticuloendotheliosis virus (REV) could protect chicks from early REV infection, one-day-old chicks with or without anti-REV maternal antibodies were inoculated with REV, and then their growth rates and antibody titers to Newcastle disease virus (NDV) and avian influenza virus (AIV), after

vaccination with inactivated vaccines, were compared. This study indicated that REV infection could cause growth retardation and severely inhibit immune reactions to inactivated vaccines against NDV and Avian influenza virus (AIV, H9 and H5) in one-day-old broilers without maternal antibodies specific to REV. Maternal antibody from breeders vaccinated with an attenuated REV vaccine effectively protected REV-challenged birds from growth retardation and immunosuppression on antibody reactions to NDV and AIV vaccines. Four weeks after vaccination, the HI titers to NDV, AIV-H9, and AIV-H5 in maternal antibody positive and negative groups were 3.36+/-2.04 versus 1.58+/-1.69 ($P<0.01$), 6.27+/-3.87 versus 0.71+/-1.60 ($P<0.01$), and 6.72+/-3.92 versus 0.54+/-1.44 ($P<0.01$). Maternal antibodies from breeders vaccinated with REV vaccine could successfully protect chicks from REV infection and effectively prevent REV-induced growth retardation and immunosuppression in antibody responses to NDV and AIV.

Keywords: reticuloendotheliosis virus; Newcastle disease virus; avian influenza virus; immunosuppression; maternal antibody

Kwonil Jung, Dae-Sub Song, Bo-Kyu Kang, Jin-Sik Oh, Bong-Kyun Park, Serologic surveillance of swine H1 and H3 and avian H5 and H9 influenza A virus infections in swine population in Korea, Preventive Veterinary Medicine, Volume 79, Issues 2-4, 16 May 2007, Pages 294-303, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2006.12.005.

(<http://www.sciencedirect.com/science/article/B6TBK-4MT5513-2/2/8980864c5a8c1e22f5f8a14ea2d67aea>)

Abstract:

Influenza A is a respiratory disease common in the swine industry. Three subtypes, H1N1, H1N2 and H3N2 influenza A viruses, are currently co-circulating in swine populations in Korea. An outbreak of the highly pathogenic avian influenza H5N1 virus occurred in domestic bird farms in Korea during the winter season of 2003. Pigs can serve as hosts for avian influenza viruses, enabling passage of the virus to other mammals and recombination of mammalian and avian influenza viruses, which are more readily transmissible to humans. This study reports the current seroprevalence of swine H1 and H3 influenza in swine populations in Korea by hemagglutination inhibition (HI) assay. We also investigated whether avian H5 and H9 influenza transmission occurred in pigs from Korea using both the HI and neutralization (NT) tests. 51.2% (380/742) of serum samples tested were positive against the swine H1 virus and 43.7% (324/742) were positive against the swine H3 virus by HI assay. The incidence of seropositivity against both the swine H1 virus and the swine H3 virus was 25.3% (188/742). On the other hand, none of the samples tested showed seropositivity against either the avian H5 virus or the avian H9 virus by the HI and NT tests. Therefore, we report the high current seroprevalence and co-infectivity of swine H1 and H3 influenza viruses in swine populations and the lack of seroepidemiological evidence of avian H5 and H9 influenza transmission to Korean pigs.

Keywords: Seroprevalence; Pigs; Swine influenza viruses; Avian influenza viruses; Korea

E. Thiry, A. Zicola, D. Addie, H. Egberink, K. Hartmann, H. Lutz, H. Poulet, M.C. Horzinek, Highly pathogenic avian influenza H5N1 virus in cats and other carnivores, Veterinary Microbiology, Volume 122, Issues 1-2, 16 May 2007, Pages 25-31, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2006.12.021.

(<http://www.sciencedirect.com/science/article/B6TD6-4MRN9RJ-1/2/4efa39d8c104ce128548c0ba41a4719a>)

Abstract:

The Asian lineage highly pathogenic avian influenza (HPAI) H5N1 virus is a known pathogen of birds. Only recently, the virus has been reported to cause sporadic fatal disease in carnivores, and its zoonotic potential has been dominating the popular media. Attention to felids was drawn by two outbreaks with high mortality in tigers, leopards and other exotic felids in Thailand. Subsequently, domestic cats were found naturally infected and experimentally susceptible to H5N1 virus. A high

susceptibility of the dog to H3N8 equine influenza A virus had been reported earlier, and recently also HPAI H5N1 virus has been identified as a canine pathogen. The ferret, hamster and mouse are suitable as experimental animals; importantly, these species are also kept as pets. Experimental intratracheal and oral infection of cats with an HPAI H5N1 virus isolate from a human case resulted in lethal disease; furthermore, cats have been infected by the feeding of infected chickens. Spread of the infection from experimentally infected to in-contact cats has been reported. The epidemiological role of the cat and other pet animal species in transmitting HPAI H5N1 virus to humans needs continuous consideration and attention.

Keywords: Cat; Feline; Avian influenza; H5N1

DaPeng Peng, SiShun Hu, Yan Hua, YunCai Xiao, ZiLi Li, XiLiang Wang, DingRen Bi, Comparison of a new gold-immunochromatographic assay for the detection of antibodies against avian influenza virus with hemagglutination inhibition and agar gel immunodiffusion assays, *Veterinary Immunology and Immunopathology*, Volume 117, Issues 1-2, 15 May 2007, Pages 17-25, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2007.01.022.

(<http://www.sciencedirect.com/science/article/B6TD5-4N1T1M3-1/2/3cce2cf13cb21aab6be6305948516e90>)

Abstract:

A gold-immunochromatographic test-strip kit is used for the detection of IgG antibodies against the nucleocapsid protein of Avian Influenza Virus (AIV). Compared with the 'gold standard', i.e. hemagglutination inhibition (HI) and agar gel immunodiffusion (AGID) assays, the gold-immunochromatographic test strip has many advantages, such as high specificity, high sensitivity, convenience, is rapid and has low cost. The gold-immunochromatographic test strip provides a unique tool for the on-site surveillance and diagnosis of Avian Influenza.

Keywords: AIV; Antibody; Gold-immunochromatography test strip

Shingo Iwami, Yasuhiro Takeuchi, Xianning Liu, Avian-human influenza epidemic model, *Mathematical Biosciences*, Volume 207, Issue 1, May 2007, Pages 1-25, ISSN 0025-5564, DOI: 10.1016/j.mbs.2006.08.001.

(<http://www.sciencedirect.com/science/article/B6VHX-4KNM6K7-1/2/31d4bce0298a0e45ffa1b290c30d48d>)

Abstract:

A mathematical model is proposed to interpret the spread of avian influenza from the bird world to the human world. Our mathematical model warns that two types of the outbreak of avian influenza may occur if the humans do not prevent the spread of avian influenza. Moreover, it suggests that we cannot feel relieved although the total infected humans are kept at low level. In order to prevent spread of avian influenza in the human world, we must take the measures not only for the birds infected with avian influenza to exterminate but also for the humans infected with mutant avian influenza to quarantine when mutant avian influenza has already occurred. In particular, the latter measure is shown to be important to stop the second pandemic of avian influenza.

Keywords: SIR model; SI model; Endemic; Pandemic; Avian influenza; Mutation

Marius Gilbert, Xiangming Xiao, Prasit Chaitaweesub, Wantanee Kalpravidh, Sith Premasathira, Stephen Boles, Jan Slingenbergh, Avian influenza, domestic ducks and rice agriculture in Thailand, *Agriculture, Ecosystems & Environment*, Volume 119, Issues 3-4, March 2007, Pages 409-415, ISSN 0167-8809, DOI: 10.1016/j.agee.2006.09.001.

(<http://www.sciencedirect.com/science/article/B6T3Y-4M2WNV0-1/2/10b44ef93c75316309184c1f369312ed>)

Abstract:

Highly pathogenic avian influenza (HPAI) caused by H5N1 viruses has become a global scale problem which first emerged in southern China and from there spread to other countries in

Southeast and East Asia, where it was first confirmed in end 2003. In previous work, geospatial analyses demonstrated that free grazing ducks played critical role in the epidemiology of the disease in Thailand in the winter 2004/2005, both in terms of HPAI emergence and spread. This study explored the geographic association between free grazing duck census counts and current statistics on the spatial distribution of rice crops in Thailand, in particular the crop calendar of rice production. The analysis was carried out using both district level rice statistics and rice distribution data predicted with the aid of remote sensing, using a rice-detection algorithm. The results indicated a strong association between the number of free grazing ducks and the number of months during which second-crop rice harvest takes place, as well as with the rice crop intensity as predicted by remote sensing. These results confirmed that free grazing duck husbandry was strongly driven by agricultural land use and rice crop intensity, and that this later variable can be readily predicted using remote sensing. Analysis of rice cropping patterns may provide an indication of the location of populations of free grazing ducks in other countries with similar mixed duck and rice production systems and less detailed duck census data. Apart from free ranging ducks and rice cropping, the role of hydrology and seasonality of wetlands and water bodies in the HPAI risk analysis is also discussed in relation to the presumed dry season aggregation of wild waterfowl and aquatic poultry offering much scope for virus transmission.

Keywords: Highly pathogenic avian influenza; Domestic ducks; Remote sensing; Agriculture intensification; Rice paddy production

Alexander Nagy, Jirina Machova, Jitka Hornickova, Miroslav Tomci, Ivan Nagl, Bedrich Horyna, Ivan Holko, Highly pathogenic avian influenza virus subtype H5N1 in Mute swans in the Czech Republic, *Veterinary Microbiology*, Volume 120, Issues 1-2, 25 February 2007, Pages 9-16, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2006.10.004.

(<http://www.sciencedirect.com/science/article/B6TD6-4M3H7HX-4/2/c8173a0678281d33668b280ff58d1a25>)

Abstract:

In order to determine the actual prevalence of avian influenza viruses (AIV) in wild birds in the Czech Republic extensive surveillance was carried out between January and April 2006. A total of 2101 samples representing 61 bird species were examined for the presence of influenza A by using PCR, sequencing and cultivation on chicken embryos. AIV subtype H5N1 was detected in 12 Mute swans (*Cygnus olor*). The viruses were determined as HPAI (highly pathogenic avian influenza) and the hemagglutinin sequence was closely similar to A/mallard/Italy/835/06 and A/turkey/Turkey/1194/05. Following the first H5N1 case, about 300 wild birds representing 33 species were collected from the outbreak region and tested for the presence of AIV without any positive result. This is the first report of highly pathogenic avian influenza subtype H5N1 in the Czech Republic. The potential role of swan as an effective vector of avian influenza virus is also discussed.

Keywords: Highly pathogenic avian influenza; H5N1; Mute swan; Bird flu; Influenza A

Jin A. Kim, Sung Hwan Cho, Hyun Soo Kim, Sang Heui Seo, H9N2 influenza viruses isolated from poultry in Korean live bird markets continuously evolve and cause the severe clinical signs in layers, *Veterinary Microbiology*, Volume 118, Issues 3-4, 20 December 2006, Pages 169-176, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2006.07.007.

(<http://www.sciencedirect.com/science/article/B6TD6-4KPP48W-1/2/704ab28cddcd70284d4e48163f43c449>)

Abstract:

H9N2 influenza viruses are endemic in many Asian countries. We demonstrated that H9N2 influenza viruses isolated from poultry in Korean live bird markets are genetically changing and could cause the clinical signs in layers. Genetic analysis showed that Korean avian H9N2 influenza viruses are distinct from H9N2 influenza viruses circulating in poultry in China and Hong

Kong. When we infected layers with H9N2 isolates, layers showed about 30% mortality and the reduction of egg productions. Considering that H9N2 influenza virus is one of potential pandemic candidates, the continuous surveillance is needed to monitor avian H9N2 influenza viruses for the poultry industry and humans.

Keywords: Avian influenza virus; H9N2; Chicken

M.P. Doyle, M.C. Erickson, Emerging microbiological food safety issues related to meat, *Meat Science*, Volume 74, Issue 1, 52nd International Congress of Meat Science and Technology (52nd ICoMST) 13-18 August 2006 Dublin, Ireland, September 2006, Pages 98-112, ISSN 0309-1740, DOI: 10.1016/j.meatsci.2006.04.009.

(<http://www.sciencedirect.com/science/article/B6T9G-4JW7FJN-1/2/b958e4329da8992820d0d523cfa64bf5>)

Abstract:

Avian influenza viruses and antibiotic-resistant pathogens have become topics of current public health interest. This paper will focus on the significance of these pathogens to the meat industry as well as other emerging microbiological food safety topics likely to impact the meat industry. These include surveillance of foodborne pathogens, microbial source tracking, risk assessment, and human populations at increased risk of infection by foodborne microbes. These emerging issues will likely lead to even greater challenges to producing microbiologically safe meat products than the industry has ever experienced. However, accompanying such challenges will be innovative solutions that provide even greater public health protection to meat-containing foods.

Keywords: Avian flu; Antibiotic resistance; Surveillance; Microbial source tracking; Food attribution; Sensitive populations

Qiao-Yang Teng, Ji-Yong Zhou, Jia-Jun Wu, Jun-Qing Guo, Hui-Gang Shen, Characterization of chicken interleukin 2 receptor [alpha] chain, a homolog to mammalian CD25, *FEBS Letters*, Volume 580, Issue 17, 24 July 2006, Pages 4274-4281, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.06.044.

(<http://www.sciencedirect.com/science/article/B6T36-4K8R2XG-4/2/e600d739bb3c95f803f0ce50a7683e06>)

Abstract:

To identify chicken IL-2R [alpha] chain (chCD25), the cDNA of chCD25 was cloned and mapped onto chicken chromosome 1. The polyclonal and monoclonal antibodies raised from the recombinant chCD25 specifically bound to the cell surface of splenic mononuclear cells (SMC) and inhibited chicken IL-2-dependent proliferation of T cells. Flow cytometry analysis revealed that chCD25 molecules could be expressed on the surface of monocytes/macrophages, thrombocytes, CD4+ and CD8+ cells as well as tissue cells. Importantly, the CD4+CD25+ and CD8+CD25+ cells were upregulated dramatically in chickens infected with H9N2 avian influenza virus. These results confirm that the cloned cDNA is the nucleotide sequence of chicken IL-2R, and suggest that chicken CD4+CD25+ and CD8+CD25+ cells may play an important role in immune responses induced by H9N2 virus, and the monoclonal antibodies to chCD25 may be useful for investigating biological functions of chicken regulatory T cells.

Keywords: Chicken CD25; Monoclonal antibody; Regulatory T cell; Avian influenza virus

G. Chowell, C.E. Ammon, N.W. Hengartner, J.M. Hyman, Transmission dynamics of the great influenza pandemic of 1918 in Geneva, Switzerland: Assessing the effects of hypothetical interventions, *Journal of Theoretical Biology*, Volume 241, Issue 2, 21 July 2006, Pages 193-204, ISSN 0022-5193, DOI: 10.1016/j.jtbi.2005.11.026.

(<http://www.sciencedirect.com/science/article/B6WMD-4HYN0FV-7/2/f2739d5adcb7a7d8fdd698533bff5b1f>)

Abstract:

Recurrent outbreaks of the avian H5N1 influenza virus in Asia represent a constant global pandemic threat. We characterize and evaluate hypothetical public health measures during the 1918 influenza pandemic in the Canton of Geneva, Switzerland. The transmission rate, the recovery rate, the diagnostic rate, the relative infectiousness of asymptomatic cases, and the proportion of clinical cases are estimated through least-squares fitting of the model to epidemic curve data of the cumulative number of hospital notifications. The latent period and the case fatality proportion are taken from published literature. We determine the variance and identifiability of model parameters via a simulation study. Our epidemic model agrees well with the observed epidemic data. We estimate the basic reproductive number for the spring wave (95% CI: 1.45-1.53) and the reproductive number for the fall wave (95% CI: 3.57-3.93). In addition, we estimate the clinical reporting for these two waves to be 59.7% (95% CI: 55.7-63.7) and 83% (95% CI: 79-87). We surmise that the lower reporting in the first wave can be explained by a lack of initial awareness of the epidemic and the relative higher severity of the symptoms experienced during the fall wave. We found that effective isolation measures in hospital clinics at best would only ensure control with probability 0.87 while reducing the transmission rate by >76.5% guarantees stopping an epidemic.

Keywords: Spanish flu; Pandemic; Influenza; Reproductive number; Switzerland

Shimon Perk, Caroline Banet-Noach, Ester Shihmanter, Shimon Pokamunski, Michael Pirak, Michael Lipkind, Alexander Panshin, Genetic characterization of the H9N2 influenza viruses circulated in the poultry population in Israel, *Comparative Immunology, Microbiology and Infectious Diseases*, Volume 29, Issue 4, July 2006, Pages 207-223, ISSN 0147-9571, DOI: 10.1016/j.cimid.2006.06.004.

(<http://www.sciencedirect.com/science/article/B6T5H-4KTVTRF-1/2/5dc945c51267121326b4fef8b35193af>)

Abstract:

The partial nucleotide sequences of the hemagglutinin (HA) genes of 72 H9N2 influenza viruses isolated from chickens and turkeys in Israel during the period 2000-2005 were genetically analyzed. The isolates possessed the three types of amino acid motif -R-S-S-R/G-L-, -R-S-N-R/G-L-, and -R-S-K-R/G-L- at the cleavage site of HA. Phylogenetic analyses showed that all Israeli isolates belonged to the same group which further divided into three closely related sub-groups. The HA genes of these isolates were related to the HA gene of A/chicken/Germany/R45/98 isolated from chicken in Germany in 1998.

Keywords: Virus de la grippe aviaire de type H9N2; Gene de l'hemagglutinine; Site de clivage; Analyse phylogenetique; Avian influenza virus; H9N2 subtype; Hemagglutinin gene; Cleavage site; Phylogenetic analysis

David E. Swayne, Microassay for measuring thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat, *International Journal of Food Microbiology*, Volume 108, Issue 2, 25 April 2006, Pages 268-271, ISSN 0168-1605, DOI: 10.1016/j.ijfoodmicro.2005.08.032.

(<http://www.sciencedirect.com/science/article/B6T7K-4J8D8T4-4/2/e4bb5e9e4ff638a6f7c014a613d3b4fc>)

Abstract:

A precise, reproducible microassay was developed to measure thermal inactivation of high pathogenicity avian influenza (HPAI) virus in chicken meat. Small pieces of breast or thigh meat (0.05 g) from chickens infected with A/chicken/Pennsylvania/1370/1983 (H5N2) (PA/83) or A/chicken/Korea/ES/2003 (H5N1) (Korea/03) HPAI viruses were tested for inactivation in the heating block of a thermocycler. Korea/03 infected thigh and breast meat had higher virus concentrations (106.8 and 105.6 mean embryo infectious doses [EID50]/g, respectively) compared to PA/83 infected thigh and breast meat (102.8 and 102.3 EID50/g, respectively). The samples

were ran through a ramp-up cycle from 25 to 70 [degree sign]C, and meat samples were removed and examined for virus infectivity at 30, 40, 50, 60 and 70 [degree sign]C, and after treatment for 1, 5, 10, 30 and 60 s at 70 [degree sign]C. The reduction in virus infectivity titers was dependent on virus concentration and no HPAI virus was isolated after 1 s of treatment at 70 [degree sign]C. A change in coloration from pink-tan to white was associated with a loss in recovery of infectious virus. The microassay provided a predictable and reproducible method to measure thermal inactivation of HPAI virus in chicken meat.

Keywords: Avian influenza; Chicken; H5N1; Meat; Thermal inactivation

A. Mannelli, N. Ferre, S. Marangon, Analysis of the 1999-2000 highly pathogenic avian influenza (H7N1) epidemic in the main poultry-production area in northern Italy, *Preventive Veterinary Medicine*, Volume 73, Issue 4, 16 March 2006, Pages 273-285, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2005.09.005.

(<http://www.sciencedirect.com/science/article/B6TBK-4HCMS6F-2/2/22a6516fbd667c4b37cde32873eb0b3a>)

Abstract:

We evaluated the effects of risk factors and control policies following the highly pathogenic avian influenza (HPAI) epidemic that struck northern Italy's poultry industry in the winter of 1999-2000. The epidemic was caused by a type-A influenza virus of the H7N1 subtype, that originated from a low-pathogenic AI virus which spread among poultry farms in northeastern Italy in 1999 and eventually became virulent by mutation. Most infected premises (IP) were located in the regions of Lombardy and Veneto (382 out of 413, 92.5%), and the eradication measures provided for in the European legislation were enforced. In Veneto, where flock density was highest, infection-control was also accomplished by means of depopulation of susceptible flocks through a ban on restocking and pre-emptive slaughter of flocks that were in the vicinities of or that had dangerous contacts with IPs. In Lombardy, such control measures were applied to a lesser extent. Infection incidence rate (IR) was 2.6 cases per 1000 flocks per day in Lombardy and 1.1 in Veneto. After the implementation of infection-control measures, the at-risk population, the percentage of flocks ≤ 1.5 km from IPs, and the HPAI-IR underwent a greater reduction in Veneto than in Lombardy. Although the proximity (≤ 1.5 km) to IPs in the temporal risk window (TRW) was a major risk factor for HPAI at the individual flock level, its effect at the population level (population-attributable fraction) did not exceed 31.3%. Viral transmission therefore also occurred among relatively distant flocks. Turkey flocks were characterised by greater IR of HPAI compared with other bird species such as layer hens, broilers, gamebirds, and waterfowl, even when located at distances > 1.5 km from IPs. In Lombardy, IR for species other than turkeys was also relatively high.

Keywords: Avian influenza; Epidemic; Italy; Log-linear model; Cox regression

A. Kijlstra, I.A.J.M. Eijck, Animal health in organic livestock production systems: a review, *NJAS - Wageningen Journal of Life Sciences*, Volume 54, Issue 1, 2006, Pages 77-94, ISSN 1573-5214, DOI: 10.1016/S1573-5214(06)80005-9.

(<http://www.sciencedirect.com/science/article/B94T2-4WFBS5K-5/2/cfb59ccbd82d6a8229a7d6c475f5431e>)

Abstract:

Organic livestock production is a means of food production with a large number of rules directed towards a high status of animal welfare, care for the environment, restricted use of medical drugs and the production of a healthy product without residues (pesticides or medical drugs). The intentions of organic livestock production have been formulated by the International Federation of Organic Agriculture Movements (IFOAM) and were further implemented by EU regulation 2092/91 in the year 2000. The consequences of these rules for the health of the animals were not yet fully anticipated at the time these regulations were made and it has become clear that in some cases the rules are not clear enough, thereby even hampering the development of the production

system. In this review we shall discuss the implications of these rules for animal health, whereby we shall focus on pig, poultry and dairy production systems. Disease prevention in organic farming is based on the principles that an animal that is allowed to exhibit natural behaviour is not subject to stress, is fed optimal (organic) feed, and will have a higher ability to cope with infections than animals reared in a conventional way. Fewer medical treatments would thus be necessary and if an animal would become diseased, alternative treatments instead of conventional drugs should be preferred. Although homeopathy or phytotherapy are recommended according to prevailing regulations, not many organic farmers use this treatment regimen because of lack of scientific evidence of effectiveness. Important health problems in organic livestock farming are often related to the outdoor access area, exposing the animals to various viral, bacterial and parasitic infections some of which may only influence the animals' own welfare whereas other ones may also endanger the health of conventional livestock (e.g. Avian Influenza) or pose a food safety (Campylobacter, Toxoplasma) problem to the consumer. Many preventive measures can be taken, such as using better animal breeds, optimized rearing conditions, pre- and probiotics, and addition of acids to the drinking water. In case of infectious disease, tight vaccination schedules may prevent serious outbreaks.

Keywords: organic production; homeopathy; infectious disease

G. Koch, A.R.W. Elbers, Outdoor ranging of poultry: a major risk factor for the introduction and development of High-Pathogenicity Avian Influenza, *NJAS - Wageningen Journal of Life Sciences*, Volume 54, Issue 2, 2006, Pages 179-194, ISSN 1573-5214, DOI: 10.1016/S1573-5214(06)80021-7.

(<http://www.sciencedirect.com/science/article/B94T2-4WFBS65-6/2/def70d02ff1c7dbd022d4ce707f519d8>)

Abstract:

High-Pathogenicity Avian Influenza (HPAI) is an extremely infectious viral disease of poultry. Public health concerns were raised when six persons died in Hong Kong in 1997 after exposure to HPAI-infected poultry. Its danger became imminent in the recent HPAI epidemic in South-East Asia when the virus expanded its geographical range via parts of central Asia to Europe, Africa and the Middle East. Wild birds are frequently carriers of influenza A viruses. Nearly all Avian Influenza (AI) viruses isolated from wild birds are low-pathogenic and cause no clinical problems in these birds. Only after low-pathogenicity viruses are introduced in poultry, in particular in chickens and turkeys, high-pathogenicity mutants emerge after a variable length of time. Biosecurity is the first line of defence against an introduction of AI into commercial poultry flocks. Any conceivable contact between possibly contaminated animals, areas around poultry houses contaminated with faecal material from wild birds and contaminated abiotic vectors on the one hand and domestic poultry on the other must be avoided. In this paper we shall discuss the worldwide occurrence of HPAI outbreaks, the existence of AI virus infections in wild birds, and possible strategies to reduce the risk of the introduction of AI viruses into domestic poultry flocks, with special reference to free ranging.

Keywords: wild birds; public health risk; Avian Influenza ecology

M.P.M. Meuwissen, M. Van Boven, T.J. Hagenaars, G.J. Boender, G. Nodelijk, M.C.M. De Jong, R.B.M. Huirne, Predicting future costs of High-Pathogenicity Avian Influenza epidemics: large versus small uncertainties, *NJAS - Wageningen Journal of Life Sciences*, Volume 54, Issue 2, 2006, Pages 195-205, ISSN 1573-5214, DOI: 10.1016/S1573-5214(06)80022-9.

(<http://www.sciencedirect.com/science/article/B94T2-4WFBS65-7/2/72648fa7a805f49324d436ab3f259f60>)

Abstract:

Every five years, the Dutch government and the poultry sector agree on how the direct costs of epidemics in poultry, should they occur, will be shared. In the agreement for 2005-2009 the

maximum amount to be paid by the poultry sector was set considerably higher than in the 1999-2004 agreement. This increase was caused mainly by the expected financial risks associated with High-Pathogenicity Avian Influenza (HPAI) epidemics. In this paper we focus on elucidating the uncertain and the less uncertain aspects of the HPAI financial risk problem. We distinguish between (1) the probability of an introduction of HPAI in the Netherlands, (2) the transmission potential of HPAI in the Netherlands, and (3) the costs and financing issues resulting from HPAI epidemics. We argue that whereas current understanding allows relatively precise answers to the question 'If there is an epidemic, how many farms will be affected and what will be the direct costs?', much larger uncertainties are associated with the questions 'What is the chance of an HPAI epidemic in the Netherlands?', 'How large will be the long-term government share in the direct costs?', and 'How large will be the indirect costs?'.
Keywords: financial risk; risk analysis; modelling an epidemic

Mohammad Q. Al-Natour, Mahmoud N. Abo-Shehada, Sero-prevalence of avian influenza among broiler-breeder flocks in Jordan, *Preventive Veterinary Medicine*, Volume 70, Issues 1-2, 12 August 2005, Pages 45-50, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2005.02.009.

(<http://www.sciencedirect.com/science/article/B6TBK-4FS2378-2/2/9adbd55c202915e7dc255ae33ead0513>)

Abstract:

Thirty blood samples were collected randomly from each of the 38 breeder-broiler farms in Jordan. Serum samples were examined using indirect ELISA for specific antibodies to avian influenza virus. The overall true flock-level sero-prevalence of avian influenza was 71% (95% CI: 55,83). Positive flocks had 2-30 sero-positive chickens and half of flocks had >20 sero-positive birds. The number of sero-positive flocks varied in the studied localities with more sero-positives in farms located within the migratory route of migratory wild fowl. The examined broiler-breeder flocks had no clinical signs, or noticeable decrease in egg production; mortalities were within the normal range (0.1-1%). The number of positive sera/flock correlated with flock size. There were a no significant (Pearsons $r = 0.21$, $p = 0.21$) correlation between positive flocks and age. A non-pathogenic AI virus infects broiler-breeder farms in Jordan. Wild local and migrating birds might promote the further spread of this virus in Jordan and other countries.

Keywords: Avian influenza; Poultry; Viral diseases; Broiler-breeder; ELISA; Age influence; Jordan

M.E. Thomas, A. Bouma, H.M. Ekker, A.J.M. Fonken, J.A. Stegeman, M. Nielen, Risk factors for the introduction of high pathogenicity Avian Influenza virus into poultry farms during the epidemic in the Netherlands in 2003, *Preventive Veterinary Medicine*, Volume 69, Issues 1-2, 10 June 2005, Pages 1-11, ISSN 0167-5877, DOI: 10.1016/j.prevetmed.2004.12.001.

(<http://www.sciencedirect.com/science/article/B6TBK-4G1GF4J-1/2/38333798451b354f754b4e10ecb788b6>)

Abstract:

An epidemic of high pathogenicity Avian Influenza (HPAI) occurred in the Netherlands in 2003. A census survey of 173 infected and 401 uninfected commercial poultry farms was carried out to identify factors associated with the introduction of the HPAI virus into poultry farms. Data on farm size, production characteristics, type of housing, presence of cattle and pigs were gathered by the National Inspection Service for Livestock and Meat from all farms included in this study. For each risk factor (RF) available for analysis, the Mantel-Haenszel odds ratio was calculated (stratified on farm size and housing type). We found an increased risk of HPAI virus introduction in layer finisher type poultry: OR = 2.05 (95% confidence interval, CI = 1.29-3.27). An explanation for this increased risk is the high number of contacts between these farms, especially via cardboard egg trays used for removal of eggs during the epidemic. Our analysis did not indicate significant differences between the infected and uninfected farms with regard to housing type, presence of

cattle or pigs. Since layer finisher type farms are assumed to be at higher risk for HPAI virus introduction, more specific control measures might be applied in future outbreaks.

Keywords: Poultry; Avian Influenza; Risk factors; Epidemic; The Netherlands

C. C. Breathnach, R. Rudersdorf, D. P. Lunn, Use of recombinant modified vaccinia Ankara viral vectors for equine influenza vaccination, *Veterinary Immunology and Immunopathology*, Volume 98, Issues 3-4, April 2004, Pages 127-136, ISSN 0165-2427, DOI: 10.1016/j.vetimm.2003.11.004. (<http://www.sciencedirect.com/science/article/B6TD5-4BMJX4M-1/2/5908e90827512c0a6b5c1d3251b032ea>)

Abstract:

Recombinant modified vaccinia Ankara (MVA) vectors expressing equine influenza virus genes were constructed and evaluated for use in equine vaccination. Two strains of recombinant MVA, expressing either hemagglutinin (HA) or nucleoprotein (NP) genes were constructed. Each influenza virus gene was cloned from A/equine/Kentucky/1/81 (Eq/Ky) into an MVA construction plasmid, and was introduced to the deletion III locus of the wild type MVA genome by homologous recombination. Recombinant viruses were plaque purified, and antigen expression was confirmed by immunostaining.

Two ponies were primed by vaccination with 50 [mu]g HA-DNA and two ponies were vaccinated with 50 [mu]g NP-DNA using the PowderJect XR research device. Six and 10 weeks later, ponies were immunized with 2x10⁹ infectious units of recombinant MVA encoding the homologous influenza antigen, equally divided between intramuscular and intradermal sites in the neck.

A marked rise in influenza virus-specific IgG_A and IgG_B serum antibody titers was detected following administration of MVA boosters with both HA and NP antigens. Influenza virus-specific lymphoproliferative responses and IFN- γ mRNA production were also strongly elicited by both antigens. This study demonstrates the facility with which recombinant MVA viruses expressing defined pathogen genes can be constructed, and provides preliminary evidence of the immunogenicity and safety of these vectors in the horse.

Keywords: Horses; Influenza virus; Vaccination; Modified vaccinia Ankara

Takashi Suzuki, Tadanobu Takahashi, Takehiko Saito, Chao-Tan Guo, Kazuya I.-P.Jwa Hidari, Daisei Miyamoto, Yasuo Suzuki, Evolutional analysis of human influenza A virus N2 neuraminidase genes based on the transition of the low-pH stability of sialidase activity, *FEBS Letters*, Volume 557, Issues 1-3, 16 January 2004, Pages 228-232, ISSN 0014-5793, DOI: 10.1016/S0014-5793(03)01503-5.

(<http://www.sciencedirect.com/science/article/B6T36-4BBM3XN-7/2/7399a5fcddc0da72eefb672ffa0113d5>)

Abstract:

The 1957 and 1968 human pandemic influenza A virus strains as well as duck viruses possess sialidase activity under low-pH conditions, but human H3N2 strains isolated after 1968 do not possess such activity. We investigated the transition of avian (duck)-like low-pH stability of sialidase activities with the evolution of N2 neuraminidase (NA) genes in human influenza A virus strains. We found that the NA genes of H3N2 viruses isolated from 1971 to 1982 had evolved from the side branches of NA genes of H2N2 epidemic strains isolated in 1968 that were characterized by the low-pH-unstable sialidase activities, though the NA genes of the 1968 pandemic strains preserved the low-pH-stable sialidase. These findings suggest that the prototype of the H3N2 epidemic influenza strains isolated after 1968 probably acquired the NA gene from the H2N2 low-pH-unstable sialidase strain by second genetic reassortment in humans.

Keywords: Influenza virus; Neuraminidase; Sialidase; Evolutional analysis

T.B. Rodenburg, M.C. Van Der Hulst-Van Arkel, R.P. Kwakkel, *Campylobacter* and *Salmonella* infections on organic broiler farms, *NJAS - Wageningen Journal of Life Sciences*, Volume 52, Issue 2, 2004, Pages 101-108, ISSN 1573-5214, DOI: 10.1016/S1573-5214(04)80006-X.

(<http://www.sciencedirect.com/science/article/B94T2-4WFBS5S-1/2/acb882d2d5245782ef2ac59de6bbadf9>)

Abstract:

Organic poultry production in the Netherlands is developing. Although consumers assume organic products to be safer and healthier, there are aspects of organic animal husbandry, like access to an outdoor run, that can result in increased risks of food safety problems. The aim of this study was to compare housing and management of organic and conventional broiler farms in the Netherlands and to study the occurrence of *Salmonella* and *Campylobacter* infections on the former. Large differences were found between the two farming systems with respect to mixed or single farming, manure storage, drinking-water system, ventilation, access to an outdoor run, and pest control. From the 31 organic flocks sampled for *Salmonella* and *Campylobacter* in 2003, 13% were positive for *Salmonella* and 35% for *Campylobacter*. Results for the summer period are missing due to an outbreak of avian influenza, so the actual number of flocks infected with *Campylobacter* can be expected to be even higher. *Campylobacter* appears to be the main risk on organic broiler farms, so that it would be interesting to study specific risk factors of infection with this pathogen on these farms.

Keywords: food safety; risk factors; outdoor run

M. A. De Marco, L. Campitelli, E. Foni, E. Raffini, G. Barigazzi, M. Delogu, V. Guberti, L. Di Trani, M. Tollis, I. Donatelli, Influenza surveillance in birds in Italian wetlands (1992-1998): is there a host restricted circulation of influenza viruses in sympatric ducks and coots?, *Veterinary Microbiology*, Volume 98, Issues 3-4, 5 March 2004, Pages 197-208, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2003.10.018.

(<http://www.sciencedirect.com/science/article/B6TD6-4B85086-1/2/931ebc55ef9fab538eec5f6568aa6078>)

Abstract:

We report the results of a 6-year serological and virological monitoring performed in ducks and coots in Italy, in order to assess the degree of influenza A virus circulation in these birds during wintering. A total of 1039 sera collected from 1992 to 1998 was screened by a double antibody sandwich blocking ELISA (NP-ELISA): seroprevalence of antibodies to influenza A viruses was significantly higher in ducks compared to coots (52.2% vs. 7.1%, respectively). The hemagglutination-inhibition (HI) assay, performed on NP-ELISA positive sera, showed that 16.9% of these duck sera and 33.3% of these coot sera had antibodies to at least one influenza virus HA subtype: ducks showed HI antibodies against most of the HA subtypes, except for the H3, H4, H7, and H12; coots were seropositive to the H3 and H10 subtypes, only. From 1993 to 1998, 22 virus strains were obtained from 802 cloacal swabs, with an overall virus isolation frequency of 2.7%. Viruses belonging to the H1N1 subtype were by far the most commonly circulating strains (18/22) and were isolated mainly from ducks (17/18). The remaining viruses were representative of the H10N8, H5N2 and H3N8 subtypes. Our data indicate some differences between influenza A virus circulation in sympatric ducks and coots and a significant antigenic diversity between some reference strains and viruses recently isolated in Italy.

Keywords: Avian influenza; HA subtype circulation; Serological survey; Virological survey; Wild aquatic birds; Sympatric waterfowl species; Italy