First International Symposium
on
Avian Influenza

A B S T R A C T S

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Avian Influenza 1981: A Silver Anniversary, a Centennial or a Millennium?

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There are many significant events recorded in the chronicle of influenza. Perhaps even more significant events have gone unrecognized, unrecorded, and unheeded. There are at least 3 events in that chronicle that relate to this Symposium. They are concerned with; 1) the recognition of a serious disease among poultry in 1878; 2) a global pandemic of influenza in human beings and the appearance of a new influenza-like disease in swine in 1918; and 3) the beginning of an avian influenza era in 1956.

During these many years, many faces of influenza have been observed. Viruses once thought to be limited to human beings and swine are now found throughout the world in many avian species. Has there been an explosion of these viruses among the avian species or is it due only to a lack of awareness on our part as biomedical scientists? Is the seemingly happy ecologic family of birds and influenza viruses the result of a very long evolutionary process?

Despite the world-wide occurrence of these viruses, we know very little about their real threat and/or potential to cause significant reductions in avian populations with significant economic losses.

It will be clear to the participants in this Symposium that there is a considerable bank of knowledge on avian influenza. While that information is indeed important, the benefits of this Symposium will not be complete unless the participants provide goals, priorities, and recommendations for international unity in addressing the problems of avian influenza.

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Influenza Avian Species - Uncomplicated Infection with Virulent Strains

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Start → Complications Associated With Avian Influenza Infections

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There is considerable variation in the pathogenicity of the numerous Avian Influenza Virus subtypes. There is no apparent correlation between the virus subtype and its ability to produce disease in the host. Reproducing the clinical disease observed in the field under laboratory conditions is very difficult. Most Avian Influenza Virus isolates are only mildly pathogenic if at all in the laboratory. The major reasons for this inability to reproduce the clinical disease in the laboratory are: 1) The absence of concurrent infections (e.g. Newcastle disease virus, P. multocida, E. coli), 2) The simultaneous use of live viral or bacterial vaccines (Newcastle or Fowl Cholera), 3) Good environmental conditions, or 4) A host whose immune system is not compromised.

Avian Influenza is usually a subclinical infection. When a turkey producer observes an excessive vaccination reaction to life Fowl Cholera vaccination, or has high mortality following an outbreak of Cholera, he will often find Avian Influenza Virus along with the condition. Mortality in excess of 50% isn't unusual if AIV infects young birds in confinement where ventilation is marginal and E. coli is invading the respiratory system.

A very susceptible period for Avian Influenza is during egg production. Physiological and environmental stress during this period play a prominant role in the degree of mortality and production loss observed as a result of the infection.

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AVIAN INFLUENZA IN THE UNITED STATES (1964-1980)

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Classical fowl plague has not been identified in the United States since 1929. The first reported isolation in the United States of less pathogenic strains was made from turkeys in California in 1964 by Bankowski and Mikami. Isolations were made from turkeys in 1965 in Massachusetts and Wisconsin and in 1966 the disease was identified serologically in turkeys in Minnesota. Since then, influenza has been identified each year in turkeys in Minnesota. The disease has been reported in turkeys in other States since 1966, Washington, Oregon, South Dakota, Iowa, Missouri, Ohio, Pennsylvania and Texas. Only two outbreaks have been reported in chickens, Alabama (1975) and Minnesota (1978). Domestic ducks have been found infected in Pennsylvania (1970) and as well as domestic geese (1975) and guinea fowl (1975) and pheasants (1980) in Minnesota.

A wide variety of Hemagglutinin (HA) and Neuraminidase (NA) antigens have been identified in isolates from domestic avian species in the United States, HAV1, HAV2, HAV4, HAV5, HAV6, HAV9, HSW1. These isolates have varied in pathogenicity under field conditions resulting in mild to moderate signs and lesions in meat birds and in flocks in production, the effects on egg production have varied widely. Although HAV1 (fowl plague hemagglutinin) has been isolated from turkeys in three States (Oregon (1971), Texas (1978), Minnesota (1980) and from pheasants (Minnesota (1980)), the pathogenicity of these isolates have been low to moderate in severity under field conditions and showed low pathogenicity under laboratory conditions.

A wide variety of influenza isolates have been isolated from migratory waterfowl in the major flyways in the United States as well as from imported exotic cage birds at USDA quarantine stations.

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Start → CURRENT WORLD-WIDE SITUATION OF AVIAN INFLUENZA IN AUSTRALIA

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Influenza virus infection of commercial poultry has only been detected once in Australia. Fowl plague caused by a virus with antigenic determinants Havl Neql was detected in 2 egg layers, 1 broiler and 1 duck breeder flocks in January and February 1976. Naturally occurring disease was observed in the chickens but not in the ducks. All infected flocks were slaughtered and extensive serological surveys throughout Australia demonstrated no further occurrence of infection.

The source of fowl plague infection was not determined although serology and cloacal swab culture were used in an attempt to demonstrate infection of wild birds.

Influenza infection of sea birds was detected in the north-east region of Australia in 1972 and 1975, when virus with antigenic determinants Hav. 6 Nav. 5, Hav. 5 Nav. 2 and Hav. 3 Nav. 6 were isolated.

Since 1978 some 45 influenza viruses have been isolated from sea and freshwater birds and domestic chickens in the north-western region of Australia. These viruses have no pathogenicity for domestic chickens.

Extensive cultural and serological investigations of birds, particularly penguins, on Macquarie Island and the Australian Antarctic Continent has not resulted in the isolation of any influenza viruses. However, antibody to fowl plague virus was detected in the sera of 10.9 per cent of adult Adelie penguins sampled on Peterson Island.

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Current World-Wide Situation of Avian Influenza - Belgium

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Start → A Review of Influenza in Canadian Wild and Domestic Birds 1962-1980.

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Influenza in Canadian poultry became a problem in domestic turkeys in the early 60's reached a peak in the late 60's and declined during the 70's to a level of near insignificance. Influenza in chickens was never recorded during this time, while duck influenza, although enzootic, has drawn little attention as a pathological problem except occasionally in the very young, and then in association with other pathogens. Turkey influenza appeared mostly in sporadic outbreaks and sofar has never established itself enzootically on farms, as is evident from the serologic varieties of viruses isolated from diseased flocks. On the other hand, several influenza surveys carried out in the Canadian wild bird fauna have shown that every one of the 12 hemagglutinin types of the latest classification scheme (1980) has been found in Canadian wild birds. Accepting the premise that influenza in domestic birds has its source of infection in wild birds, a hypothetical vaccination scheme of turkeys would require a vaccine encompassing most or all HA types, a difficult and costly proposition further weakened by the short-lived immune response of turkeys to influenza antigens. Ontario poultry pathologists propagate the concept of strict separation between domestic turkeys and chickens from all wild or feral birds. The reduction in turkey influenza in Ontario, which contrasts with an increase of influenza reported from the US, is attributed to the acceptance of this concept, coupled to established normal sanitation principles of modern poultry husbandry, by the principal Ontario turkey breeders.

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Current World-Wide Situation of Avian Influenza - France

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Start → Avian influenza in Hong Kong

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Approximately 36 million domestic poultry were raised in Hong Kong for local consumption in 1980. This comprised only 60% of requirements, the balance being supplied mainly as live birds from neighbouring Guangdong Province and also from Guangxi Province, China.

Since November 1975, continuous market surveillance of ducks, geese and chickens and local duck farm studies have resulted in the isolation of 41 different antigenic combinations of influenza viruses from Chinese poultry and 21 from Hong Kong poultry. Whereas swabs taken from Chinese poultry comprised only 44% of the total samples, they yielded 85% of the isolates, the most common isolate being H4N6 (Hav4Nav1). Although ducks constituted about 20% of the poultry sold in Hong Kong, because of their considerably higher virus isolation rate, they yielded 96% of the influenza isolates. The isolation of these viruses showed a cyclical and seasonal trend, being greater in the warm, humid summer months.

All birds sampled were apparently healthy including those from which H7N1 (Hav1N1) and H7N2 (Hav1N2) were isolated; H7N2 infection of experimental poultry was asymptomatic. Surveillance of local duck farms on which H3N2 (Hav7N2), H7N1, H7N2 and other antigenic combinations occurred confirmed the asymptomatic nature of infection and indicated (1) faecal-water-oral transmission of virus (2) maintenance of virus by regular (monthly) introduction of ducklings onto the virus-contaminated pond and (3) birds >70/80-days-old were essentially free of detectable virus.

Whilst only limited data are available for isolates from domestic quail and pigeon (and even less from feral and migratory birds), it seems likely that, as no outbreaks of disease attributable to influenza have been recorded in Hong Kong during the period of surveillance, avian influenza is of limited pathogenicity in the local poultry.

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Start → Studies on the Ecology of Avian Influenza Viruses in Israel.

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Israel is a unique place for the studies on the ecology of avian influenza viruses because of its geographical, ecological and economical peculiarities, i.e. its location along the main flyway of feral birds migrating from Eastern Europe to Africa and highly developed poultry industry. Systematic studies in this field were established in Israel in 1978. Since then tracheal and cloacal swabs were taken from 1409 birds including 473 domestic poultry: turkeys (332), chickens (56) and ducks (85); and 936 feral birds: mallard ducks (47), teals (31), pintail ducks (4), coots (64), moorhens (12), rock partridges (126), cattle egrets (99), pigeons and doves (137), starlings (282), quails (35), larks (20) and other various species of migrating birds (79). A total of 29 influenza viruses has been isolated. The majority (24) of the isolates were derived from the feral birds: mallard ducks (16), pintail ducks (1), coots (4), rock partridges (2) and starlings (1). Five isolates were derived from the domestic poultry: turkeys (2), chickens (1) and ducks (2). Besides, three unidentified hemagglutinating agents (isolated from turkeys in the past - 1971, 1973 and 1978 - and preserved in a viable form up to now) were identified retroactively as influenza viruses. Five serologically different influenza A viruses have been identified: H7N7, H7N2, H10N4, H11N3 and H5N2. Of these combinations, H5N2 was found in all the three retroactively identified isolates from turkeys and was not found in the recent isolations. H7N2 combination being more frequent was isolated from the turkeys, chickens, mallard ducks and rock partridges and H7N7 combination was isolated from the starlings. The latter isolates which are serologically similar by hemagglutinin antigen to fowl plague viruses (the isolate from the starlings is serologically identical to A/FPV/Dutch/27(H7N7) prototype strain by both hemagglutinin and neuraminidase antigens) appeared to be avirulent to chickens, turkeys and ducklings (the studies on pathogenicity were performed by Dr. D. J. Alexander).

One of the studied cases has offered evidence suggesting immediate epizootiological connection between occurence of influenza among the mallard ducks and influenza outbreak in a turkey farm. The case is that about 200 ducks were found dead in fields located about 1.5 km from the turkey farm in which a month later the influenza outbreak was observed. From each of the 15 ducks taken at random for investigation, influenza viruses were isolated which were serologically identical to the isolate from turkeys in hemagglutination inhibition, neuraminidase inhibition and double immunodiffusion tests.

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CURRENT SITUATION IN ITALY

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After a brief hystorical overview 1878-1972, the situation during the years 1973-80 will be described.

An increased incidence of respiratory disease with serious economic losses was observed in commercial turkeys in North-East Italy during 1973-79. The main area studied was the province of Verona, where numerous strains of the prevalent Hav_6N_2 serotype were isolated. The clinical disease and the epidemiological picture will be described. The disease spread more and more from 1973 to 1979, usually with a major incidence during the winter months. In 1979 the disease continued until September. No more episodes of Hav_6N_2 were identified thereafter.

All these strains were of low virulence by laboratory tests ad rather resistant to pH 4.

During the period 1973-79 only one strain different from the prevalent serotype was isolated in turkeys: Hav_2N_2 (1977).

In 1980, one small outbreak appeared in turkeys in Bergamo due to Hav_5N_2. A new Hav_6Nav_1 appeared in a small otubreak in turkeys in Verona in 1980.

In other species, a few strains were isolated: Hav_5N_2 in hens related to the said outbreak in turkeys in 1980, a Hav_6N_2 in guinea fowls in 1979 and a Hav_2Neq_2 in quail (1980). This last serotype was prevalent in Lombardy and Veneto in turkeys, quails and other birds during the years 1965-68.

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Start → Current Situation of Avian Influenza in Poultry in Great Britain

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Influenza virus infection of chickens was last reported in Great Britain in 1959 when a virulent virus, A/chicken/Scotland/59 (H5N1), was isolated.

During 1963-1978 the following viruses were isolated from turkeys: A/turkey/England/63(H7N3), A/turkey/England/66(H6N2), A/turkey/England/69(H3N2), A/turkey/Scotland/70(H?N7), A/turkey/England/N28/73(H5N2), A/turkey/England/110/77 (H6N2) and A/turkey/England/647/77(H7N7). In spring 1979 a series of outbreaks of influenza A infections occurred in turkeys. Ten sites in Norfolk and one associated site in Suffolk were affected with viruses of H7N2, H7N3, H7N7 and H1N1 subtypes. A further outbreak occurred in Hertfordshire where virus of H10N4 subtype was isolated. On two of the Norfolk sites(under same management) virulent virus of H7N7 subtype was isolated and the flocks slaughtered under"fowl plague" legislation. Surveys on turkey flocks using virus isolation and serological techniques were carried out throughout 1979 and 1980 but revealed no evidence of spread of virus to unaffected flocks.

Prior to 1979 no isolation of influenza A virus had been reported from commercial ducks since the isolations made in 1956 and 1962. However, between August 1979 and March 1980 ten influenza A viruses, of subtypes H6N2 (three), H4N6(four), H4N1(two) and H3N8(one), were isolated from ducks as a result of investigations into respiratory disease and high mortality seen on a duck farm in Norfolk. The viruses all had low virulence for chickens and ducks. More recently 35 influenza viruses were isolated from 60 pools of 10 cloacal swabs taken from ducks at a Norfolk slaughter house.

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Occurrence of Avian Influenzaviruses Type A in Germany

by

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During recent years infections caused by influenzaviruses Type A have not played a major role in diseases of domestic poultry in Germany. Overt outbreaks of clinical influenza have not been recorded.

Attempts to recover influenza A viruses from imported psittacine birds (South East Asia, West Africa, South America) were unsuccessful. A number of different influenza viruses were, however, isolated from feral fowl, mainly ducks and coots. Altogether 61 type A influenzaviruses of various antigenic configurations were recovered from fecal swabs, among them 6 strains with Hsw1N1(H1N1) antigens, one with Hav1Neq2(H7N8) antigens and a number of strains possessing Hav4Nav1(H4N6) antigens.

Of special interest were the avian derived Hsw1N1(H1N1) strains, since similar isolates were made from pigs with severe clinical signs of respiratory distress in Belgium in 1979/80. These porcine isolates showed a close antigenic relationship to the avian Hsw1N1 (H1N1) viruses. In hybridization experiments a base sequence homology of 90% was shown with segment 8 of fowl plague virus (C.Scholtissek, unpublished results). Since the avian Hsw1N1 viruses are infectious for pigs, and infection by contact is possible, it is postulated that avian viruses can cross an assumed species barrier and vice versa.

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Epidemiology of Avian Influenza and Sources of Infection Domestic Species

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INFLUENZA IN FERAL BIRDS

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The epidemiology of influenza in feral birds is still obscure in the main. Responsible for this is primarily the multimillion size of the avian population comprising a great number of species that lead widely different ways of life--there are the migratory, synanthropic, aquatic and small singing birds. Moreover, the period of roughly 15 years during which this problem has been accorded intensive attention is relatively short for the epizootological links of avian influenza to have been recognized and the full signigicance of feral birds in the natural history of influenza understood. Nevertheless, a summary of the results obtained during the years of study allows preliminary concepts concerning some aspects of influenza epizootology in avian species to be found.

Influenza virus has been demonstrated in widely diverse species of freely flying birds, among which water fowl have been its most frequent hosts, while small and singing birds only occasionally. These differences are apparently associated with the manner of transmission and spread of the virus, which is shed with cloacal excrements. The portal of entry can be both the cloaca and the respiratory tract. Most of the influenza strains isolated were obtained from healthy birds without any signs of disease; only exceptionally epizootics accompanied by massive deaths were reported. The most probable source of infection of water fowl is faecally contaminated water in shallow reservoirs, from which virus has repeatedly been isolated. The manner of spread of the virus in nature among different kinds of birds is not known and the possibility that the infection may be transmitted to breeds of domestic birds has not been confirmed, but epizootological reasons--outdoor breeds, common water sources, etc.--strongly suggest this. It is impossible to claim, on the basis of investigations carried out so far, that feral birds could act as a source of infection for the lower vertebrates or man. On the contrary, some strains for a long time known only in man and lower vertebrates have only recently been demonstrated in feral birds.

The influenza strains prevalent in feral birds are very diverse and include most of the known subtypes. The greatest numbers of strains have been isolated on the territories of North America, the Soviet Union, South-East Asia and Europe. However, this aspect of distribution of the virus may be seriously biased and may be merely reflecting the localities of most intensive research. With respect to time, the numbers of isolates and numerosity and antigenic composition of strains have been observed between individual years in longitudinal studies.

Although there remain many controversial and unresolved points, one is entitled to say that feral birds represent an important link in influenza virus ecology. They harbor the largest influenza virus gene pool in nature; they are a host population where recombinants with distinct combinations of antigens and pathogenic features are formed; and, last but not least, they disseminate these strains among geographically, mutually remote regions of the world.

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Start → Isolation of Influenza A Viruses from Exotic Birds in Great Britain

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Influenza A viruses have been isolated from the carcases of exotic birds obtained from two sources:

1. Dead birds arriving at Heathrow (London) Airport.

Between May 1976 and October 1978 dead birds from 307 consignments were examined. Forty-eight influenza viruses of H3N8 (Hav7Neq2) were isolated between May 1976 and March 1977 and 14 of H4N6 (Hav4Nav1) subtype between March 1977 and October 1978. In the two years after October 1978 no influenza isolations were made from more than 300 consignments examined.

2. Birds dying in quarantine

Between March 1976 and January 1979 29 influenza A viruses (and 32 paramyxoviruses)were isolated from birds dying in quarantine after importation into Great Britain. Eight of the influenza A viruses were of H3N8 (Hav7Neq2) subtype and 21 of H4N6 (Hav4Nav1) subtype. No H3N8 viruses were isolated after Spring 1977. During 1979 five influenza A viruses were isolated: two were of H4N6 subtype, two of H1ON7 (Hav2Neq1) and one of H7N1 (Hav1N1) subtypes. Since September 1979 no influenza viruses have been isolated from birds dying in quarantine although in 1980 paramyxovirus isolations (28) from this source were greater than in previous years.

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Start → Economic Impact of Avian Influenza in Domestic Fowl in the United States

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Avian Influenza has been experienced in a number of areas of the United States resulting in serious economic loss. Minnesota is the only area where accurate estimates of financial loss have been made available. Based on a survey of producers, the losses totalled 4.2, 1.2, and 1.3 million dollars in 1978, '79, and '80 respectively. Market turkeys, breeder turkeys and laying hens were affected. There were 5 or 6 different strains of virus involved in the outbreaks each year. Some individual flocks were affected by two different strains. Financial loss due to the disease includes mortality, morbidity, condemnation, medication, downtime, loss of egg production, and loss of fixed costs and profit.

Control of the outbreaks by producers also requires depopulation, isolation and sanitation procedures to eliminate the virus from their premises. The loss of production that results causes additional loss in that the cost of maintaining facilities that are not being utilized must be added to the cost of subsequent production. These costs include labor, utilities, depreciation, taxes, insurance, etc. Many other factors such as vaccination, isolation, management, poult cancellation, production and market rescheduling, loss of processing plant, hatchery and feedmill production time also result in additional costs which must be added to the cost of subsequent production. These depopulation costs may amount to as much or more of a financial loss as the original cost of the outbreak as reported in the survey.

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Start → Economic Impact of Avian Influenza in Domestic Fowl on International Trade.

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U.S. poultry and poultry products reached another record last year totaling $600 million. Fresh and frozen U.S. poultry meat is now exported to over 100 countries valued at $386.5 million in 1980, with another $14 million of poultry specialty products. Live poultry and egg exports contributed another $116.8 million.

The major U.S. poultry and egg markets include Japan, the Caribbean countries, Venezuela, Hong Kong, Singapore, and the European Community. In more recent years, the Middle East Countries have become an important market accounting for nearly $50 million in trade in 1980.

Many barriers to trade in poultry and egg products still exist around the world. Some are in the form of non-tariff barriers, such as licensing or quotas, and some are in the form of hygiene regulations and animal health restrictions.

One of the most significant barriers that U.S. producers have encountered in developing markets around the world is restrictions placed on poultry and egg products originating from countries that have live-virus type vaccine programs to control Newcastle disease.

At present, U.S. poultry exports to Northern Ireland and Ireland, Denmark, Sweden, Norway, and New Zealand are limited to fully cooked poultry products since these countries have declared themselves free of Newcastle disease. Australia is also free and accepts only canned (sterilized) poultry products. It is interesting to note that only Denmark, from the above-listed so-called "Newcastle free" countries, is a major producer and exporter of poultry and egg products.

The U.S. poultry and egg industry has become very dependent on the export markets over the years. Some producers/exporters and some regions of the U.S. are more dependent on exports than others. As a result, producers and industry/government leaders are more and more concerned about the threat of disease outbreaks, such as the large outbreak of exotic Newcastle disease which occurred in California in 1972-74. During the past few years, the USDA has spent $87 million controlling the disease in poultry and birds.

What would consequences be for U.S. exports of poultry and eggs if a major outbreak of Newcastle disease occurred in U.S. poultry flocks? They would be disastrous, particularly if viewed by other countries as a national outbreak. Exports to our major markets would come to virtually a full stop, with the possible exception of fully cooked poultry products. Confronted with this situation, U.S. officials would most probably push even harder a concept that is taking on more and more interest--the concept of regionalization.

Veterinary officials of the major meat-producing countries are currently giving serious consideration to the "regionalization concept" for diseases such as African swine fever, hog cholera, and bluetongue in bovine animals. Very little thought, unfortunately, has been given to such a concept as it relates to poultry.

Some U.S. laws, such as the one dealing with controls on foot-and-mouth disease, prohibit consideration of a regionalization concept versus a "free country concept." However, no such constraints are placed on U.S. veterinary officials in dealing with most other diseases.

In view of the volume of poultry and eggs moving in international trade, a more pragmatic approach to disease control and disease acknowledgement needs to develop. Countries need to take a serious look at the "regionalization concept" to control and certification.

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ECONOMIC IMPACT OF AVIAN INFLUENZA IN DOMESTIC FOWL

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The economic impact of the fowl plague outbreak in Victoria in 1976 was felt by government and the poultry industry.

The costs to government arose from providing compensation to owners for depopulation and cleaning of infected properties and for mounting survey and monitoring procedures to ensure rapid eradication. These costs amounted to $250.000.

The costs to the poultry industry arose out of loss of interstate and overseas trade. The nature of the poultry industry in the control area ensured that there was little direct loss to producers. If there had been a major breeding property or a large hatchery, the economic effects would have been considerable.

There was direct economic loss to producers throughout the remainder of the State that was very considerable in individual circumstances. As an example, one hatchery suffered almost 100 per cent loss of business for the month and a half of restrictions. No attempt was made to quantify these losses.

Considerable economic loss was occasioned in the marketing of eggs and egg products. To assist in covering for loss of markets and loss from sales at reduced prices, the marketing authority increased charges to producers. Egg receivals rose due to loss of hatchery sales and handling and storage costs consequentially increased. Total direct costs to the marketing authority was about $275.000.

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The Role of Influenza Virus Hemagglutinin in Infectivity and Pathogenicity

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The structure of the influenza virus hemagglutinin (HA) as coded for by the viral genome determines whether a host proteolytic enzyme will be capable of performing posttranslational cleavage of the molecule. This proteolytic activation is an essential requirement for the ability of the virus to penetrate and therefore for infectivity. The mechanism of penetration involves fusion of the virus envelope with the cellular membrane. Consequently, the formation of virus particles with cleaved HA is a precondition for multiple cycle replication and spread in the host. Comparative analyses performed on a large number of avian influenza virus strains have demonstrated that indeed, only those viruses which are produced in an infectious form in a wide range of different host cells are pathogenic. The findings pointing to the prime significance of the HA for pathogenicity apply only for naturally occurring viruses. In studies with recombinants of influenza A viruses obtained *in vitro* it became evident that pathogenicity depends besides the HA also on an optimal gene constellation which is determined by the parental viruses used for the reassortment. Therefore, the term "optimal gene

constellation" could not be defined so far. Some findings will be presented which could shed light on this phenomenon. It appears that in the avian host, only viruses with an optimal gene constellation are selected and survive. If in addition, to carrying such an optimal gene composition, the viruses possess HA which is cleaved in different types of host cells and therefore allows rapid spread, they are pathogenic.

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Start → Recent Advances in Antigenic and Genetic Analysis of Influenza A Viruses.
V.S. HINSHAW*, R.G. WEBSTER and W.J. BEAN, St. Jude Children's Research Hospital, Memphis, TN.

Influenza A viruses in domestic poultry represent a costly disease problem for commercial producers, especially in turkeys and ducks. Unfortunately, we do not know the source of these viruses or the antigenic and genetic composition of the viruses that cause problems. The viruses are either maintained in domestic flocks or are introduced from outside sources, such as feral birds. Studies, in conjunction with the University of Minnesota, have demonstrated the presence of antigenically indistinguishable viruses in turkeys, feral ducks and sentinel ducks in the same area. To establish that these viruses originate from the same source, however, it is necessary to examine all eight genes and gene products of these viruses in greater detail. Studies on these viruses are still in the preliminary stages, so the answer is not yet available; however, the successful application of the techniques being used can be demonstrated by results from studies on influenza A viruses from seals. In this case, a virus antigenically related to fowl plague virus was isolated from dead harbor seals (Phoca vitulina) in the U.S. Antigenic analyses using both heterogeneous antisera and monoclonal antibodies showed that the surface antigens and the nucleoprotein of the seal virus were most closely related to recent avian isolates. Studies on the RNAs, using competitive hybridization assays, showed that all eight RNA segments were most closely related to the RNAs from avian viruses. These studies indicated that the seal virus originated from avian viruses. Similar approaches are being used for detecting the origin of viruses in the domestic birds - i.e., detailed antigenic and genetic comparisons of isolates from other species, both avian and mammalian, with those appearing in these birds. Information from these studies should enable us to answer the questions as to whether feral birds, such as ducks, are the source of viruses appearing in domestic birds and whether a virus with a particular gene constellation is responsible for the annual outbreaks of disease.

OFFICIAL ABSTRACT FORM

Start → Avian Influenza Diagnostic Procedures, J.E. PEARSON* and D.A. SENNE, Diagnostic Virology Laboratory, P.O. Box 844, Ames, IA

The chick embryo is used for the isolation of influenza viruses at the National Veterinary Services Laboratories (NVSL). The type A ribonucleoprotein (RNP) antigen of each viral isolate is identified with the immunodiffusion (ID) technique. The hemagglutination-inhibition (HI) and neuraminidase inhibition (NI) tests are also used to characterize the envelope antigens. The ID, HI and NI tests are used for detection of antibody in serum samples. Useful epidemiological data is provided by the envelope and RNP antigens; however, these antigens are not related to pathogenicity. Therefore, the virulence of each isolate is determined by the inoculation of susceptible birds. Classical fowl plague strains of influenza are usually lethal for experimentally inoculated birds.

Depending on the origin of the specimen, different approaches to influenza diagnosis are used at NVSL. Specimens submitted from domestic poultry are inoculated into embryonating chicken eggs by the allantoic route. At 4 days post-inoculation, the amnioic-allantoic fluid is tested for hemagglutinating (HA) viruses. If a HA virus possessing type A RNP is isolated, it is characterized using the NI and HI tests. The NI test is performed first using antiserums prepared from recombinant viruses that have the HO hemagglutinin with each of the specific neuraminidases. The antiserums selected for the HI test are prepared from antigens that have a different neuraminidase than the virus being identified. Serum samples are screened on the ID test and the positive samples tested using the NI and HI tests. Early detection of new strains of influenza can be made by testing serum samples.

Specimens received from birds that are being held in quarantine prior to entry into the United States are checked for virulent influenza and Newcastle disease viruses. Embryonating chicken eggs are held for five days after inoculation. Amnionic-allantoic fluid from the embryos that die are tested for HA viruses. A second passage is made if no virus is isolated on first passage. The HA viruses are tested against Newcastle antiserum with the Newcastle HI test.

All the HA isolates from import birds and the influenza isolates from domestic poultry are inoculated into 4 to 6-week old chickens and turkeys by the posterior air sac route. The inoculated birds are held for 10 days and observed for clinical signs of disease. Since 1971, when avian influenza diagnostic work was started at NVSL, all the HA isolates other than Newcastle disease virus have not been pathogenic for the chickens and turkeys inoculated. However, many influenza isolates have been isolated from turkey flocks with severe signs of disease compatible with a diagnosis of influenza.

From January 1, 1976, to December 31, 1980, there have been 8 different hemagglutinin-neuraminidase subtypes isolated from domestic poultry. During the same period, 5,016 hamagglutinating isolates were made from quarantined birds. Many of the HA isolates have been identified as paramyxoviruses.

Official Abstract Form

Start →

Immunization Approaches to Avian Influenza

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The antigenic diversity of the avian influenza isolates necessitates caution when considering immunization as a practical means of preventing or reducing losses in poultry. Fortunately, the large number of these antigenically differing strains isolated from the avian species also possess a wide range of virulence that may provide candidate viruses for viable vaccines. This same antigenic spectrum and range of virulence among avian isolates, however, creates special problems when consideration is given to the use of inactivated antigen as an immunogen for the protection of poultry.

Experimental data will be presented to support and to question the advisability of dependence upon either the viable or nonviable vaccine approaches to the reduction of losses from avian influenza.

OFFICIAL ABSTRACT FORM

Start *PRICE, Robert J., USDA, APHIS, VS. Presented is a discussion of the regulatory actions taken to insure that adequate quantities of vaccine(s) of the appropriate serotype(s) are available for use in controlling outbreaks as well as a description of methods used in developing standards for the evaluation of Avian Influenza Vaccines.

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Start →

International Responsibility for Control of Avian Influenza

J. E. LANCASTER
Port Hope, Ontario, Canada

The establishment of an internationally acceptable terminology and definition of fowl plague is of primary importance.

If avian influenza virus can be defined in a manner comparable to velogenic, mesogenic and lentogenic types of the virus, then international responsibility could be directed to the velogenic form of the virus.

This responsibility would require countries to adopt the term velogenic avian influenza or its equivalent, as a named reportable disease instead of the term fowl plague as currently used.

The control of avian influenza could be based on the present recommendations for the control of fowl plague and Newcastle disease as described in the "International Zoo-sanitary Code" of the Office International des Epizooties, Paris. These control measures should include the basis on which a country or an area within a country can be defined as free from velogenic avian influenza in the species of bird, or their products, involved in international trade. Global spread of the disease should be lessened by clearly defined and acceptable import-export requirements.

Countries have a responsibility to examine the need for diagnostic procedures, laboratory facilities and effective vaccines against the velogenic viruses.

Future progress in the control of avian influenza depends on national and international exchange of information.

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Start → Is an International Regulation of Avian Influenza Feasible?

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France

Avian influenza is known world-wide as a disease which is caused by different antigenic types of virus that may be virulent, of low virulence, or avirulent, and are capable of infecting many species of domestic and wild birds.

The disease, misnamed "Fowl Plague", is a potential cause of embargoes in the poultry trade because there are no uniform international regulations.

Research advancements achieved in birds over the past few years, mainly emphasized studies of the ecology of influenza viruses and the possibility of re-assortment of genetic information between human and animal influenza A viruses, and are concerned with the antigenic structure of the virus. Less attention was given to the evaluation of the pathogenic effects of the virus in birds, including the economic impact on the poultry industry.

Nevertheless, some information regarding virulence of Avian Influenza Virus (AIV) in domestic birds is available and may constitute basic markers for drafting international regulations for avian influenza for the poultry trade, including hatching eggs, live birds (especially one-day old chicks), and poultry consumer products.

Measurement Criteria for AIV Strain Virulence and Definition of the Disease

No correlation has been established between the antigenic type of AIV and virulence; also, no good correlations have been established between virulence and many of the tests used for Newcastle disease virus: e.g. mean embryo death time, stability of hemagglutinins, etc. According to Allan, intravenous and intracerebral index tests used for Newcastle disease virus, may be proposed for measurements of virulence and the consequent classification of AIV isolates into three types: Velogenic strains (pathogenic isolates), Lentogenic strains (apathogenic isolates), and Mesogenic strains (intermediate isolates).

According to this classification, avian influenza must be defined as a disease caused only by velogenic or mesogenic strains of AIV. This excludes infections by lentogenic strains of AIV which may be confused with a clinical disease caused by infections with lentogenic AIV strains concurrently with other avian pathogens. Consequently the term "Fowl Plague" should be discarded because AIV other than a "Fowl Plague" strain of virus may cause high mortalities and non-pathogenic strains of AIV are incorrectly designated as "Fowl Plague".

National Rules to Prevent and Eradicate the Disease

It appears that free-flying birds, especially feral ducks and migratory waterfowl, are the major sources of infection. Breeder flocks may also be a source of infection, as vertical transmission may be possible because the embryo is not killed by the virus. Furthermore, during the hatching process, vertical transmission cannot be excluded.

Rules to prevent the disease and to control the infection must include:

-- No mixing of breeders of different species on the same farm
-- Keeping breeders in closed houses or protected by a wire-netting to avoid contact between wild and domestic birds

Start → G. Bennejean, cont.

-- Blood testing by the agar gel precipitation test of 1% of the breeder flocks to control avian influenza infection when birds are more than 4 months of age, and retested at 90 day intervals.

When poultry flocks are suspected to be infected by a pathogenic strain of AIV the entire area around the infected premises must be considered as "infected" until the national authorities prove that the virus involved is not a velogenic (or mesogenic) strain of AIV or have eradicated the infected birds (by slaughter and destruction).

If eradication of the birds is not decided, or if the decision is made too late and it is proven that a velogenic (or mesogenic) strain of AIV is involved, then the area is still to be considered as "infected".

The size of the area depends on the virulence and ability of the strain of virus to disseminate. This should be correlated to the number of flocks of susceptible birds in the affected area (between ten square kilometers and 100 square kilometers). The area is once again considered to be clean of infection if no new outbreaks occur during the two weeks following the last case.

If during the quarantine period other farms are infected outside of the area, a portion of the country should be considered as "infected". Possibly the entire country should be considered "infected" if the virus is disseminated in several areas of the country.

In the event of mild forms of the disease, caused by mesogenic strains, the opportunity for applying the rules above would depend on the ability of the virus strain to disseminate rather than on the relative pathogenicity of the strain.

Regulation Concerning International Poultry Trade

Hatching eggs and one-day old chicks

The hatching eggs and one-day old chicks must originate from flocks serologically tested and free of infection of AIV. The farms and hatcheries must be located in an area free of infection by a velogenic (mesogenic) strain of AIV during the last 2 months.

Poultry meat

Flocks and abattoirs must be located in an area without any case of acute disease caused by a velogenic (mesogenic) strain during the last month.

Conclusion

International regulation of avian influenza is feasible, but requires development of research on the best methods to evaluate the pathogenicity of the strains involved.

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Start →

Regulatory Problems Associated with Avian Influenza

JOHN K. ATWELL
Deputy Administrator
Veterinary Services, APHIS, USDA

The Animal and Plant Health Ispection Service (APHIS) is responsible for protecting the poultry populations from serious poultry diseases such as highly virulent avian influenza. In order to control or eradicate a disease such as avian influenza, we must know the necessary epizootiological facts about the disease, have dependable diagnostic tests to identify the disease and its pathogenicity, have ways to evaluate the probable cost and probability of success, and be able to assess the expected economic impact on the poultry industry as a whole. All animal health officials should have a common understanding of the diseases which are being controlled to prevent undue disruption of marketing procedures.

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Start → Plaque-forming Ability in MDCK Cells and Structure of the Haemagglutinin of Influenza A Viruses Which Differ in Virulence for Chickens.

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Six influenza A viruses, showing different degrees of virulence for chickens in intravenous pathogenicity index(IVPI) tests were examined for plaque size, infectivity and cleavage of the haemagglutinin (HA) polypeptide when grown in MDCK cells.

Ck/Germany/34(H7N1), IVPI 3.00, produced large plaques (2mm diameter) in MDCK cells and neither the size of these nor the infectivity titre were enhanced by the addition of 10µg/ml trypsin to the overlay. In the presence of trypsin pt/N.Ireland/73(H7N1), IVPI 0.00, produced large plaques (3mm) in MDCK cells, but in the absence of trypsin only very small plaques (0.5mm) were seen and the titre was reduced 1500-fold. The PFU titre of ck/Australia/75(H7N7), IVPI 1.74, was unaffected by the presence or absence of trypsin. However the plaques produced by ck/Aust./75 were considerably smaller (1mm) than those produced by ck/Germ/34 but were not enhanced by trypsin. The other three viruses examined: ty/England/384/79 (H10N4), IVPI 1.34; pkt/Eng./138/75(H3N8), IVPI 0.92; ty/Eng./69(H3N2), IVPI 0.85, all produced plaques in MDCK cells at titres that were enhanced by the presence of trypsin. However,the degree of enhancement (about 30-fold) was considerably less than seen with pt/N.I./73. Ty/Eng/69 produced small plaques in MDCK cells in the absence of trypsin comparable to those produced by pt/N.I./73; whereas plaques produced by ty/Eng/384/79 and pkt/Eng/138/75 were comparable,in size, to those of ck/Aust/75. For these three viruses a threefold increse was seen in plaque size in the presence of trypsin.

Polyacrylamide gel electrophoresis analysis of the structural polypeptides of the six viruses grown in MDCK cells in the absence of trypsin revealed only ck/Aust/75 and ck/Germ/34 to have a cleaved HA polypeptide.

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