



Epidemiology of H5N1 Avian Influenza in Asia and Implications for Regional Control

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Executive Summary

This study has examined the epidemiology and control of highly pathogenic avian influenza H5N1 (HPAI) in each of the countries affected by the 2003-5 Asian epidemic. Outbreaks reported to OIE between December 2003 and 11 February 2005 total 3095 - 13 in Cambodia, 50 in the People's Republic of China, 169 in Indonesia, 5 in Japan, 19 in the Republic of Korea, 45 in Laos, 10 in Malaysia, 1064 in Thailand and 1764 in Vietnam.

By examining the evolution of the epidemic geographically over time, considering available risk factor information, and considering the molecular epidemiological evidence available, it is concluded that either three or four epidemics have occurred concurrently, and that correct understanding of the epidemic processes requires them to be examined in that framework, to identify common factors responsible for their emergence. The 2003-5 epidemics appear to have arisen due to the establishment of H5N1 infection in wild birds in about 2000 to 2002, following earlier evolution of multiple genotypes from the precursor H5N1 virus first identified in geese in China in 1996. This virus appears to have arisen by reassortment among earlier progenitor viruses, which infected a number of different bird species. Although alternative explanations of the mechanism of establishment of epidemics in the different regions of Asia in 2003-5 have been proposed, they are difficult to reconcile with all of the evidence presented in this report and the accompanying country profiles. Viral genotype Z, various subtypes of which have been responsible for a very high proportion of the total outbreaks during the epidemic, emerged by 2002 and largely replaced earlier genotypes. It seems likely that this is due to higher infectivity of this genotype both within and between species. The subtype circulating in Southeast Asia has also shown high virulence for a range of species, including humans.

Once the epidemics were seeded into various countries in the region, continuing transmission has depended principally on movements through marketing channels within and possibly between countries, which have caused the large scale of the epidemics, and made control very difficult in countries which have complex poultry trading patterns. The epidemics can only be fully understood by seeing them in ecological terms, with interchange of infection between reservoirs of infection in some species of domestic birds (principally ducks, geese, and quail) and probably wild birds (principally the Family Anatidae), which then cause spillover infection into chickens and other domestic poultry, and from there into a range of mammals. Some species, such as humans, are currently only aberrant hosts which do not transmit infection to any degree, but are at risk of reaching spillover host status due to viral evolution, which could result in a global influenza pandemic.

Control by stamping out has been successful in countries where the outbreak was small and surveillance provided adequate assurance of success, but has not worked alone in other affected countries. Countries which have used vaccination to deal with large outbreaks have achieved better control of their outbreak than those which have not adopted this method, but future control will depend on integrated strategies linked to specific poultry production compartments within the country. These should be based around surveillance systems to identify important local and regional transmission

pathways for H5N1 infection, leading to integrated risk-based control programmes based on vaccination, enhanced biosecurity (especially separation of reservoir and spillover species), management of risks in the marketing system, and selective application of movement control.

All of these findings are based on outbreak reports and virological information. There have been no structured epidemiological investigations of the epidemic, and hence key questions which are necessary for effective control remain to be answered. This requires some additional investment in tightly focused research.

Report Objectives

The objectives of this report are:

1. For each of the countries affected by the avian influenza H5N1 epidemic of 2003-2005, to provide a profile which describes relevant features of the country, its bird populations and the pattern of disease occurrence in the country. From this information, conclusions are drawn concerning the adequacy of the information obtained, and the factors likely to have influenced the epidemiology of H5N1 infection in the country.
2. To conduct example epidemiological analyses on selected countries, where sufficient information could be obtained to evaluate the evolution of the epidemic using geographical methods and temporal analyses.
3. To identify risk factors which appear to have influenced the evolution of the H5N1 epidemic in Asia, at national level and across the whole region.
4. To provide a synthesis of current understanding of the epidemic, and an evaluation of alternative hypotheses which have been proposed to explain events.
5. To assess the relationship between infection in birds and human exposure.
6. To evaluate the adequacy of surveillance for avian influenza in the region, and identify aspects which require enhancement and harmonization.
7. To make recommendations on further action which should be taken in relation to the epidemiological investigation and control of this major epidemic.

Historical Background on Avian Influenza and Emergence of the H5N1 Strain

There is widespread agreement that the pattern of avian influenza infection which has occurred during the 2003-5 Asian epidemic so far represents a disturbing new evolutionary development in the behaviour of the influenza A virus, the full ramifications of which may not yet have unfolded. What remains the subject of intense debate is how the epidemic has spread so far and so fast, and how best to control it. Overview descriptions of avian influenza in general (Webster 1992; Alexander 2000; Webster and Hulse 2004) and the situation in Asia specifically (Alexander 2003) give valuable background information for this paper.

Until the 1950s, poultry production throughout the world was undertaken largely in small individually-owned flocks, similar to those owned by many families in Asia today. Avian influenza (under its former name of fowl plague) was reasonably common in outbreak form, and in some situations was endemic in poultry populations. However as the poultry industry evolved and expanded rapidly in the second half of the twentieth century, the disease became rare. Only 21 outbreaks have been recorded worldwide from 1959 to the start of the Asian epidemic. All were H5 or H7 (of the 15 haemagglutinin subtypes found in birds), and in only six incidents was the outbreak of significant size. The rest affected relatively few farms, and control was fairly easily achieved. Two previous outbreaks involved H5N1 viruses, both in Great Britain (1959 and 1991), and both were small in size. It is noteworthy that almost all countries in which epidemics were diagnosed were countries with high per capita GNP and with levels of national veterinary service organization at the high end of the scale. It is most unlikely that these were the only countries to be affected, but they were the ones in which the disease was diagnosed.

The H5N1 virus which has caused the Asian epidemic had emerged by at least 1996, when it caused an outbreak of influenza in geese in Guangdong, with high death rates, and it may have already been circulating in the region some years earlier (Chen, Deng et al. 2004). It came to international attention in 1997 when it caused an outbreak of severe disease in poultry in Hong Kong SAR, because for the first time a true avian influenza virus caused serious human disease, with 18 people clinically affected and six deaths. Subsequent serological evidence has indicated that additional people were infected with the virus without showing clinical signs, and there was evidence of occupational exposure (Bridges et al, 2002). Previously it had been considered that true avian viruses could not cause serious disease in humans, but since 1997 the H5N1 virus has caused further severe cases of human disease, and avian viruses of H7 and H9 subtypes have caused human infection, with one fatality in The Netherlands due to an H7N7 virus.

The 1997 outbreak in Hong Kong SAR was controlled by slaughtering the entire poultry population. Subsequent monitoring confirmed that the repopulated flocks were free of H5N1 infection. However between 1997 and 2003, despite successful eradication each time, a further series of outbreaks occurred - though at a declining rate as control measures were intensified (see Hong Kong SAR profile for details). There were indications that infection was also present in one or more countries in the vicinity, but no reports of clinical disease in poultry. In 2003 there were two confirmed cases of H5N1 infection (and a third possible case) in a Hong Kong SAR family during travel to mainland China.

In 2002 and 2003, H5N1 viruses were isolated from wild birds in Hong Kong SAR, mostly in areas where bird density was high. These were the first recorded isolations of H5N1 from wild birds, although more recently the virus has been isolated from a range of wild bird species in a number of countries.

In early December 2003, an outbreak of H5N1 infection began in the Republic of Korea. In the following weeks, outbreaks of H5N1 disease in poultry were reported by seven

further countries. During this first phase of the epidemic, 35 human cases of H5N1 disease were reported, with 23 deaths.

In July 2004, a second wave of disease began in poultry in Thailand and Vietnam, and still continues with recent extension to include Cambodia. There have also been human cases in all three countries, with one case of likely human to human transmission (Ungchusak 2005). Most or possibly all of the other human cases can be reasonably attributed to poultry exposure, although in some cases human to human transmission cannot be excluded. Malaysia also reported the disease for the first time during this second epidemic wave.

Over the last decade the virus has shown a high rate of evolution, and unusually wide and expanding host range. Its future behaviour is of continuing concern both as an animal pathogen and as a potential source of a human pandemic virus.

This report brings together available information on the epidemic to the end of 2004, and seeks to identify features which might help manage the disease in future.

The report identifies some of the constraints to controlling country epidemics but it is not the intention or purpose of the report to criticize management procedures or veterinary infrastructures in any countries. Experience with large scale epidemics in other countries has shown on many occasions the difficulties associated with achieving effective control of complex and well-established diseases, and H5N1 avian influenza in Asia presents an exceptionally challenging control problem. Those challenges are growing worse at present, and this report is intended to provide an objective and unbiased appraisal of why that has happened, and provide proposals on what actions should now be taken.

Methods Used to Gather Data for This Report

A wide range of techniques has been used to obtain information for this report, because the information available so far is an incomplete jigsaw - many countries are involved, they have diverse administrative systems and technical capacity, the supporting scientific activity is spread around even more countries and much of the work is as yet unpublished. There remain many gaps in our knowledge of important aspects of the problem.

In preparing this overview report and the country profiles which support it, our aim is to present the best available synthesis of what is currently known, to make an epidemiological interpretation of the processes which are most likely to be involved, and to seek further information and views in order to enhance the robustness of the evidence on which control decisions can be made. The overview report is concerned with the regional issues, and relies on the country profiles as sources for specific details. It is not practical in a report of this nature to provide references for every statement either in the main overview report or in the country profiles, but publicly accessible sources are cited where they are of importance, and other statements are generally based on a range of sources as described below.

The sources used are described in more detail at the end of this report. In brief, FAO consultant reports provided a valuable information source, and visits were made to

Indonesia and Vietnam by Dr Jackson and to Thailand by Professor Morris to obtain information. As much data relevant to epidemiological analysis as possible were gathered from national sources in all countries and a number of experts were also consulted on various issues. Information on wild birds was collated from a range of sources, and about 200 relevant scientific papers were reviewed to find important parts of the jigsaw. Sources such as ProMED have also been very useful.

Request for feedback and extra information

Efforts have been made to verify items where possible, but inescapably in considering an epidemic disease of this nature, some statements represent informed judgments of the weight of evidence on a point, rather than verified factual data or scientific findings. It is hoped that people who may have access to information which corrects or updates our summaries will provide this to the authors, so that any necessary adjustments can be made. Inevitably, some of the statements made will be controversial, because there are many strongly held but conflicting opinions both about what has happened and why. The purpose of this report is to assist decision-makers, and to inform a wider audience. To achieve that, it is necessary to put forward assessments of each of the important issues, and allow these views to be debated constructively in the interests of controlling avian influenza in poultry and reducing the risk of a human pandemic arising. Readers are requested to provide feedback where they disagree or have better information, but to accept that this report attempts to synthesize current understanding across all relevant areas, including aspects where information is very sparse.

Evaluation of Epidemiological Surveillance Procedures in the Region

Information on populations at risk

One of the major problems in undertaking epidemiological investigation of epidemics is that the data usually focuses on affected “cases”, with limited or no information being available on the unaffected units within the population. In other words, this is numerator data with no valid denominator. This is not just a problem of avian influenza or Asia. It is a global issue which affects almost all outbreak investigations of significant scale.

While analysis of case data alone can provide valuable understanding on simple issues such as the scale and temporal pattern of the epidemic, it is prone to produce conclusions of uncertain value when any comparison or more detailed analysis is undertaken. For example, simple case data may show that the epidemic is concentrated in one production system, species or region, but if these high risk categories are also the most common, they may in fact be at substantially lower risk of becoming affected than other less common categories, and may mislead with regard to important risk factors. Thus population at risk data, measured as a count of the number of units (of equivalent definition to the cases) which remained unaffected by the disease, is important for epidemiological analysis, but not widely available in most countries.

Some countries, such as Hong Kong SAR, the Republic of Korea and Japan, had very good systems in place for recording census information and digitized geographic location of farms in outbreak areas. During the outbreak they meticulously collected neighborhood population at risk data¹ for affected areas. Hence it was possible to conduct case control studies in Hong Kong SAR during outbreaks, to identify risk factors for H5N1 disease in poultry flocks. However most other countries in Asia have not collected sufficient data on populations of poultry-owning units of various types, to enable comparisons to be made of incidence between different categories of flocks, and other in-depth epidemiological analyses to be undertaken.

Census data for poultry is notoriously difficult to obtain because of difficulty in identifying all poultry owners and creating a valid sampling frame, seasonally fluctuating population sizes and within enterprises, and in some cases reluctance of owners to divulge animal numbers if they fear such information may be used for taxation.

Nevertheless, poultry census information is collected in most countries but misgivings about the quality of the data were suggested to us on several occasions, with various explanations of why reported numbers may not match reality at all well. Census information for numbers of households with poultry and distributions of enterprise size used for analytical purposes in this report could not be verified and its use was based on an assumption that historical data would reasonably accurately reflect the current situation. The larger integrated poultry enterprise sector undoubtedly records detailed population and other information within individual companies, but it is not publicly available for analysis.

It is not essential to undertake a complete census in order to estimate populations of poultry at risk in various categories of flocks within affected areas, and sample surveys are quite adequate for the purpose of epidemiological analysis, as long as they are appropriately structured. They could be carried out in conjunction with other surveillance activities, and would greatly assist with epidemiological interpretation of the epidemic.

Information on bird movement in trade

Countries with sophisticated data management systems and import health certification based on risk analyses were able to review imports that came through legal channels. However for many countries information cannot be readily retrieved from the paper based movement permit systems, and judgments on the nature and scale of legal cross-border movements have had to be made largely by informal means. With the exception of birds entering Hong Kong SAR (where a comprehensive surveillance system has been used for several years, and progressively enhanced), permits issued for live bird movement in many cases give virtually no assurance of freedom from avian influenza.

¹ For example, in the Oita outbreak in Japan, the veterinary investigators reported that within a 5km radius of the infected place there were 230 houses with up to 10 pet chickens and a total pet bird population of 1,300 birds.

Unsupervised movements of birds between countries in the region are known to be extensive and quite variable over time as prices and circumstances change. While each individual movement may be small relative to the legal shipments, the total volume of this trade is probably substantially larger across most of the borders, and the risk of transferring avian influenza infection in each movement is higher. There are quite a number of known cases where H5N1 infection has been detected in illegally moved items, ranging from duck meat and live birds moved within Asia to pet birds smuggled into Europe. However only a tiny fraction of such movements would have been detected.

Movements within countries in the region are far more complex, and in most countries there is little objective information. Movement patterns also differ greatly between influential populations such as ducks, meat chickens, fighting cocks, minor poultry species, and day-old chicks. Research in Hong Kong SAR and the People's Republic of China (PRC) has shown the crucial role which live bird markets play in maintenance and dissemination of avian influenza viruses in countries where birds are sold live to consumers, yet patterns and scales of movement of different species through live bird markets is poorly documented in most of the countries affected by H5N1. For the purposes of this report, information from the studies in Hong Kong SAR has been a valuable resource, but Hong Kong SAR is far from typical of affected countries, and additional information beyond the current limited evidence is urgently needed, as discussed later in the report.

National level outbreak surveillance

It is axiomatic that disease control programmes cannot operate effectively without surveillance, and good management decisions require good surveillance data. Data collected from surveillance activities is used to progressively improve and modify control programmes, to make them more cost effective and to reduce financial hardship on individuals and animal industries.

Some countries were overwhelmed by the number of outbreaks in the first quarter of 2004 and fully occupied with responding to the immediate tasks, with few resources (either money or staff) left to begin a structured surveillance and investigation programme. However over a year after the epidemic began, surveillance is still very patchy and far too focused on disease reporting and case-finding rather than targeted forms of surveillance.

There are immense difficulties for effective case-finding in countries where most householders keep backyard or small commercial flocks. Probability theory applied to the steps from disease occurrence in a village to delivery of a diagnosis by a quality-assured laboratory procedure, indicates that the probability of detection of any individual disease incident is low in poorly resourced conditions. The separate probability steps entail recognition of a problem by the farmer, reporting to an animal health worker, a farm visit by this person and his recognition of a disease incident, then reporting to a veterinarian, a visit by the veterinarian, his recognition of the incident, taking correct and adequate samples, notification to superiors, sending appropriate and samples of sufficient quality to a laboratory, arrival of samples under proper conservation to the laboratory, and finally laboratory capabilities and their timely reporting. Southeast Asian countries with

large networks of animal health workers who are active in subsistence village farming have potential for local surveillance and control, but infrastructural problems hamper operational timeliness, efficacy and success. Data flow is cumbersome and mainly by hard copy at all steps in the process. Provincial autonomy in particular hampers disease control and disease information flow.

In countries where disease outbreaks are common and poorly controlled, farmers are used to dealing with adverse events on their farms and when faced with deaths in birds will manage the financial risk in a way that suits them best. That may well be by disposing of birds before (or after) they die by selling them. When outbreaks principally affect village chickens, reporting may be substantially delayed and the disease may have already spread widely before controls can be implemented.

All of the countries under consideration have a rapidly developing and diversifying poultry sector. There is still a large village based component, but medium and large scale commercial production has been expanding rapidly over the last twenty years, producing a major expansion of total bird numbers. This scale of development has not been matched by corresponding development in veterinary capacity and specialized skills to service industry needs. Epidemiology personnel would benefit greatly from training at advanced levels in risk analysis, mapping and descriptive epidemiology, analysis of field data, use of spreadsheets and databases and some basic research. Provincial veterinarians likewise would benefit from specialized training in data management and disease investigation. Without suitable skills to support a risk factor approach, there is a tendency to fall back on traditional control approaches, which will not deal effectively with H5N1 infection.

It is essential to move away from simple case-reporting and case-finding to a structured surveillance programme which focuses on quantifying transmission routes and risk factors, and implementing control programmes which are tailored to reducing transmission under the specific conditions of the country. This requires a greater depth of analysis and interpretation of the dynamics of H5N1 infection than has been possible to apply in most affected countries so far, with the resources available.

Influenza viruses in wild birds

Most of the sampling of wild birds has either been from dead and diseased birds found or captured in locations considered likely for various reasons to have H5N1 infection present, or from opportunistic sampling of accessible populations where the infection status of the population being sampled is completely unknown, and the prevalence of infection in different species, age groups etc is very uncertain and probably very variable.

We have reported positive wild bird results in the various country profiles, and will consider likely explanations for the various conflicting and sometimes confusing findings later in this report.

Unofficial data sources

In an outbreak of this magnitude and complexity, a lot happens which never reaches official documents. Part of this is disseminated through informal exchange of information, opinion and “disease rumours” (using this in its technical epidemiological sense of unsubstantiated disease reports through unofficial channels). In the H5N1 epidemic, many of the disease rumours have subsequently been substantiated by supporting evidence and the rumours have provided a valuable early alert to new disease foci.

However other reports have proved to be false or have remained unsubstantiated. In addition, many items which have circulated as claimed fact have proved to be pure conjecture or even distortion of factual data to present an impression which does not accurately represent the real situation.

Unofficial sources have been used in this report to supplement official sources, and to act as a prompt for seeking to check the validity of particular items of evidence. However such sources have not been used as factual evidence unless substantiated by other sources, either at the time or later. As surveillance improves for a disease and information about developments is released more promptly, rumours tend to die away.

Summary of Epidemiological Evidence about the Asian Epidemic

Definitions of Terms Necessary to Describe the Epidemic

Some of the terms needed to discuss the epidemic have been used with different interpretations by various authors, and therefore the definitions used here need to be made clear. In this report, the terms virulence, pathogenicity and infectivity are used in accordance with the meanings ascribed to them by J. M. Last in “A Dictionary of Epidemiology”, Oxford University Press, 1995.

Virulence is defined as “*The degree of pathogenicity; the disease-evoking power of a microorganism in a given host. Numerically expressed as the ratio of the number of cases of overt infection to the total number infected, as determined by immunoassay*”.

Thus:

$$\text{Virulence} = \frac{\text{number of cases of disease}}{\text{number infected}}$$

Pathogenicity is defined as “*The property of an organism that determines the extent to which overt disease is produced in an infected population, or the power of an organisms to produce disease. Pathogenicity of infectious agents is measured by the ratio of the number of persons developing clinical illness to the number exposed to infection.*”

Thus:

$$\text{Pathogenicity} = \frac{\text{number of cases of disease}}{\text{number exposed}}$$

Infectiousness is defined as:

- “1. A characteristic of the disease agent that embodies capability to enter, survive and multiply in the host. A measure of infectivity is the secondary attack rate.
2. The proportion of exposures, in defined circumstance, that results in infection.”

Infectivity is defined as “A characteristic of a disease that concerns the relative ease with which it is transmitted to other hosts. A droplet spread disease, for instance, is more infectious than one spread by direct contact. The characteristics of the portals of exit and entry are thus also determinants of infectiousness, as are the agent characteristics of ability to survive away from the host, and of infectivity.”

Thus:

$$\text{Infectivity} = \frac{\text{number of infecteds}}{\text{number exposed}}$$

Using these definitions, pathogenicity can be thought of as the product of virulence and infectivity.

Because of the nature of the test used to assess avian influenza isolates what is evaluated is virulence, rather than pathogenicity. Strictly the strains should be distinguished as high or low virulence on the basis of the standard intravenous “pathogenicity” test (IVPI). However current terminology has been established for so long that it is unlikely to be changed. In this report strains of avian influenza will be described as having high or low pathogenicity based on the IVPI, in accordance with international practice, but the epidemiological evaluation will use the terms according to the correct definitions.

Both virulence and infectivity of H5N1 strains in Asia have varied over the decade from when the first isolates were made in the mid-1990s to early 2005, as a sequence of new genotypes has emerged, and then each dominant genotype has been replaced by others with different characteristics. Throughout the ten years since H5N1 emerged, all strains have exhibited high virulence for chickens, and some recent strains have shown high virulence for humans in a few countries. Early strains had low virulence for ducks, whereas more recent ones have shown variable but generally higher virulence.

Infectivity of early strains in the 1990s appears on the evidence to have been low to moderate, but more recently infectivity has been higher, as measured by the rate of transmission between hosts. This is likely to be principally due to changes in the relative importance of different viral excretion routes and the total quantity of virus excreted by an individual, but may also be influenced by factors such as greater involvement of some species in the infection process than previously, and changes in virus survival in the environment.

The Origins and Evolution of the H5N1 Virus

It is known that birds infected with H5N1 were entering Hong Kong SAR from the People's Republic of China from at least as early as 1997, and that a highly pathogenic H5N1 was infecting geese in the People's Republic of China at least by 1996, when the A/Goose/Guangdong/1/96 virus was isolated (Guo, Xu et al. 1998), now considered to be a precursor of the subsequent evolutionary tree of viruses. There are hints that viruses within the H5N1 group were first evolving in the agricultural ecosystem of southern China earlier in the 1990s. However this cannot be definitively confirmed. The virus appears to have arisen by reassortment among earlier progenitor viruses including H9N2 and H6N1, both of which have been found principally in quail in Hong Kong SAR market studies (Guan, Peiris et al. 2002). There are three different lineages of H9 viruses, one isolated from quail and the other two from chickens, ducks and aquatic birds. The one of quail origin appears to have contributed the viral replicating mechanism to the H5N1 virus which caused the 1997 outbreak in Hong Kong SAR.

The H9 viruses appear to be undergoing a period of evolution and adaptation (Choi, Ozaki et al. 2004), having become much more common in poultry over the last decade than they were previously. Intriguingly, the adapted H9N2 virus began to be isolated from aquatic species from about the same time that H5N1 began to be isolated from wild birds. There is no indication that H5N1 virus was present in wild birds until some time in the period 2000-2002, with isolations from wild birds commencing in 2002, and building up since that time both in Hong Kong SAR (Ellis, Bousfield et al. 2004) and in other parts of Asia, as reported in the country profiles.

Therefore whereas the typical history of HPAI viruses is that they originate in wild birds as LPAI viruses, transfer to domestic poultry and progressively gain pathogenicity in domestic birds through a series of infection cycles until they become HPAI, it seems most likely that this virus arose through a recombination process between viruses in the "influenza epicentre" region of Asia (Shortridge and Stuart-Harris 1982) involving interchange of viruses among a number of species of domestic birds, with possible involvement of wild birds. Both H5N1 and H9N2 evolved contemporaneously, and showed distinct parallels in their evolutionary pathways. They also have strong similarities in the six internal genes of the virus (Lin, Shaw et al. 2000). Both have a wide host range, including humans as a host of these true avian viruses for the first time. H5N1 has shown a capacity to maintain and even enhance its ability to cause severe disease in a broad spectrum of species, spillover hosts (chickens and other domestic poultry), aberrant hosts (humans, domestic and large cats, etc) and even species which are towards the reservoir end of the host range (domestic ducks). In contrast, H9N2 has been more prevalent, but has caused little disease.

Following the initial emergence of H5N1 in the early to mid-1990s, the virus then began to evolve into a range of genotypes within the H5N1 group, which differed in some of their important characteristics. Influenza viruses typically evolve much more rapidly in spillover hosts such as chickens and turkeys than they do in reservoir hosts such as wild

water birds (Suarez 2000). The evolutionary process for H5N1 involved geese, and most likely domestic ducks, quail and possibly some other species as well, with exchange of infection between the species and reassortment of parts of the viral genome to produce various new genotypes (Webster and Hulse 2004), with the evidence suggesting that there are also differences in epidemiological characteristics among sub-lineages which fall within the same genotype.

The strain which caused the 1997 outbreak in Hong Kong SAR has not been detected again since the poultry population of Hong Kong SAR was entirely slaughtered out to control the outbreak, but other strains were detected in Hong Kong SAR during later incidents involving domestic poultry (Guan, Peiris et al. 2003) and more recently wild birds only (Ellis, Bousfield et al. 2004). At first five separate genotypes were circulating in Hong Kong SAR live bird markets, with isolations predominantly in aquatic poultry (ducks and geese), but progressively increasing in terrestrial poultry as the emerging strains became more adapted to these species (Guan, Peiris et al. 2002). All five genotypes readily infected quail experimentally, and in the live bird markets the two other viruses considered to be precursors of the 1997 H5N1 virus, H9N2 (G1) and H6N1 (W312), were actively circulating. Two of the emergent strains displaced as the predominant viruses in geese the parental Gs/Gd-like virus from which they had all evolved. All five H5N1 viruses were highly pathogenic for chickens, with genotypes A, B and E particularly so. In contrast to typical findings for human influenza viruses, all five genotypes were lethal for mice without prior adaptation and four could spread to the brain, demonstrating their pathogenic potential in humans.

In the period from 1999 to 2002, there were H5N1 viruses circulating in aquatic poultry in the People's Republic of China (Cauthen, Swayne et al. 2000), where they could be isolated from healthy ducks (Chen, Deng et al. 2004). The viruses tested were highly pathogenic for chickens, and showed progressively increasing pathogenicity for mammals. However a study of live poultry markets in southern China in 2000 and 2001 failed to find any H5N1 viruses, although other influenza viruses were circulating (Liu, He et al. 2003).

In 2001, a cross-sectional study (Nguyen, Uyeki et al. 2005) over two days of live poultry markets in Vietnam found H5N1 viruses in healthy geese (2 isolates), which were highly pathogenic in experimental chickens, but not in ducks. This shows that an H5N1 virus was circulating in Vietnam at the time without any major outbreaks of disease being reported. The viruses were very similar to those circulating in Hong Kong SAR and mainland China at about the same time, but were quite distinct from H5N1 viruses affecting poultry and people in Vietnam in 2004.

From 2002 onwards, eight new H5N1 genotypes were circulating in Hong Kong SAR (V, W, X₁, X₂, X₃, Y, Z and Z⁺), but genotypes A, C, D and E and the precursor virus Gs/Gd were no longer found (Li, Guan et al. 2004). The poultry outbreak of H5N1 which occurred in Hong Kong SAR between January and March 2002 involved three different genotypes of virus. The X genotype affected a single farm and did not spread, whereas contemporaneous outbreaks due to Z and Z⁺ both spread among farms to cause multiple secondary cases.

Then in 2003 genotype Z became the dominant type in samples from both Hong Kong SAR and mainland China, with single isolates of Z⁺ in Hong Kong SAR and V in mainland China (Li, Guan et al. 2004). Genotype Z has been the type isolated from Indonesia, Thailand and Vietnam (Li, Guan et al. 2004), but the Indonesian isolates were different from those in Thailand and Vietnam. Genotype V was isolated from cases in Japan during the epidemic (Mase, Tsukamoto et al. 2005) and this virus was closer to the Indonesian isolate than it was to those from Thailand and Vietnam. The virus isolated in the Republic of Korea showed over 99% homology with the Japanese isolates, suggesting a common origin. Among the four outbreaks in Japan, there was close homology of the viruses, but it was concluded that infection entered at least three of the four farms from separate sources, considered most likely to be wild birds (Japan MAFF. 2004). All four isolates showed lower virulence in mice than the strain isolated from human cases in the 1997 Hong Kong SAR outbreak, indicating a lower virulence for mammals, which may explain the lack of human cases in Japan, despite the fact that human exposure has recently been reported as occurring in that outbreak, and immune response to H5N1 was demonstrated in exposed people. Whether the Indonesian isolate also has lower virulence for mammals has not yet been reported.

The Japanese isolate was also obtained from 9 dead crows collected from the field in the areas of the outbreaks, and crows were reported to be dying of the disease. However in laboratory studies crows became infected when experimentally challenged with the virus, but did not develop disease. A range of other wild birds collected from the field in Japan and tested for H5N1 were not found to be positive. However on experimental challenge mallard ducks were susceptible to infection but suffered no illness and none died. Budgerigars, starlings and sparrows became infected and diseased. However miniature pigs did not become infected.

Spatio-temporal Pattern of the Epidemic

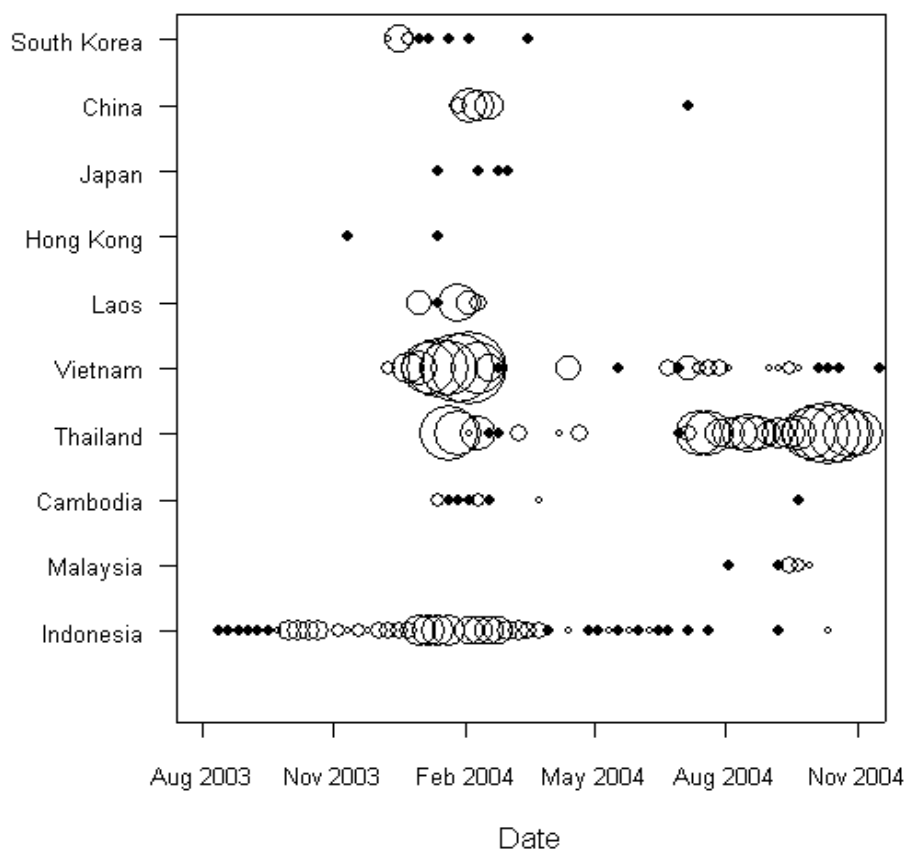


Figure 1 Temporal pattern of H5N1 avian influenza outbreaks by country, with single outbreaks shown as ♦, and multiple outbreaks shown as circles, with the size of the circles denoting number of outbreaks per week.

The H5N1 epidemic in Asia 2003-5 has been exceptional with respect to its geographical dissemination and its apparent extremely rapid spread over such a large area. It has also been notable for the lack of any consensus about how and why it spread so widely, and what measures should be taken to control it. Figure 1 shows the weekly incidence for all affected countries, with the size of the circle denoting the number of cases for that time period.

During the second half of 2003 as shown in Figure 1, the only cases of H5N1 infection which had been officially reported were the index farm case in the Republic of Korea, and

positive surveillance testing of cages and dead birds in Hong Kong SAR live poultry markets. These were the last isolations from domestic poultry in Hong Kong SAR – after vaccination of chickens commenced the only incidents in Hong Kong SAR in 2004 were both single isolations from wild birds.

So the reporting of geographically widespread H5N1 outbreaks in poultry from seven additional countries and multiple human cases of avian influenza from two countries over the next two months gave the appearance of a dramatic and explosive region-wide outbreak. This would have implied rapid movement of infectious material between countries and spread within the countries.

Now that it has been possible to reconstruct the pattern of the outbreak more comprehensively, the picture which emerges is significantly different. The elements of this picture will now be described using information from the country profiles accompanying this report, plus published papers. Published papers are cited in the text, and all other specific pieces of information used can be found in the country profiles.

Apart from the various incidents in Hong Kong SAR between 1997 and 2003, the earliest confirmed disease outbreaks in the current epidemic occurred in Indonesia in August 2003. From an examination of the epidemic curve and the geographical distribution, it is possible that the epidemic in Indonesia had two or more initial foci, with an introduction into West Java some time before August 2003 with the early development of geographically dispersed but relatively localized foci, and later introductions into other areas, either from outside Indonesia or as secondary transfers from the initial focus. The temporal curve suggests an initial epidemic seeded in mid-2003, and a second larger epidemic wave starting in late 2003. This pattern of small mid-year outbreaks and larger outbreaks from December to about March is seen also in Thailand and Vietnam over two successive years.

There is a need for further molecular epidemiological studies on Indonesian isolates to determine whether one or multiple genotypes or sub-types were present, but on the evidence so far available the outbreaks in Indonesia were due to a different sub-type of Z genotype from the one which occurred in Thailand and Vietnam. Based on both the haemagglutinin and the matrix protein genes, duck and chicken isolates from Indonesia lie some genetic distance from the Thai and Vietnamese isolates, and the fact that there were no recorded human cases in Indonesia suggests that it may have differed either with respect to infectivity for humans, or virulence for humans. This is comparable with the Japanese V genotype isolate (with which it showed some genetic affinity), which showed lower mammalian virulence than the Hong Kong 1997 virus. The virus in Indonesia does not appear to be epidemiologically linked to the virus which was circulating in Thailand and Vietnam, since it differed both in genetic subtype and epidemiological characteristics from the viruses examined from those countries.

Thus it seems unlikely that the virus was transferred from either of these countries through movements of poultry or other owned birds. The most plausible explanation for the introduction of this virus into Indonesia is that there were one or more introductions in

wild birds, which infected domestic poultry. The multicentric distribution of early cases supports this, and the early spatial distribution does not appear to match well to movement of owned birds. Most of the disease outbreaks over the course of the epidemic occurred in Java, where the northern areas of the island tend to be principally chicken/duck production, while the drier areas towards the southern coast are more commonly chicken/quail combinations. The geographical distribution of disease outbreaks in Indonesia does not indicate any marked difference in epidemiological pattern between the two ecological zones. In contrast to the early phase, the pattern of dissemination during the later stages of the Indonesian epidemic is consistent with distribution principally through the marketing system, although it is possible that some foci originated from new introductions by wild birds. This could only be resolved through more extensive epidemiological studies and associated molecular characterization of viruses.

Indonesia has made extensive use of vaccination in addition to heightened biosecurity and movement restrictions, and appears to have brought its previously widespread epidemic under control by this method.

The outbreaks in Japan and the Republic of Korea were identified next. These have now been shown to be due to viruses which differed from those in all the other national epidemics for which genotyping data is available, and showed over 99% homology between viruses isolated in the two countries. Thus they represent an independent epidemic not directly related to other outbreaks in the region, and the evidence very strongly favours the hypothesis that they were both due initially to introductions in wild birds. Given the rare isolation of V genotype in the period leading up to the two outbreaks (only once, in the People's Republic of China in 2003), the geographical proximity of outbreaks between Japan and Korea and the almost simultaneous occurrence of the outbreaks, a wild bird link between the two seems quite plausible. In the Republic of Korea the index case has all the features of a wild bird-derived infection, while later cases appear to have arisen from movement of domestic poultry. Of the four outbreaks in Japan, three appear to have independently arisen from wild bird introductions, while the fourth may either have been from this source, or from farm to farm transfer. In the Japanese outbreaks, crows (and possibly other resident wild birds) were infected and affected by disease, and wild birds were still being found infected at least a month after all infected poultry had been killed. Whether the crows became infected from migratory birds or from domestic poultry cannot be determined. In the Republic of Korea, it was considered that one farm became infected from wild birds, and H5N1 was isolated from two Korean magpies associated with separate infected farms.

The outbreaks in Thailand, Lao PDR, Cambodia and Vietnam appear to be interrelated at least to some degree. The viruses isolated from poultry and human cases in Thailand and Vietnam are all Z genotype, and almost identical at a genetic level. No molecular typing has been reported for isolates from Lao PDR or Cambodia. The early geographical distribution of reported cases in both Thailand and Vietnam is consistent with multicentric origin of the outbreaks, suggesting multiple sites of introduction in wild birds, with later perifocal dissemination around the introduction sites through poultry

marketing and possibly some dissemination to new areas by resident wild birds. In Vietnam, our analysis of the more micro-scale distribution of the epidemic indicated a spatial association with bodies of water. This could not be directly evaluated for Thailand. Early outbreaks in Vietnam were concentrated in the valleys of the Red River and Mekong River, but later the infection became more disseminated throughout the country.

In Thailand, the first wave occurred in a patchwork of geographical areas concentrated in the north, with a smaller focus in the far south – neither of which are areas where commercial poultry production is important, and neither is an area with strong emphasis on duck-raising. The fact that the viruses isolated in Thailand and Vietnam early in the epidemic are virtually identical suggests that they came from the same primary source. However the range of isolates which have been examined genetically is relatively limited, and there may well have been other viruses circulating within these two large outbreaks, but they were not included among the small set of isolates selected for genotyping.

In both Thailand and Vietnam the first epidemic wave peaked between late 2003 and early 2004 then declined in severity, with a second wave starting in July 2004. In Thailand this second wave has a substantially different geographical distribution from the first, occurring principally in the central region and lower north, with some detections more recently in the north east and east. The area where the second epidemic wave has occurred is the main duck growing area of Thailand, and also the region which is most involved in supplying poultry to Bangkok so has a stronger market orientation than areas more distant from Bangkok, which were affected in the first wave.

Outbreaks commenced in Cambodia in December 2003, continued until April, then disease occurred again in September 2004 and January 2005. One human case of apparent Cambodian origin occurred in January 2005. Reported outbreaks occurred in Lao PDR from December 2003 or January 2004 through to March 2004, and there have been no subsequent reports. The reported outbreaks were principally in areas of commercial poultry production, and it remains unclear whether there were outbreaks in village chickens in other areas. No human cases have been reported from Lao PDR.

It is possible that the epidemic in this region commenced in one of the countries from an introduction of owned birds from the area within China where genotype Z was circulating, and spread from there to the other countries in this group through marketing channels, rather than originating independently from wild birds. With the data we currently have available we cannot confidently distinguish which of these two explanations is correct. However if marketing channels are the explanation, they would have to be multi-country ones, yet the outbreaks are principally in native chickens and ducks, not in larger scale commercial poultry units, and these affected populations of traditionally produced birds are not ones for which there is extensive cross-border trading or purchase of breeding stock, which could produce multicentric outbreaks of the kind experienced in these countries. Although H5N1 virus was present in Vietnam in 2001, it was genetically quite different from the one which caused the epidemic in this group of countries, and it did not produce any reported disease outbreaks between 2001 and 2003.

Since genotype Z was first identified in China and Hong Kong in 2002, and took some time to become the dominant genotype with high pathogenicity, it is unlikely that genotype Z was circulating undetected in this group of countries for more than a few months before outbreaks were first detected in late 2003.

The spatio-temporal and molecular evidence therefore favours an initial significant role for wild birds in producing the first outbreaks in one or more of these countries, although market channels are certainly also important spread mechanisms which may have been very influential, and possibly even the sole cause of this early part of the epidemic. Movement of fighting cocks also appears to have played a role. H5N1 virus has also been isolated from various species of wild birds in Thailand over recent months, showing that infection is present in wild bird populations, although at this stage of the epidemic it may not be of great relevance to control policy.

All of the reported human cases in the current epidemic have occurred in Thailand, Vietnam and most recently in a person from Cambodia. Whereas genotype Z did not cause any human cases in Hong Kong during the short periods it was circulating in the markets and farms there a year earlier, the pathogenicity for mammalian hosts which the virus is displaying in Thailand, Vietnam and Cambodia suggests that it is a virus with significantly different epidemiological characteristics from those in other countries affected by the epidemic, and that it has perhaps evolved to increase its virulence for humans.

The situation in the People's Republic of China requires further clarification. H5N1 viruses have been isolated regularly from domestic poultry in the People's Republic of China since 1996, and the first isolation in 1996 from geese was associated with severe disease. However there were no further reports of disease until January 2004, when 49 outbreaks occurred over two months, and a single further outbreak in June 2004. No further outbreaks have since been reported. The People's Republic of China supplied substantial quantities of vaccine used by other countries during the 2004 outbreaks, and is believed to have had over 20 plants producing vaccine in early 2004, with the number later being reduced to nine. No human cases have been reported, but H5N1 virus was isolated from asymptomatic pigs. Malaysia reported no cases until the second epidemic wave occurred in nearby areas of Thailand, and this infection appears to have been a cross-border transfer.

An important issue is the list of at-risk countries which did not become infected. Taiwan Province of China and the Philippines have both undertaken intensive surveillance, but have not detected H5N1 infection in their flocks, despite extensive trading links with other countries in the region. They do however lie on the fringes of the main water bird migration routes. Myanmar has also not reported cases, but the reason for this is uncertain since adjacent areas of Thailand had extensive outbreaks. Similarly, the Democratic People's Republic of Korea has not reported any avian influenza H5N1

outbreaks², although it lies between two countries which have reported outbreaks. Australia and Papua New Guinea have also not had outbreaks, although in the past Australia has had 5 outbreaks of H7 disease between 1976 and 1997, out of 21 outbreaks reported worldwide between 1959 and the start of the Asian epidemic. All of those outbreaks are thought to have had an association with wild bird exposure. However in contrast to this earlier experience with H7 viruses, it would appear that H5N1 virus has not caused disease in the more southern parts of the bird migration range.

Species-specific infection information for poultry and other owned birds

All species of domestic poultry were affected during the epidemic. In the countries with mixed populations of birds, village chickens and ducks were the principal focus of clinical disease, although a range of other species were also involved. Outbreaks appear also to be predominantly in smaller flocks. A notable feature is the apparent under-representation of outbreaks from larger scale commercial flocks.

Ducks are of particular importance in the epidemiology of avian influenza, and the virulence of H5N1 for ducks has been increasing (Chen, Deng et al. 2004; Sturm-Ramirez, Ellis et al. 2004).

Species-specific infection information for other birds and mammals

An important feature of the epidemic and the virus involved was the wide range of species in diverse taxonomic groups of birds and mammals which showed clinical disease (Perkins and Swayne 2003). Deaths in water birds³ in a zoo and a nature park due to an H5N1 virus had occurred in Hong Kong SAR in late 2002 (Ellis, Bousfield et al. 2004) and at that time virus was isolated from a dead tree sparrow (*Passer montanus*) and a dead feral pigeon (*Columbia livia*). Subsequently, a series of isolations of H5N1 have been made from wild birds in Hong Kong SAR, some of which belong to migratory species. During the current epidemic, multiple species of captive exotic birds died at the Phnomn Tamao Wildlife Rescue Centre in Cambodia in late 2003 and virus was isolated from wild bird deaths in Hong Kong SAR and Japan (Japanese crows). There were two separate isolations of H5N1 from Korean magpies near to infected places and Japanese crows and sparrows were thought to be involved in transmission in Japan. Wild birds from six species in diverse bird groups have been found infected in Thailand.

A controversial issue has been whether wild migratory birds have played a significant role in the epidemiology of the outbreak, as they have done in initiating outbreaks in other parts of the world. As the evidence has accumulated, it is increasingly difficult to explain the pattern of the Asian epidemic without attributing a role to both migratory and resident

² Influenza in commercial chicken farms caused by H7 avian influenza A virus was confirmed in March/April 2005.

³ Assorted species of geese, ducks and swans, captive Greater Flamingo (*Phoenicopterus ruber*) Little Egret (*Egretta garzetta*) at two waterfowl parks and from two dead wild Grey Heron (*Ardea cinerea*) and a Black-headed Gull (*Larus ridibundus*) in Hong Kong.

wild birds. A proposed mechanism by which the various populations may have interacted to produce the Asian epidemic is described in the Epidemiological Synthesis section.

Infection, but not disease, has been identified in pigs in the People's Republic of China (Anonymous 2004).

Large cats were first reported as infected with H5N1 in the People's Republic of China in 2002. Deaths also occurred subsequently in captive tigers, leopards and lions at the affected wildlife centre in Cambodia and high mortalities and prevalence of disease were reported in a captive large cat establishment in Thailand, involving tigers and clouded leopards. Disease has also occurred in domestic cats, which have also been shown to be susceptible experimentally (Kuiken, Rimmelzwaan et al. 2004).

Human cases have also occurred in Hong Kong SAR (though not in the current epidemic), Thailand (Chotpitayasunondh, Ungchusak et al. 2005), Vietnam (Hien, Liem et al. 2004) and most recently in Cambodia, although the person was diagnosed in Vietnam.

Thus H5N1 has shown the widest host range of any of the HPAI viruses, and has been pathogenic in most of the species. It was the first true avian influenza A virus to cause serious disease in people with fatalities.

Risk factor information

There have been no direct epidemiological studies reported describing a risk factor analysis for the H5N1 outbreak in Asia. There have been studies of risk factors in relation to the early 2002 outbreak in Hong Kong SAR, including links to infection in southern China (Kung et al, University of Hong Kong/Massey University EpiCentre joint research paper in preparation), and one study of risk factors for human infection in the 1997 Hong Kong SAR outbreak. There have also been two recent studies of risk factors for H5N1 infection in Thailand and the region as a whole (Gilbert and Slingenbergh 2004; Gilbert, Wint et al. 2004).

In the absence of direct studies of factors responsible for spread of infection within countries, a list of factors likely to be influential has been prepared from a consideration of the published literature and information gathered in the course of this study, to produce a list of putative risk factors. These factors were examined for each country along with possible mitigating factors. A summary matrix table with risks scored for individual countries is presented in Table 2. The scores in this assessment are put forward as best judgments from the available evidence, and are offered as a basis for discussion to assist countries in defining appropriate controls. Country profiles have been used as far as possible to provide the estimates, but in some cases information is sparse, and further guidance is requested.

The scores assigned are 0 = zero risk, 1 = very low risk, 2 = low risk, 3 = moderate risk, 4 = high risk and 5 = very high risk.

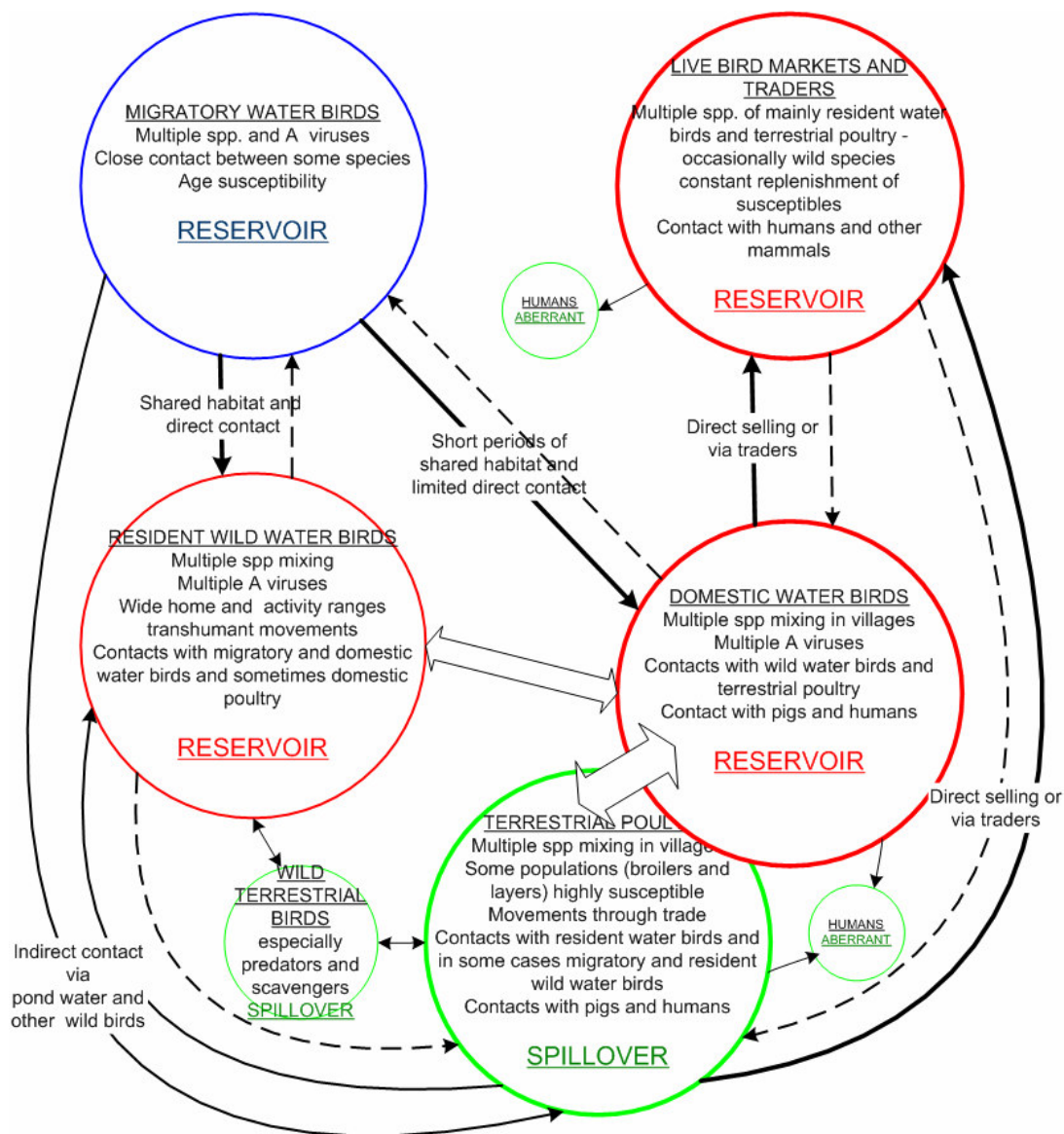
Some risk factors carry higher risk for introduction of infection into the country, while others are linked more strongly to transmission within the country.

It is noteworthy that the summary assessments based on sums of the component scores match well to the apparent difficulty of achieving full control of H5N1 in the various countries. It is suggested that these factors could be used to assist in planning of control strategies based on risk factors, as discussed later in this report.

Table 1 Matrix of risk factors and countries affected by H5N1 with risk factors scored on a scale of 0 to 5 with 0 = zero risk, 1 = very low risk, 2 = low risk, 3 = moderate risk, 4 = high risk and 5 = very high risk

Risk factors	Korea	Japan	Hong Kong	Malaysia	China	Indonesia	Thailand	Cambodia	Lao	Vietnam
Legal movement of birds across country borders	1	1	3	1	1	1	2	2	2	2
Illegal movement of birds across country borders	1	1	1	3	4	4	4	5	5	5
Uncontrolled within-country bird movement	1	1	1	3	5	5	5	5	5	5
Live bird markets	1	0	3	3	5	5	5	5	5	5
Movement of people and contaminated items	1	1	2	3	4	5	4	5	5	4
Wild resident water birds	3	3	2	2	5	5	4	5	5	4
Migratory water birds	5	5	5	5	5	5	5	5	5	5
Fighting cocks	0	0	0	2	1	3	5	3	3	5
Non-reporting by owners of birds	1	1	1	2	4	4	4	4	4	4
Non-recognition of disease by owners of birds	1	1	1	2	4	4	4	5	5	4
Limited financial compensation	0	0	0	?	5	5	5	5	5	5
Major cultural festivals with peak consumption	0	0	2	?	4	2	2	2	2	3
Dead bird disposal	1	1	1	1	2	2	2	4	4	4
Sale/consumption of sick and dead birds	0	0	1	1	2	2	3	4	4	4
Mixture of species on farms	2	2	0	3	5	5	5	5	5	5
Movement of song birds into/within the country	1	2	1	1	4	2	2	1	1	3
Likelihood of endemicity	1	1	0	2	5	4	5	5	5	5
Total score	20	20	24	34	65	63	66	70	70	72
Risk Rating	Low	Low	Low	Low	med-high	med-high	high	high	high	high
Total H5N1 outbreaks Dec 2003 to 11 Feb 2005	19	5	0	10	50	169	1064	13	45	1764

Epidemiological Processes Involved in the H5N1 Epidemic



TRANSMISSION PATHWAYS

Figure 2 Proposed epidemiological relationships in Asian H5N1 avian influenza epidemic

The above diagram summarizes our proposed synthesis of available information, and the overall hypothesis we put forward to explain the Asian epidemic of H5N1 influenza, and the preceding occurrence of H5N1 viruses in Asia over the last decade. The diagram will be referred to throughout the following part of the report.

Views on the causal processes involved in the current epidemic are very diverse, and most of them are consistent with only limited parts of the available evidence. We have therefore conducted a detailed examination of all the information we have been able to accumulate, to produce an epidemiological evaluation of all the available evidence, and propose a coherent explanation for all of the findings to date. These views are put forward to stimulate informed debate and wise decision-making in the containment and control of HPAI.

Reservoir, Spillover and Aberrant Hosts

In diseases which have complex ecological relationships, such as avian influenza, a very important concept is that of different types of hosts having different roles in the disease.

A reservoir host is one which maintains infection, and usually either does not get disease, or there is only mild disease, or only young animals are clinically infected while adults are immune or only subclinically infected. In the case of avian influenza, wild waterfowl are the reservoir hosts for influenza A viruses. In the specific case of H5N1 infection, domestic ducks and/or geese and possibly quail have probably acted as reservoir hosts at various stages of the evolution of the virus.

A spillover host is one which is susceptible to infection if exposed, and excretes the agent so may transmit infection to other hosts, but would not maintain infection within the species in the long term unless there is constant or intermittent replenishment of infection from a reservoir host species. So if exchange of infection with reservoir hosts is eliminated, infection will sooner or later die out in spillover hosts. Commonly, spillover hosts suffer much more severe disease than reservoir hosts do, and disease affects a wider range of age groups than in reservoir hosts. Viruses such as avian influenza are likely to evolve much more rapidly in spillover hosts than in reservoir hosts (Suarez 2000). Chickens are a clear-cut example of a spillover host for avian influenza in general, and H5N1 in particular. There can be cascades of spillover hosts, with infection in one such host species spilling over into a second, and so on. Thus in the Japanese H5N1 outbreak, crows may have been a spillover host from migratory birds, which infected chickens, or a spillover host from chickens, or both. However, if the reservoir host source is removed, the spillover cascade will eventually dry up. Controlling a disease in spillover hosts will only work for the short term, unless further transmission from reservoir hosts is prevented. In general, the more serious the disease in the spillover host, the higher the mortality rate, and the faster the hosts die after infection, the less they contribute to maintenance of infection. So such steps as killing wild birds which show disease is inappropriate from a disease control viewpoint as well as a conservation viewpoint.

An aberrant host is one which is only rarely infected, commonly suffers severe disease, and usually does not excrete sufficient virus to transmit to other hosts. Aberrant hosts are therefore unimportant in the epidemiology of the disease, but may be severely affected. At present humans are an aberrant host for H5N1, and the major concern is that the virus may change sufficiently that humans will become an important spillover host, in which H5N1 may transmit worldwide, before infection with that particular viral genotype peters out in humans, as it always eventually does with influenza A viruses.

The separation between these host types is not fixed, and a host species may move from one category to another due to a change in the genetic character of the virus, or a change in host ecology. Humans are currently an aberrant host for H5N1, but if a pandemic strain emerged which could transmit readily between people, humans would move to the category of spillover host, purely due to a change in viral genetics. Some hosts may also be borderline between two categories, either for all influenza A viruses, or for a particular viral genotype. Nevertheless, the separation of hosts into these ecological categories is important for accurately describing infection dynamics in Asian ecosystems.

Wild birds as hosts of H5N1

The primary reservoir hosts for influenza A viruses are the Family Anatidae, the main group within the Order Anseriformes. These are the various species of waterfowl - ducks, geese, swans and related web-footed birds. The shorebirds and gulls in the Order Charadriiformes also act in this role, though probably to a lesser extent (Kawaoka, Chambers et al. 1988; Melville and Shortridge 2004).

Numerous bird species within these two groups are migratory within Asia, estimated to be about 3 billion birds in total. They breed in the high northern latitudes, in areas such as Siberia, flying northward principally between March and May, and breeding from May to September. Once the chicks are fledged and able to fly, the birds make the return journey to their winter range, which are species-dependent areas ranging in location from low northern latitudes to southern latitudes (as far as Australia and New Zealand).

Southward movement begins in July, and builds up during the following months, with birds fully occupying their winter ranges between November and March. Some populations separate into sub-groups which fly on either side of the Himalayas to south-east Asia and the Indian subcontinent, while others largely fly down the coastal side of the Himalayas, or across open sea. Some shorebird species fly direct between Siberia and their winter ranges (as far as New Zealand) without stopping, while waterfowl typically fly in short hops, stopping daily to feed and rest, and sometimes stopping at intermediate points for longer periods. We will use the term “**activity range**” to describe the area used by a species during the course of one complete annual cycle.

The Asian Waterbird Census administered by Wetlands International (www.wetlands.org/iwc/awc/awcmain.html) has extensive information and references on the populations of species in the region and their migration patterns, including summary reports and maps of annual activity ranges. The most recent collated summary is for the period 1997-2001. Figure 2 shows the East Asian Flyway monitored by the Census, and it can be seen that the flyway for Anatidae matches quite closely to the area affected by H5N1 outbreaks in poultry. However no single species fits this overall pattern, with each species using different parts of the flyway and having a different total activity range, as shown in the Census distribution maps. Even those maps are crude approximations to where the birds fly, being based on limited counting of birds at monitoring sites on specified observation dates.

Thus the overall flyway can be seen as the summary of a series of layers for the distribution of each of the many species making up the group. Information in the Census is available for 110 species.

Some species of birds are variably migratory, such as the Peregrine Falcon, some individuals of which are migratory while others are sedentary. Other water birds move between different regions within a region according to season, feed supply and water availability. We have called these **transhumant**, since the term normally means seasonal movement of livestock between regions, and the effect of this seasonal rotation of bird species is similar. Yet other bird species are **resident** or **sedentary**, maintaining a range which does not move significantly over the course of the year.

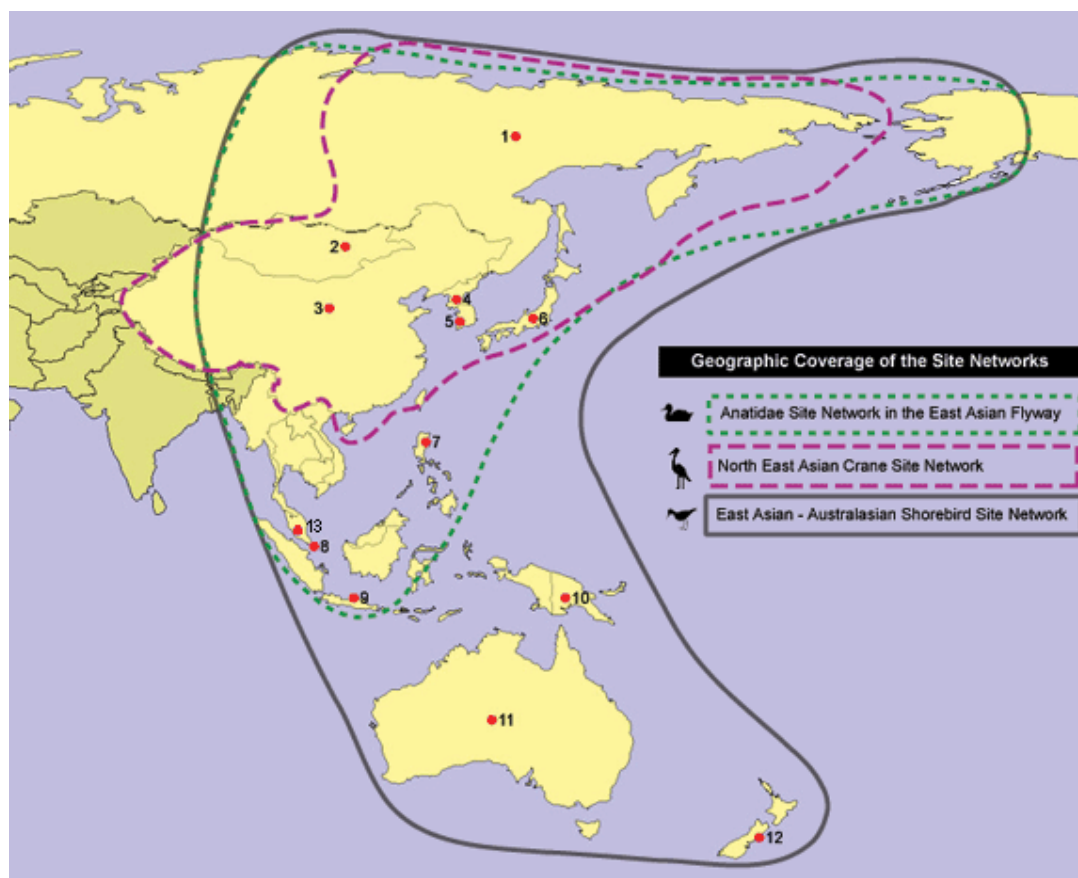


Figure 3 Flyways Monitored by Asian Waterbird Census – Anatidae range shown by dashed green line

It is generally accepted that the migratory Anatidae have been the principal source of spillover infection of influenza A viruses in chickens, turkeys and other poultry, but the usual pattern is that the viruses circulating in wild birds are LPAI, then an LPAI virus is transferred from the wild birds into chickens, and becomes progressively more pathogenic through successive infection cycles in the spillover hosts.

As outlined earlier, H5N1 virus may have originated in part by this process, but it contains genetic material of goose and quail origin, and by the mid-1990s was circulating in domestic ducks and geese, with spillover infection in chickens and other domestic poultry, and a small number of aberrant infections in humans. There is no evidence that the virus was present in wild birds for about another five years. During that time it was evolving quite rapidly into multiple genotypes, which differed in pathogenicity and duration of virus excretion for domestic ducks, and probably also for other domestic poultry – since the dominant genotypes kept changing over a few years.

Although there has been debate about whether H5N1 has become established in migratory birds (Melville 2004; Melville and Shortridge 2004), it is clear that it is circulating within at least some species of wild birds, including migratory species. An important difference between H5N1 and other avian influenza viruses is that H5N1 probably evolved to become HPAI in domestic birds and then subsequently was transmitted into wild populations, rather than emerging first in wild populations. It may well therefore behave quite differently in wild birds from other influenza viruses, being more virulent, and perhaps occurring at much lower prevalence (thereby making it hard to detect in wild populations).

In 2003, an H5 avian influenza A virus was isolated by the Laboratory for Investigation and Surveillance of Emerging Zoonotic Diseases (Novosibirsk, Russia) from a wild mallard duck on lake Chany in the south of Western Siberia. The area where the birds live is a rather sparsely populated area with many lakes and is a crossroad for migrating birds. The isolate was sequenced to determine relatedness to other H5-type avian influenza A viruses in Asian poultry. The A/mallard/Chany/9/03 avian influenza A virus was related to, but was not identical to current avian influenza A (H5N1) viruses circulating in domestic poultry in Asia. The A/mallard/Chany/9/03 avian influenza virus has haemagglutinin sequence similarity in the 90—95% range to the current Asian influenza A (H5N1) virus, which is similar to sequences of other Eurasian H5-type avian influenza viruses, including A/duck/Potsdam/1402-6/86 (H5N2) and A/turkey/England/50-92/91 (H5N1).

The A/mallard/Chany/9/03 avian influenza virus is not the same virus as the Asian H5N1, and is not direct evidence of infection and spreading of the current Asian H5N1 avian influenza virus by migratory birds (ProMED reports 28/10/04 and 30/11/04). However, the isolation of an H5N1 virus in this area is an important finding, irrespective of its relationship to current Asian strains, since it indicates long term persistence of H5N1 virus in wild birds and infection of breeding populations. Isolates were also obtained from wild birds in Hong Kong SAR in 2002 and later (Ellis, Bousfield et al. 2004), Hong Kong SAR profile). Since the start of the epidemic more active surveillance of wild birds has been conducted, and the virus has been isolated from a growing range of species, including migratory, transhumant and sedentary types. Further details are provided in the various country profiles.

One of the challenges which is commonly made to the possible role of migratory waterfowl is that isolation rates are very low for H5N1 and low for other H and N types – although a mix of different influenza A viruses can regularly be obtained from such birds in various parts of the world (De Marco, Foni et al. 2003; De Marco, Foni et al. 2003; Delogu, De Marco et al. 2003; Fouchier, Olsen et al. 2003; Ellis, Bousfield et al. 2004). This is exactly what would be expected in reservoir species, which have comparatively low transmission rates for each of the viruses, and hence maintain them continuously circulating in the population through faecal/oral cycling of virus. Typically infection prevalence would peak in juveniles as they lose their yolk sac immunity and go through an infection cycle, commonly at about the age they would be migrating southward. This helps explain why infection of other birds has occurred during the period of southward migration and over-wintering in tropical areas, but not during the northward migration.

The migratory birds themselves are probably not directly responsible for most of the transfers to domestic poultry, but rather spread infection to a spectrum of spillover bird species outside the reservoir taxa, such as demonstrated for crows in Japan and magpies in the Republic of Korea. Such birds have no immunity from previous exposure, are susceptible to infection and in some species to disease. Epidemiological experience with other diseases suggests that such spillover species will excrete considerably more virus than reservoir species, and have direct contact with domestic poultry, hence passing infection on to them and initiating focal H5N1 outbreaks.

Typically infection will die out in wild populations of spillover species unless reinforced by further transfers, so population control in spillover species which are dying of the disease is not a useful measure to control avian influenza. However an understanding of this “infection cascade” between species is important in the design of control strategies.

Infection patterns in different species of domestic poultry

The first identification of H5N1 was in geese (Guo, Xu et al. 1998), and domestic ducks and geese have remained very important both in the occurrence of disease, and in the evolution of the virus. Ducks have been more fully studied than geese, and both virulence and duration of virus excretion have increased over time (Webster, Guan et al. 2002; Chen, Deng et al. 2004). Where ducks and geese can interact with other poultry species, they are an important source of infection, and ducks in particular have commonly been infected during the epidemic, and appear to have been a particularly important source of exposure for other domestic species.

Minor domestic poultry species also represent a significant issue in relation to the transmission process in markets in Asia, where they have traditionally been kept in cages adjoining those containing chickens. Most of the commonly kept species can become infected. Quail and silky chicken are capable of maintaining infection with influenza viruses and to a lesser degree pheasant, guinea fowl, pigeon and chukar can contribute to the maintenance of infection. In studies of Hong Kong SAR markets, although these species were present in much smaller numbers than chickens, H5N1 was isolated from a number of these species although they were kept in separate cages (Kung et al, University of Hong Kong/Massey University EpiCentre joint research paper in preparation). They

may also be important in the evolution of new strains, and adaptation of such strains to chickens. Some of the minor species are marketed at younger ages than chickens, and may stay in the market stalls longer because demand is lower, so they may contribute disproportionately to the circulation of H5N1 within the poultry markets.

Fighting cocks are very traditional in a number of countries in the region. They are a high risk group for transmission because they move (and are traded) more widely than other birds, they are deliberately mixed under stressful conditions, and husbandry practices are conducive to spread between birds, and between birds and owners. They require special consideration in control programmes.

Pet birds can also become infected with influenza viruses. In particular, passerine birds (including the cage birds in this group) can maintain infection, mainly with H3 and H4 subtypes. This has led to suggestions that laughing thrushes, which are traded widely in Asia, could be involved in regional transmission of the disease (Melville and Shortridge 2004). However the epidemiological pattern of disease occurrence does not support this and H5N1 has not been isolated from this species, nor have there been reports of unusual mortality. Psittacine birds do not commonly become infected with avian influenza viruses, but do seem on the evidence to be quite susceptible to H5N1 infection.

Mammalian spillover and aberrant hosts

An unusually wide range of mammal groups has been identified as infected with H5N1, as described above and in country profiles, but all infections can reasonably be attributed to oral exposure except for the single human to human transfer. In all other cases, there is no clear evidence of onward transmission although it may have occurred for example in tigers. Thus most or all of the mammals which have become infected can be classed as aberrant hosts.

The pig is of particular interest because it has cell receptors for both avian and human influenza viruses. However an important sparing factor for emergence of a possible pandemic strain is that infection of pigs has occurred only rarely, and “swine flu” has not become a feature of this epidemic, as it had been in the 1918 and some later influenza epidemics. A Japanese study in which miniature pigs were challenged with H5N1 failed to cause infection (Anon, 2004). This low susceptibility may well have contributed to the fact that a mammal-adapted virus has not yet emerged from H5N1. However the virus group has shown exceptional genetic plasticity and continuing vigilance is required to minimize the risk of this occurring.

Because humans remain an aberrant host of this virus, the case fatality rate in hospitalized people is extremely high. However these cases may represent “the tip of the iceberg”, and infection may be significantly more common in humans than the clinical data would suggest. A study of various categories of people in Hong Kong SAR in 1997-8 (Bridges et al, 2002) showed that household contacts of human cases had the highest prevalence of H5 antibodies (12%), poultry workers next (about 10%) with intensity of exposure to birds showing a positive association with seroprevalence, and government workers who participated in the cull of infected birds showing 3%, a similar level to that in people who

cared for or traveled with human cases. People who had no identifiable exposure had lower levels (0% in blood donors, 0.7% in health care workers). Information from the Japanese outbreak in 2004 has recently shown that substantial numbers of people were exposed, though no severe human case of H5N1 infection occurred in Japan.

In the 2003 H7N7 epidemic in The Netherlands, there were 83 confirmed cases of human H7N7 infection in workers involved with poultry and disease control, and their families. Most suffered only conjunctivitis while some had mild influenza-like illness. There was one fatal case of generalized infection in a veterinarian who was involved in the control programme. There was evidence of possible transmission of infection from two poultry workers to three family members, who developed mild illness.

In many regions of Asia where there is close contact between people and poultry, the quantity of virus to which people are exposed during an outbreak will be large, and measures to reduce the virus load are an important part of control.

Survival of influenza viruses in the environment

Influenza A viruses have exceptionally long survival times outside the living host, when in a favourable environment. When excreted by water birds they can survive in lake water for many months at 17 degrees C, and for even longer at 4 degrees C. Infectivity of this virus for hosts is variable, depending on strain and environmental factors. In less favourable environments, such as in faecal material, the virus survives no more than 24 to 48 hours.

Thus virus survival is a very significant issue for transmission within wild bird populations, where water contamination may continue to provide a source of infection for extended periods, especially in the northern breeding grounds. Under village conditions in Asia virus survival in the environment can also be important, but its role depends on temperature, the nature of fomites which may carry virus, and density of birds. Maintenance of infection in wild and domestic bird populations is considerably more important than environmental sources in spreading infection to new locations.

Exposure of people to virus occurs through direct handling of infected birds and consumption of raw products or contamination of fomites with virus from uncooked poultry. Highly pathogenic H5N1 virus has been isolated from multiple surveillance samples taken from duck meat imported into the Republic of Korea from the People's Republic of China, confirming extended survival (Tumpey, Suarez et al. 2002). It is not stated whether the product was chilled (most likely) or frozen, but virus survived three cycles of freezing and thawing before isolation was attempted.

Virus also survived for days to weeks in pig meat contaminated with H1N1 virus when the meat was chilled or frozen, and in tissues from pigs experimentally infected with the same strain. It is likely that virus will also survive in poultry meat, but this issue would benefit from further investigation since the level of risk and the duration for which product from infected birds would remain infectious are both unclear. It is very relevant to the issue of transmission mechanisms. Virus does not survive normal cooking

procedures, so exposure through consumption can only occur through cross-contamination of items consumed raw, as a result of virus on chicken contaminating hands or other food, or consumption of raw products such as duck blood.

Transmission mechanisms between hosts

A crucial part of replication of influenza A viruses is cleavage of the precursor HA protein in newly formed viral particles, which is an essential step in making the new particles infectious, so that they can infect additional cells. This step is mediated by a host enzyme rather than being achieved by the virus itself. Some strains of avian influenza virus have specific amino acids close to the cleavage site of the precursor HA protein which limit the host enzymes which can split the molecule to those which are found in the respiratory and digestive tracts. These strains are of low pathogenicity in chickens. Strains which have different (basic) amino acids at these same sites can be split by enzymes which occur in a much wider range of tissues, and these are highly pathogenic, since they can multiply throughout the body. This increases the transmission potential of the virus.

There appear to also be differences between strains in predominant excretion routes arising directly from the genotype of the virus. Most avian influenza viruses are principally or entirely excreted in faeces, so are mainly spread by contamination of food resulting in oral intake, probably causing infection through the oropharyngeal mucosa. This is the natural process in reservoir hosts, and most spillover hosts. Some viruses are also excreted by respiratory aerosol and therefore can much more readily spread from host to host by droplet infection (as in most human influenza viruses). In the outbreak in Hong Kong SAR in early 2002, aerosol transmission may have occurred over moderate distances, but it is difficult to resolve whether such transfers are due to short distance aerosol spread, or to movement of fomites or similar items, leading to oral exposure.

These factors are very important in the epidemiological processes which can potentially lead to pandemic strains, and in the rate and pattern of spread of avian influenza between flocks, and between different species in places such as live bird markets.

Epidemiological Synthesis of the H5N1 Epidemic Process

It would appear that the 2003-5 epidemic is in fact at least three separate events.

1. The **Indonesian epidemic** began about August 2003, and continued until at least October 2004, peaking in late 2003 and early 2004. Although the small number of virus isolates evaluated belong to the Z genotype, they differ significantly from the Z genotype circulating in Thailand and Vietnam. Although there was considerable scope for human cases to occur, none have been reported.
2. The **outbreak in Japan and the Republic of Korea** began in December 2003/January 2004 and was successfully eradicated in both countries by stamping out only diseased poultry flocks, with the last cases occurring in March 2004. These outbreaks were due to closely related viruses of V genotype, which had only been found on one previous

occasion, in the People's Republic of China. Infection occurred in people exposed to the virus in Japan, but there was no evidence of disease.

3. The **epidemic in Thailand, Lao PDR, Cambodia, and Vietnam** began in late 2003, peaked first over the winter period December 2003 to March 2004, then died down, but a second wave of outbreaks commenced in July 2004, and has reached a peak late in the year, with transfer of infection also into Malaysia. This outbreak is due to Z genotype, and isolates examined early in the epidemic from Thailand and Vietnam were very closely related. All human cases of disease have occurred in these countries.

The **epidemic in the People's Republic of China** occurred over the same period as those in Japan and the Republic of Korea, and outbreak locations were very widespread, especially in the south-east. No genotyping data is available, so it is not possible to determine whether this epidemic is related to either epidemic 2 or 3, or was due to one or more different viruses. It would be very helpful to have genotyping data on the Chinese epidemic.

It is striking that the seasonal pattern of disease is broadly consistent across the three epidemics, and has repeated in two successive years in epidemic 3. In the period 1997 to 2003, five H5N1 incidents occurred during December-March periods of various years.

This period has three important features. It is the coolest time of the year at least in some countries of the region, it is the time when major festivals occur (such as Chinese New Year and Tet, which cause peak demand for poultry and extensive movement of poultry and people), and it is the time when migratory birds are in winter residence in countries which were affected by the epidemic.

Figure 2 shows how it is suggested that the epidemic occurred. From all of the evidence available, the most plausible explanation of the overall epidemic is that it was initiated principally by migratory birds seeding infection into local wild and domestic bird populations, with one or more separate foci established in each of the epidemic areas. Alternative explanations have been carefully assessed, but are difficult to reconcile with all of the evidence. Early spread within countries and possibly in some cases to nearby countries occurred through movement of poultry, once infection had been seeded into one or more locations.

If infection entered migratory birds in about the period 2000 to 2002, then buildup of infection in the breeding populations would have occurred by the 2003 breeding season, when H5N1 virus was isolated in the breeding areas of Siberia. When these birds moved south in the second half of 2003, they carried infection with them. It would appear that two or more genotypes of virus (V and Z) and some subtypes were circulating in these populations. Examination of the activity range maps for various species of Anatidae suggest that different species were probably responsible for initiating infection in the various countries or groups of countries, and that there was exchange of infection among species of Anatidae while they were in the northern breeding grounds.

However birds from the same breeding grounds over-winter in the Indian sub-continent, and to some degree in Europe. The Netherlands had an epidemic of avian influenza commencing in February 2003, but this was H7N7. No countries in East Asia have reported H5N1 disease, although they did report other viruses. It seems likely that the species and sub-populations of species which used the coastal flyway route became infected, but those which used the inland flyway had low or zero prevalence of H5N1. This would not be surprising, since birds tend to follow the same flyway for life, and infection entered wild birds along the coastal route, so has probably been retained so far in this sub-population. This may not always remain the case.

It is not necessary to think of infection being linked to one or a few species of Anatidae, but rather to be circulating in the mixed breeding populations and to infect some groups of birds in a number of species, which perhaps transferred infection to other migratory species during the southward flight.

Infection would have been transferred from migratory birds to resident wild water birds, to some terrestrial wild birds, and perhaps in some cases direct to domestic poultry. This type of infection process is consistent with the geographical pattern of outbreaks in the various countries. It is noteworthy that predator and scavenger species are unexpectedly prominent among the wild terrestrial birds from which H5N1 has been isolated, suggesting that they became infected by eating diseased birds, and become infected in that way.

Once infection has become established in domestic poultry (both aquatic and terrestrial) it can maintain for extended periods if sufficient susceptible birds are available, without requiring further introductions from reservoir species. In general, the epidemic has occurred disproportionately in outdoor populations of birds, such as village chickens and ducks, with relatively limited infection in large scale poultry units in countries where they co-exist. Japan and the Republic of Korea had outbreaks in some large flocks, but there were water bird links in flocks which were not infected from known market movements. Outbreaks in the People's Republic of China tended to be in quite large flocks, but there is no epidemiological information on which to evaluate how they may have been exposed.

Live poultry markets and poultry trading appear to have progressively taken over from wild bird contact as the mechanism for further dissemination of infection, with ducks playing an important role in transmission in many but by no means all situations. The mix of species in markets and farms is the key to continuing circulation of virus, and while ducks have been important in many affected areas, in other areas species such as quail appear to have helped maintain infection. Fighting cocks have also been a high-risk group because of factors described earlier.

It is unclear from the available data whether migratory birds played a significant role in the second wave of infection in Thailand, Vietnam and later Cambodia, which commenced in July 2004 and built up towards the end of the year. The geographical pattern in Thailand and Vietnam over that period, and evidence of other factors as demonstrated in country profiles, suggest that over the course of the second wave domestic poultry of various species were maintaining and disseminating infection very

effectively (with ducks generally considered particularly important), and that wild birds were at most an exacerbating factor, not a primary causal factor as they were in the first wave of the epidemic.

Human exposure undoubtedly occurred in all areas, but only caused recognizable disease in Thailand, Cambodia and Vietnam. Evidence from both the 1997 incident in Hong Kong SAR (Bridges, Lim et al. 2002) and the 2004 outbreak in Japan show that infection of people with these particular viruses was much more widespread than disease, but there is no publicly available evidence to evaluate this for the virus circulating in Thailand and Vietnam.

It is very important that further information be gathered on human exposure, since it is of great importance in understanding the pattern and extent of this exposure, which in turn is very influential on the risk of a pandemic strain emerging.

Assessment of control strategies adopted by countries

Experience in the various countries is very helpful in assessing the merits of different control strategies.

Hong Kong SAR provides a demonstration of the fact that successful control is possible using an integrated control approach, progressively adjusting measures to achieve effective control.

During the early incidents in Hong Kong SAR, stamping out was used very effectively. As the incidents continued to arise, separation of species in the markets, the use of rest days to reduce virus exchange within the markets, and the banning of some birds (such as quail, ducks and geese) from live marketing improved control substantially but did not completely stop new outbreaks. Ultimately vaccination and strict biosecurity was introduced, and it is very noteworthy that Hong Kong SAR has gone through the entire 2003-5 epidemic without a case in commercial poultry or people, despite the extensive infection in nearby countries, importation of live poultry over most of this period from China, and fairly regular detection of infection in wild birds in the urban area. This is a remarkable achievement under the circumstances, but is a high cost approach suited to a region with a very large and dense human population and a very small and tightly controlled poultry population.

The cost of the strategy used in Hong Kong SAR is well beyond the capacity of a number of the other countries in the region to adopt, taking into account their far larger and less tightly controlled populations of poultry, but nevertheless the principles which have protected Hong Kong SAR can be adapted for use in other countries in the region.

Stamping out is the classical method of control for avian influenza, and it was completely successful in Hong Kong SAR, Japan and Korea in situations where the number of farms affected was small, and tight control could be exerted over transmission risks. In all three

affected areas infection was present in wild birds, but this did not prevent eradication or subsequent maintenance of freedom from infection.

In other countries which used the policy, stamping out reduced the size of the epidemic but could not eradicate infection when it was geographically dispersed, and being seeded to at least some degree from wild birds. Once infection has become endemic in medium-term reservoir species such as domestic ducks, stamping out alone is unlikely to succeed, and a new more integrated approach must be adopted.

Among these countries, vaccination was applied to control infection in Indonesia and China, and to prevent infection in Hong Kong SAR. The evidence is circumstantial and not based on scientific trials, but vaccination appears to have been very beneficial in reducing the virus load to which populations were exposed, shortening outbreaks and preventing long term maintenance of an infected state. Countries which adopted vaccination have had better overall results in controlling their outbreaks than countries which did not vaccinate, in the face of a substantial epidemic.

The effect of heightened biosecurity (including restrictions on marketing and movement of birds, separation of high risk species from lower risk ones during marketing, etc) is difficult to assess separately from other factors. In Hong Kong SAR there is evidence that the use of rest days (when markets were completely emptied of poultry, and the markets re-opened with new birds) was beneficial, and epidemiological studies of live bird markets both in Hong Kong SAR and in other countries have demonstrated that they provide probably the single most effective virus exchange mechanism, which can greatly intensify interchange of influenza viruses among birds, and to people. Hence they have the potential to assist in the emergence of new genotypes with higher disease-causing potential.

Assessment of surveillance strategies adopted by countries

Surveillance has been principally based on disease reporting by owners and some active case-finding efforts. Where case-finding has been intensified, it has generally found substantially higher numbers of cases than were being detected through routine levels of case-finding, indicating that levels of detection substantially understate the true level of occurrence of H5N1 infection in the affected countries. Proving that countries are free of infection under such circumstances is epidemiologically challenging.

Japan and Korea undertook extensive surveillance after detecting infection, and both countries were able to detect and stamp out infected farms, and to detect infection in wild birds, which (as far as could be determined) progressively disappeared once the infected flocks had been stamped out. Both countries have now gone almost a year with no new evidence of infection.

Hong Kong SAR operates an intensive laboratory-based surveillance system for local farms and imported live birds, which has been effective in detecting incursions of H5N1 infection, and also undertakes surveillance activities in wild birds.

Other countries do not have adequate resources to mount laboratory-based surveillance of equivalent intensity. However there is now a need to shift from case-finding to structured surveillance strategies which will allow management based on risk factors rather than simpler “search and destroy” approaches, which will only work when infection is limited in distribution, and can be detected by simple means. When species such as ducks and quail can be infected and transmitting HPAI virus, without showing clinical signs of disease, this strategy alone will not work.

Through national efforts supported by international organizations, especially the Technical Cooperation Projects mounted by FAO, crucial infrastructure has been put in place to enhance capacity for both diagnosis and surveillance.

The next step is to shift surveillance away from simple case-finding (which is poorly effective once infection is well established and not transferable between countries) to a risk-factor based approach, which focuses on identifying factors which exacerbate or reduce spread, and developing strategies for managing these risk factors. These risk factors are likely to operate similarly across the currently infected and high-risk countries, so there is considerable scope for sharing of approaches and information, and learning from the experience of others.

Proposal for a Risk-Based Approach to Control of H5N1 Virus

In the initial phase of the epidemic, stamping out offered the best hope of a quick resolution, and succeeded in Japan and Republic of Korea. However once infection became widely established in different bird types and market networks, especially where some of the birds showed no evidence of disease, other measures became necessary.

The best prospect of controlling H5N1 infection in the currently infected countries lies in adopting an approach built around surveillance measures to identify where and how transmission is occurring (assessing risk), and then adopting control measures which progressively reduce the various risky processes and influential transmission pathways.

Risk factors of current importance in the epidemiology of H5N1

At this stage of the epidemic, of the 17 factors listed in Table 1, live bird markets (especially with mixed species) and uncontrolled within-country bird movement are considered to be the most important influences on maintenance of the epidemic. Bird movement relates not just to poultry for consumption, but special categories of birds such as fighting cocks. Illegal movement of birds may in some situations be an important part of the transmission process, and may actually be increased by control measures, through forcing these movements to occur by stealth. Legal or illegal movement of birds between countries may create problems in previously free countries, but in the infected countries is unlikely to change the overall disease situation very much, unless infection is introduced to completely new areas or bird types.

Peaks in bird movement and consumption associated with festivals may increase transmission among poultry, but its most serious risk is that of increasing the exposure of the human population.

Mixing of species during rearing and production is also considered important, because it allows interchange of infection between different types of birds, which can produce outbreaks and may also increase the rate of change of the viral genome (Suarez, 2000).

Movement of people and contaminated items is also of importance, although less influential than movement of birds.

Most of the other factors listed are less important now than they were in the early stages of the epidemic.

Managing risk in bird marketing systems

In the currently infected countries, live bird markets and other traditional marketing systems appear to represent a key factor in transmission of H5N1. Such systems are so widespread in the affected countries, and so important to the people, that making fundamental changes in approach are unlikely to alter the course of the epidemic.

Therefore what is needed is to use a standard assessment method to classify these systems into risk levels, and then develop different management strategies for each risk level to reduce the scale of transmission and in conjunction with other measures, eliminate it.

This will involve such measures as assessing different types of markets, and determination of risk ratings for each market, so that different strategies can be adopted to suit the conditions of the market. This is more beneficial than steps such as closing large numbers of markets, which will drive trading underground. Assessment of markets will first involve simple check lists to evaluate catchment and distribution areas for birds passing through the market, whether birds are brought only for a day or stay in the market for extended periods, scale of market turnover, specific species being traded, whether species are mixed in close proximity, etc. Once this initial classification has been used to assess risk, high risk markets may be monitored by culturing faeces from under cages for H5N1 virus, and adjusting procedures in the light of results.

Managing risk in production systems

Field surveillance should also seek to identify types of villages etc at risk, using geographical and other information, to focus efforts in key areas to reduce transmission so that infection progressively dies out in areas as transmission falls below the threshold required for maintenance of the epidemic. This will require the use of enhanced approaches to designing and operation of surveillance activities, which should as far as possible be coordinated between the various countries for mutual benefit. FAO has already committed to providing training in such methods to countries of the region.

Vaccination is a very important method of managing risk during production, and has been beneficial in some countries. Vaccination does not have to be universal, but may in the first instance focus on high risk groups, with the aim of reducing transmission from these, so that onward flow of infection is reduced. There is continuing debate about the merits of vaccination as a control procedure, with issues raised on both sides of the debate

(Webster and Hulse 2004) but very limited field data available which would answer the various concerns. In current circumstances, reducing virus excretion is an urgent requirement, so the concern that vaccination may not protect entirely against infection or transmission is less important than reducing virus excretion overall.

Enhancing biosecurity

A range of measures can be used to enhance biosecurity, but the exact measures are likely to differ between environments. Separation of different species (ducks from chickens, for example) is one relatively simple measure which is likely to have considerable benefits, but a range of measures can be adopted in different situations.

Integrated control

There is no universal solution to control of avian influenza H5N1, which is a complex task and differs between countries, regions, and industry compartments. It is however possible to build a phased programme for each country, to identify risks, then develop a series of steps to progressively manage those risks. At present there is too much emphasis on single actions, when what is needed is an integrated approach targeted specifically to the situation in the country, based on risk management for the most significant transmission pathways.

Conclusions and Recommendations

FAO has already produced two important publications dealing with diagnosis and surveillance of avian influenza, and with control and prevention. A global research strategy to underpin future control has also been developed at a meeting at the Australian Animal Health Laboratory in October 2004, reported in ProMed.

The following comments do not repeat any of this detailed material, but identify major points for action, arising out of the epidemiological findings described earlier.

National diagnostic and surveillance procedures

Diagnostic capability in the region for avian influenza has been substantially enhanced by national efforts, with international organization coordination and support. However surveillance methods are still seriously inadequate to allow confident national and regional decisions to be made. The following actions should be undertaken:

- Shift surveillance effort from simple case-finding to risk-based identification of factors influencing maintenance of infection, and integrate surveillance procedures into control strategies, so that they are tightly linked.
- For each infected country, identify the transmission pathways which are considered to have been responsible for infection dissemination so far in the epidemic, and develop a surveillance strategy which will allow each of these pathways to be monitored, and changes in incidence to be assessed. Use a risk factor-based approach to this process.

- For example, use market surveillance and interviews to identify high-risk markets, bird types and bird sources, and build control strategies around this information.
- For each previously infected country which no longer has active infection, determine what risk-based surveillance measures would minimize the time to detection of a new incursion, within the limits of available resources.
- Apply molecular epidemiological investigation methods more comprehensively, to clarify epidemiological processes which are influencing the evolution of the epidemic.
- Assess the level of human exposure under various circumstances, in order to evaluate changing risks of emergence of a virus capable of human to human transmission. Use this information to help guide the allocation of resources to different elements of the control strategy.
- Develop rapid and standardized methods of routine analysis of surveillance data which will demonstrate important changes in the H5N1 situation, and promptly supply this information to field personnel.
- Harmonize collection and presentation of surveillance and control data across countries in the region, so that information can be interpreted in a compatible way across the region.
- An important step in building this approach is to structure the approach around the concept of *production compartments*, as adopted by World Organization for Animal Health. These are separate production systems which do not overlap epidemiologically to any substantial extent, and hence surveillance and control can be managed separately for the various compartments, thus improving efficiency of control. In this context, integrated commercial chicken production systems form one low-risk compartment, while mixed village populations of chickens and ducks represent a second high-risk compartment. FAO surveillance and control is built around four poultry compartments. Surveillance and control strategies will generally be different for each compartment.

Evaluation of merits of alternative control methods

For currently infected countries, stamping out alone is unlikely to change the course of the epidemic, and may lead to increased hiding of infection. Steps should therefore be taken to reassess control policies in order to focus on reducing the quantity of virus circulating, protecting unaffected population groups, and progressively reducing transmission rates in affected populations.

- The most valuable tools available at present are vaccination, market management, risk-based local control action, movement control at key locations, and enhanced

biosecurity through measures such as separation of reservoir and spillover species during production and marketing.

- The first step in developing strategies is to identify the production compartments and their geographical distribution, then to build control strategies suited to each, and evaluate the effect of the strategy under field conditions using surveillance to monitor success.
- Although there is continuing debate about the pros and cons of vaccination, the immediate priority is to protect the human and domestic poultry population from highly virulent strains by reducing the amount of virus circulating. The evidence supports vaccination as a tool for achieving this. Vaccination can be selective in order to block transmission in key populations and geographical locations, and does not have to be applied universally.
- In currently infected countries, markets and marketing systems are important in disseminating infection, and strategies need to be designed for each of a limited number of market types, since some markets are very high risk transmission sites, while others are of minor importance. Treating all markets as equivalent is epidemiologically unsound, and simple gathering of data about risk factors at each market will allow appropriate management strategies for each market type to be developed.
- Movement control is of value in some situations, but needs to be used with care, to avoid transferring movements “underground”. Special populations such as fighting cocks may justify different strategies from other poultry.

Implementation of nationally appropriate control programmes

In conducting this assessment of the epidemiological situation, it has been difficult to be confident in many cases of the exact nature of control procedures being applied in different countries and regions within countries, and hence it has been impossible to compare control policies between countries in any detail.

- There is a need to strengthen the degree of cooperation between countries in applying control programmes both because the action of each country in the region will impinge on its neighbours, and because overall control across Asia would be substantially enhanced if countries could make better use of the experience of others, and of the growing depth of understanding of factors influencing the epidemic, as information becomes more widely available. Producing this report has been a major challenge precisely because information was not readily available and had to be gathered from many different sources, but once the information was brought together in one place, many of the questions which had been debated extensively were answered from the evidence already gathered, but not previously collated in one place.

- It is recommended that vigorous efforts be mounted to build more coordinated approaches to this important problem, so that it is treated as an Asian regional problem rather than a country by country problem – since it is clearly pan-Asian in nature.

Research tasks

There are a number of research activities which are crucial to achieving more effective control of H5N1 infection, as laid out in the global research strategy referred to earlier. Without additional information in these various areas, control decisions will continue to be made without adequate scientific basis, which entails a high risk of failure. Avian influenza is likely to be an Asian and potentially a global problem of serious proportions for some years to come, and effective control is being seriously hampered by the lack of a number of pieces of information which would not be costly or difficult to obtain. It is noteworthy that despite the importance of this epidemic, there have been no structured epidemiological studies conducted in any of the affected countries and reported publicly. Hence inferences have to be drawn from data gathered for management of disease control activities, rather than for their epidemiological value, and all conclusions drawn must be tempered by this substantial limitation. It is to be hoped that structured studies will be conducted.

Priority tasks include the following:

- Develop and validate serological tests which can be used to monitor past exposure to avian influenza viruses in ducks, geese and pigs.
- Produce a rapid virus detection method for field use.
- Undertake additional investigations of the roles of wild birds in the overall infection process.
- Measure virus survival under Asian conditions, and assess the measures which are required to block transmission on various important types of fomites, such as bird cages.
- Undertake participatory epidemiological studies to clarify the role of marketing systems in example countries on transmission of infection, and use this to adjust marketing arrangements.
- Validate the use of currently available vaccines in ducks.
- Investigate alternative vaccine delivery systems which could reduce the cost of vaccinating important populations of birds.

Analysis of epidemiological pattern and modeling of control strategies

In preparing this report we have undertaken a range of epidemiological analyses, but concerns about data quality have prevented us from undertaking more powerful analytical approaches. There is an urgent need to enhance epidemiological data gathering activities on this disease, and to apply additional analytical approaches in order to pinpoint loopholes in control effectiveness and improve the precision of control actions, building on a stronger and more robust avian influenza surveillance strategy, which should go beyond just H5N1.

This would then allow modeling of control policies to be conducted, in order to refine future control strategies.

Required steps include:

- Bring together experts to assess in depth the role of wild birds, and how key questions on this subject could best be answered.
- Undertake investigations to determine factors responsible for re-emergence of infection in areas which have apparently been cleared of infection.
- Test and compare different disease surveillance systems, using a risk-based approach.
- Use modeling of infection dynamics in the Asian ecosystem to identify and evaluate potential enhancements to current control strategies.
- Evaluate the effectiveness of integrated avian influenza control strategies under realistic field circumstances.

International policy

Control decisions by individual countries are currently constrained by issues relating to trade and tourism, yet the H5N1 epidemic has done serious damage to both for some affected countries. There is a need to reassess international risk management strategies in relation to avian influenza, in order to achieve global protection of the human population, while avoiding scientifically unnecessary restrictions on trade in poultry products.

Description of data gathering methods used in this study

The following sources have been important to us, but we have also drawn on our own experience of the region over the last 30 years, and on discussions with people who have relevant knowledge and experience, both from inside the affected region and other parts of the world.

Consultant reports

A large number of consultants with various types of expertise have visited the area and prepared reports, which we have reviewed. Food and Agriculture Organization Technical Cooperation Programme consultant reports for Lao PDR (Dr Ray Webb), Cambodia (Drs Dorothy Geale and Thomas Rawdon), Vietnam (Drs Helen Benard, Laurie Gleeson and Ron Jackson), Indonesia (Drs Harvey Westbury, Larry Allen and Ian Douglas), Thailand (Dr David Swayne) and the People's Republic of China (Dr Les Sims) were used extensively in this report. These reports contained useful information about in-country conditions, laboratory capabilities and the poultry industries but for the most part contained relatively little specific epidemiological information which could be used in our evaluation.

A report prepared by Vétérinaires Sans Frontières (Delquigny, Edan et al. 2004) provided useful information about village and small-scale poultry enterprises in Vietnam. Special

visits were made to Indonesia, Thailand and Vietnam as part of our consultation and data-gathering activities.

National data sets

This epidemic has been very damaging to a number of the affected countries with respect to trade, tourism and food supply. This has complicated the provision of information, and created uncertainty about the interpretation of some of the information.

Wherever possible, information for country profiles and descriptions of the course of the epidemic were taken from official records and other verifiable sources of information. Data of questionable quality are identified as such throughout the report. The World Organization for Animal Health recorded information for individual countries in its standard disease report format and this appears on its website. However full accounts of all outbreaks were seldom available there and the details varied between countries from brief summaries to detailed descriptions of individual outbreaks.

In some cases, essential information was only available in the national language, and in these cases we obtained help from native speakers with veterinary skills to interpret the web sites which were the public sources of outbreak information. A full account of the outbreaks with details of investigations and conclusions was available for the Republic of Korea, but translation to English was required. English translations also had to be obtained for the excellent account of outbreaks posted on Japan's Ministry of Agriculture website at <http://www.maff.go.jp/tori/index.html>. Information for the People's Republic of China was obtained from the web site www.agri.gov.cn and essential information was translated. Full accounts of outbreaks and virus isolations were available in published scientific literature for Hong Kong SAR, and OIE updates for Hong Kong SAR gave further information.

Publicly available information for a number of other countries was incomplete and difficult to interpret. In some cases it was further complicated by the high degree of autonomy held by provincial administrations, so that only limited summary reports were provided to national veterinary authorities. Some countries did not report all outbreaks, or grouped outbreaks as single incidents covering a substantial area, and some only reported laboratory-confirmed outbreaks from which H5N1 virus was isolated. Lack of standardized forms of reporting created difficulties, and interpretation of dates of events was particularly difficult. It was seldom clear whether a date applied to date of first signs of disease, date of farmer reporting, date of investigation, or date of confirmation. In some cases, multiple incompatible dates were found in different sources for the same significant event, without clarification.

For most of the countries examined, information in the public datasets was of a preliminary notification nature and only a few countries expanded their reports with epidemiological and laboratory details. In some cases it was difficult to determine whether all available information was made available for external assessment. Few countries had provincial or national veterinary capability for detailed epidemiological investigations of outbreaks or comprehensive analysis of epidemiological data.

Country data sets therefore need to be interpreted with due consideration of their quality, the environment in which they were generated and the purposes for which they were intended. Despite their shortcomings, analysis and close examination of country datasets was a useful exercise. The analyses indicated likely biases in the data related to enterprise type and geographical locations and gave insights into the levels of under-reporting, especially when datasets from neighbouring provinces and countries were compared. Interpretation of data sets from countries in which vaccination was widely used were further complicated by unspecified scale and locations of vaccination activity, but were still useful for mapping progression of the disease and for hypothesis generation.

Examination of the data sets from the various countries showed that while spatial mapping, some spatio-temporal analyses, and descriptive epidemiological analyses could usefully be conducted, extreme caution would be required in undertaking multivariate analyses because of data biases and other limitations. There was a serious risk that the use of more complex techniques would produce spurious results, because of these problems.

A recurrent theme was outdated methods of data storage, and difficulties associated with movement of data between administrative levels. There was often reliance on paper systems with manual transfer of data to different administrative levels, although in many cases files were simply stored and were largely unread. It is unfortunate that the opportunity the epidemic presented to identify and quantify risk through detailed analyses was lost. The constraints that veterinary services operate under in some countries are numerous and occur throughout all levels of administration and the lack of personnel with advanced skills in descriptive and analytical epidemiology was evident throughout the region. There are enthusiastic and energetic people working on the problem but a continuing shortage of the skills and resources necessary to understand and manage the epidemic.

Similar problems were encountered with data sets from government agencies other than veterinary departments in most of the affected countries and point to lack of communication between government departments. In many cases, separate animal and human census data are collected by agriculture and statistics or census departments. Countries with large networks of animal health workers are uniquely placed to obtain good animal census data, but such countries commonly face problems in ensuring that data which is collected is comparable across the whole country, and is made available for interpretation and analysis at national level. Census departments face huge difficulties in countries with large rural populations and up to date information on human populations is difficult to obtain. For this report, official animal and human census data were mostly obtained from country census and agriculture department records stored in spreadsheets and year books.

Obtaining geographical information system data files for the affected countries presented special difficulties and gave an appreciation of the difficulties faced by veterinary services in almost all countries. Most countries have government agencies that use GIS

but there is often poor communication between various department users. Sometimes there appears to be an unwillingness to share but more often it seems to be simply not being aware of the involvement or capabilities of other government departments for GIS mapping and analysis. A consequence of multiple agencies working independently is inconsistencies in GIS data sets that can be further compounded by extensive boundary changes which have been made in recent years at all administrative divisions (province, district, sub-district and village). The most common inconsistencies are in spelling of place names and use of outdated files, and these and other problems combine to make mapping a frustrating exercise. Disease mapping is a rapidly advancing technical field in veterinary epidemiology and special training in up-to-date techniques would be very helpful to users in the region who currently struggle with use of software and are unable to take full advantage of the data. GIS could have application in displaying vaccination coverage and determining surveillance strategies, in addition to its established use for disease mapping. There is also a need to go beyond simple mapping, and apply spatial and spatio-temporal analysis techniques to the outbreak data to interpret rather than merely display the data.

Regional and global sources

Various organizations and people have contributed to the information available about H5N1 infection, at both the Asian regional level and at global level. There are a number of active research groups in Asia (for example, those in Hong Kong SAR, the People's Republic of China and Japan) which have been working on avian influenza for years or decades, and have collected information which is extremely valuable in understanding the current epidemic. These groups have also been active in assisting countries during the epidemic, with training and provision of specialized services such as molecular epidemiological characterization of viruses. These groups commonly collaborate with research groups in other regions, which have also contributed extensively to the understanding of the epidemic. A number of these groups have provided valuable information to us, including as yet unpublished findings, which help clarify aspects of the epidemic.

At both regional and global levels, the major international bodies WHO, FAO and OIE, have also all been very active both before and during the epidemic, in providing resources and collating information which can be used to help assess the current situation.

Large integrated poultry companies are now regional or global rather than national or local, and they represent an important component of both bird numbers and commercial bird movements around the region. They presumably have information which would be of value in assessing various alternative explanations of the evolution of the H5N1 epidemic, but due to commercial sensitivity in-house data from that source was not available. It would be helpful if data from these companies could be drawn on more effectively, while providing effective protection for privacy of company data.

Direct information gathering

Information has been gathered from as many verifiable sources as possible including the Internet. ProMed was particularly useful for early warnings of events and occasionally

presenting new points of view but the information was not always accurate and was sometimes difficult to verify. The ProMed site dedicated to avian influenza⁴ reduced the time spent searching for information and was arguably the best collection of disease records.

Massey University EpiCentre personnel involvement in Vietnam provided an opportunity to collect more detailed outbreak data in that country. Visits were also made to the Indonesian Directorate General of Livestock Services to gather outbreak data at first hand and to the Thai Department of Livestock Development to obtain up to date information.

Wild bird information

There is an extensive network of people involved in data gathering concerning wild bird populations and their movements in the Asian region. The Ramsar⁵ website provided information about migratory and resident bird populations and movements, and gave valuable insights into the wide variety of wild water bird species in the region and their seasonal abundance. The Asian Waterbird Census administered by Wetlands International (www.wetlands.org/iwc/awc/awcmain.html) provided valuable information, including distribution maps, and some national bird observer groups have more detailed information which was useful to understand smaller scale issues. A recent review (Melville 2004) was helpful in bringing issues together. An East Asian-Australasian flyway map⁶ provided more detail than those displayed throughout the country profiles. Information has also been gathered directly from people involved in wild bird work in various ways, to gain the benefit of their specialist skills and experience.

Published literature

There is a very large volume of peer-reviewed published scientific literature on avian influenza, both generally and specifically about H5N1 in Asia over the last few years. A wide range of papers has been drawn upon in preparing this evaluation, but this is an assessment rather than a scientific review. Hence key scientific papers are cited in the report, but many other papers have been used in the course of preparing the report, but are not cited directly in order to keep the focus of the document on major findings rather than turn this report into a review of the literature.

⁴ <http://www.promedmail.org/pls/askus/f?p=2400:1000:3937628031536444622>

⁵ <http://www.ramsar.org/>

⁶ <http://www.tasweb.com.au/awsg/eafw.htm>

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