Systematic review: Evaluating the evidence of an association between exposure to swine or poultry in agricultural settings and human infection with zoonotic influenza viruses.

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Table of Contents:

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Systematic review: Evaluating the evidence of an association between exposure to swine or poultry in agricultural settings and human infection with zoonotic influenza viruses.

1. BACKGROUND:

The ability of influenza A viruses to cross the species barrier from animals to humans and also cause epidemics and pandemics in humans makes them important to public health. Most experts believe that the next influenza A pandemic virus will emerge from an animal source, either through gradual adaptation to a human host or through genetic reassortment of human and animal influenza A viruses.[1-4] Consequently, public health officials are concerned about outbreaks of influenza among domestic poultry or swine where humans are exposed and infected with zoonotic strains of influenza A virus.

Influenza A viruses are known to be endemic in aquatic wild birds. As a fully adapted host, these birds do not show signs of disease from infection with most influenza A viruses.[5] This is generally not of public health concern, however if introduced into domestic animals and birds such as swine and poultry, the virus can evolve rapidly and farm workers could become exposed.[6] Influenza among both humans and swine is transmitted primarily by respiratory droplets,[7] whereas influenza among birds is spread mainly by contact with infected fecal material. [8] Influenza virus can be introduced into domestic poultry flocks or swine herds through contact with wild birds, directly, or indirectly via fecal contamination of farm water source(s) or farm equipment such as cages. Humans infected with influenza virus can also introduce influenza into swine populations. Once introduced into domestic poultry or swine populations, influenza can spread rapidly among animals, particularly closed animal populations such as in commercial swine and poultry barns.[9, 10] Recent experience in Canada with influenza A outbreaks among poultry on large commercial farms suggest that virus may also spread to adjacent flocks through airborne transmission on dust particles or feather debris.[9]

In the event of an influenza outbreak on either a swine or poultry farm in Manitoba, farm workers may be at risk for exposure to influenza viruses through their work. Contact with an infected animal or contaminated surfaces, could expose humans to zoonotic influenza virus, which could in turn, result in human infection and perhaps illness. There is also a theoretical possibility that if a person were co-infected with a zoonotic strain and a human strain of influenza A virus, the two strains could mix, possibly resulting in a new strain. Such a new strain may have the ability to spread among people.[3, 5, 11]

1.1 Manitoba's Swine and Poultry Industries:

Manitoba is one of the top 3 swine producing provinces in Canada, with approximately 8 million pigs being born in the province each year.[12] The swine census at any given time in Manitoba is approximately 2.9 million versus a human population of 1.1 million. The swine population is highly mobile, with pigs being transported from Manitoba to other locations in Canada and the U.S. for various stages of growth and development.[13] According to Statistics Canada, Manitoba's swine population is growing at twice the

national rate, and in 2002, the two-year increase in Manitoba's pig crop was 30%, the highest in the country.[14] As for poultry, Manitoba has 4.14% of the total number of poultry producers in Canada, with approximately 400 to 450 commercial poultry operations. This includes approximately 124 meat chicken operations, approximately 172 egg producing operations, approximately 65 hatching egg operations, and 66 turkey operations.[15]

Manitoba poultry and swine operations are concentrated in the southern portion of the province, as is the vast majority of the human population. Manitobans do not live in close proximity to animals as compared to people in many developing countries. Our domestic animals are not, for the most part, raised in the open where regular contact with wild birds is possible. Biosecurity measures such as ensuring wild birds cannot gain access to poultry barns and that visitors wear clean boots which are cleaned upon exiting a commercial poultry barn are recommended routine farm practices.[16, 17] These measures are recommended to prevent disease transmission between barns on a farm and between farms.[16] It is not known to what extent or how consistently these guidelines are followed in Manitoba and there is no regulatory enforcement of biosecurity standards. The domestic poultry outbreak in British Columbia in 2004 is an indicator that biosecurity measures do not guarantee zero risk of an avian influenza virus gaining access to domestic poultry flocks.[9] Therefore, if wild birds with avian influenza gain access to domestic flocks or swine, an outbreak of avian influenza could occur and could spread between barns or farms.[18] Such an outbreak could pose a risk to the health of regular farm workers and temporary workers involved in any veterinary disease eradication activity.[19, 20]

1.2 Influenza Background:

There are three types of influenza viruses, A, B, and C. Influenza A and B viruses are much more similar to each other than to influenza C viruses, and influenza B and C viruses are not as prevalent in human populations as are influenza A viruses and do not cause as much morbidity and mortality among humans as do influenza A viruses. Influenza B and C viruses are human specific viruses and are not found in avian hosts; they are generally not considered to be important animal viruses. However, in 1999, influenza B virus closely related to human strains was isolated from a harbour seal.[21] An associated retrospective study demonstrated prevalence of antibodies in 2% of seals after 1995.[21] Influenza C viruses have been isolated from pigs and dogs.[5] All three types of influenza viruses can cause epidemics in humans, with annual epidemics of influenza A occurring and only sporadic outbreaks of mild disease occurring with influenza B and C viruses. Little is known of influenza C virus behaviour in humans,[22] and it is generally not of epidemiological interest. Influenza B viruses typically affect young children. Mutational and evolutionary rates of change among influenza A viruses is far greater than that of influenza B viruses. This phenomenon and the presence of an animal reservoir of influenza A viruses are believed to be key reasons why only influenza A viruses cause occasional pandemics among humans.[2, 3, 5, 23, 24]

The International Committee on Nomenclature of Viruses (ICTV) established the ICTV Universal System of Virus Taxonomy in 1966. In 1980, the nomenclature system for influenza A subtypes was revised. This is important to note when reviewing older studies, to avoid confusion regarding subtypes described.[25] A comparison of the two naming systems is described by Kendal[25] and is outlined in Appendix 1 for reference. The revisions were prompted by the results of immunodiffusion testing, which revealed 12 hemagglutinin subtypes and 9 neuraminidase subtypes of influenza A.[25] Since that time, 4 additional hemagglutinin subtypes have been discovered, and we know that the hemagglutinin and neuraminidase proteins combine to form different subtypes of influenza.[6] The nomenclature system used today is based on the hierarchical levels of family, (in some cases also subfamily), genus, and species. Lower levels include subspecies, strain, and variant.[26] "The standard nomenclature for influenza viruses includes the influenza type, the host of origin (excluding humans), the place of isolation, the strain number, the year of isolation, and finally the influenza A subtype in parentheses (e.g., A/Duck/Vietnam/11/04 (H5N1))."[27, p.3]

Influenza viruses are negative, single-stranded RNA viruses, of the *Orthomyxoviridae* family. Influenza A and B viruses have eight segments and influenza C has seven.[24] In influenza A and B viruses, eight gene segments encode for ten proteins.[6] Of these, three are polymerase proteins (PA, PB1, and PB2), two are matrix proteins (M1 and M2), two are non-structural proteins (NS1 and NS2), one is a nucleocapsid protein (NP), and two are surface proteins, the hemagglutinin (HA or "H") and the neuraminidase (NA or "N").[6] The HA and NA surface proteins are used to identify influenza A virus subtypes.[28] Broadly described, the hemagglutinin is responsible for virus fusion and receptor binding activities and the neuraminidase is responsible for receptor destroying activities.[24] The hemagglutinin is the major surface antigen of the influenza virus [5]; neuraminidase is less abundant than hemagglutinin.[25] The hemagglutinin is highly mutable and is the major target of host immune response.[5] As such, the hemagglutinin plays a key role in certain laboratory diagnostic techniques.

Several avian and mammalian species are capable of becoming infected with influenza A viruses, e.g., whales, seals, horses, mink, wild ducks, shorebirds, gulls, passerine birds, poultry, pigs and humans.[5, 29] Camels have also been experimentally infected[30]. Since 2003, a few instances of influenza A/H5N1 infection have been identified in cats, both domestic as well as large cats in captivity, e.g., tigers and leopards, the latter in association with feeding on fresh chickens.[31] The same virus has also been detected in palm civets, a stone marten and various species of birds, including wild swans, ducks, geese, and birds of prey.[31]

Human infections of zoonotic strains of influenza A virus has been identified in association with human exposure to pigs,[32, 33] domestic poultry,[5, 34]ducks,[35, 36] and from a seal.[37] To date, only H1, H2 and H3 types of influenza A virus have caused epidemics and pandemics in humans. [6] H1(H1N1, H1N2);[33]H3 (H3N2);[38] H5 (H5N1);[39] H7 (H7N2,[40] H7N3,[19] H7N7[20]); and H9 (H9N2[41]) types have been isolated from humans in association with exposure to animals, but the evidence of direct transmission from animals to humans varies. To date, subtypes H5N1, H7N7, and H1N1 have caused

mortality in humans.[6, 8, 19, 20, 36, 40, 42-45] "Of the 16 HA subtypes, two that can evolve into highly pathogenic strains in poultry (H5 and H7) are of great concern to agricultural authorities, including the World Organization for Animal Health." [6,p. 453] The pandemic potential of these viruses make them also of concern to public health.[46, 47]

1.3 Mutation, Recombination and Reassortment:

All mammalian lineages of influenza A viruses have originated from aquatic birds.[29] Mammalian influenza viruses are under strong selection pressure to change, whereas avian influenza viruses are considered to be in evolutionary stasis. After transfer to mammals, influenza viruses undergo rapid evolution.[6] In both type A and B influenza viruses, this evolution mainly occurs in the viral surface glycoproteins in response to immunological pressure and also in each of the 8 RNA gene segments.[5] There are three key mechanisms which contribute to this evolution: mutations (antigenic drift), reassortment (antigenic shift), and RNA recombination.[6] Both the hemagglutinin and neuraminidase components of influenza A viruses are subject to antigenic drift and antigenic shift, however this occurs less often in the neuraminidase component.[25] The segmented nature of influenza viruses is important for facilitation of reassortment and recombination.[5]

Antigenic drift is a continuous process in mammalian influenza viruses, resulting in annual influenza outbreaks among humans. Antigenic drift occurs less frequently or is less well characterized in influenza viruses of swine and equine origin and even less so among those of avian origin.[6] Influenza viruses are prone to frequent mutation (antigenic drift) as they do not have 'proof reading' mechanisms and cannot repair replication errors.[6] Important mechanisms causing variation in influenza viruses are: mutations, including substitutions, deletions, and insertions.[5]

Genetic reassortment is a continuous process in nature, whereby gene segments of influenza viruses are exchanged. All subtypes of influenza A have the potential to contribute to the emergence of a pandemic strain through this process.[6, 48] Evidence gathered through molecular biologic analysis of viral nucleic acid of influenza viruses "supports the hypothesis that animals (particularly birds and pigs) may have been the source for (and possibly are a continuing reservoir of) the hemagglutinin and other genes found in viruses from previous human pandemics."[8, p. 196] Pigs have receptors for both avian and human influenza viruses and can be infected with all of the avian subtypes tested (H1-H13), and are thus believed to be an intermediate host for the reassortment of influenza viruses.[5] Pigs have also been implicated in interspecies transmission of influenza, between swine, avian and human hosts.[42]

Reassortment of viral RNA segments can occur when a host is dually infected with two different influenza viruses, e.g., human and avian. Experts believe pigs are important in the reassortment of influenza viruses due to the fact that they have receptors for both human and avian influenza viruses.[4, 6] Reassortment produces a new virus. Reassortment is important for rapid diversification of influenza A viruses and for pandemics in humans.[5] If this results in a virus with surface antigens to which the human population

does not have neutralizing antibodies, the human population will not be protected. This phenomenon is called antigenic shift and could result in an influenza virus with an ability to spread from human to human, potentially causing a pandemic, as happened in 1957 and 1968.[30,p. 140] H3N2 viruses circulating among swine in North America have undergone reassortment on several occasions.[6, 7, 10, 49, 50]

Intramolecular recombination is rare in negative stranded viruses such as influenza A viruses; however it is another mechanism for producing rapid evolutionary changes. "Recombination results in a single RNA segment containing genetic material from two different sources."[6,p.456] Recombination can occur when RNA of the influenza A virus recombines with that of the cell it infects e.g., a cellular mRNA sequence inserted into the hemagglutinin gene, or with other segments within the RNA of virus itself.[6] Recombination events are believed by experts to contribute to genetic diversity of influenza viruses, which can promote virulence shifts.[5, 6]

During investigations into the 1997-1998 Hong Kong influenza A/H5N1 outbreaks involving poultry and a small number of humans, it was found that a percentage of market birds were infected with H9N2. This virus was compared to the H5N1 virus and similarities were found, particularly the replicating genes. The internal genes PB1 and PB2 of an H9N2 virus isolated from a quail (A/quail/HongKong/G1/97) were found to be closely related to H5N1 viruses isolated in Hong Kong. This finding suggests that the 1997 H5N1 viruses were reassortants which may have obtained the internal gene segments from the quail virus. It is also possible that the reverse happened, that the quail virus obtained internal genes from the H5N1 virus, however evidence suggests the former.[51] A deletion was also found in the neuraminidase gene of the 1997 Hong Kong H5N1 virus, which has been implicated in virus adaptation to land based poultry.[27] The avian influenza H5N1 virus isolated in 2004 has undergone several reassortment events since the virus emerged in 1997. More humans have been infected since the virus re-emerged in 2003. All of this suggests this particular virus is capable of becoming more efficient at infecting humans.[52] This has led scientists to question if H9N2 itself has pandemic potential.[43]

Recombination was implicated in the virulence shift which occurred in the H7N3 avian influenza poultry outbreaks in British Columbia in 2004.[53] The significance of this finding is that it demonstrates that recombination events can occur in Canada. In a 2002 H7N3 outbreak among poultry in Chile, a 30-nucleotide insert was suspected to have been caused by recombination between the hemagglutinin and nucleoprotein genes of the low pathogenic avian influenza virus, but the exact mechanism by which this happened is not clear.[54]

1.4 Influenza in Humans:

Influenza is transmitted among people via respiratory droplets spread by coughing or sneezing.[22] These droplets are several microns in diameter.[22] Direct and indirect contact with items contaminated with these droplets, and hence the virus, e.g., unwashed hands, door handles, is another mode of influenza transmission.[22]

The expression of influenza in humans ranges from asymptomatic infection to respiratory illness to systemic disease including multi-system organ complications.[22] Death from influenza usually occurs from primary viral or secondary bacterial pneumonia.[22] The incidence of clinical features and complications of influenza varies among groups of people due to factors such as "the age of the patient, prior infection with an antigenically related strain, intrinsic properties of the virus, the presence of chronic medical conditions such as heart or lung disease, renal failure and disorders of immunity, and also pregnancy and smoking." [22, p. 221]

Influenza in humans can be categorized into two epidemiological forms: interpandemic and pandemic influenza.[2, 3, 22] Epidemics of interpandemic influenza occur every year. In the northern hemisphere, annual influenza epidemics occur from October to April, and in the southern hemisphere, from May to September. These epidemics occur due to antigenic drift in influenza A and, to a lesser extent, B viruses. The surface glycoproteins of influenza B viruses are more antigenically stable than those of influenza A. This explains why influenza B occurs mostly in the young, e.g., school-aged children.[22] Cross reacting antibodies in the population afford some protection against viruses circulating each flu season,[22] however, annual vaccination is recommended to prevent complications of influenza in those most at risk.[2] Each year, vaccine must be produced to protect people against those strains predicted to circulate and have the greatest impact on the population.[2, 3] Pandemics of influenza occur approximately every 30 years [2, 3, 55] There were three pandemics in the last century: 1918-1919, 1957-1958, and 1968-1969.[2, 3, 55] Pandemic influenza is characterized by a new or recycled subtype of influenza A virus to which the human population has little or no immunity.[2, 3, 55]

Historically, when influenza A pandemics occur among humans, the pandemic strain becomes the dominant global circulating strain in subsequent influenza seasons. It is considered unusual that both H1N1 and H3N2 types of influenza A virus have been circulating in the human population since 1977 when H1N1 re-emerged after having been replaced by H2N2 in the 1957/58 pandemic, and H2N2 subsequently being replaced by H3N2 in 1968.[23]

1.4.1 Pandemic Pathways:

To be considered a pandemic influenza A virus, the virus must be one to which the human population has little or no immunity and it must have the ability to spread in a sustained manner among humans.[2, 3, 27, 55] A pandemic threat may arise suddenly and would rapidly result in a public health emergency.[2, 3, 8]

There are two key pathways through which pandemic influenza A viruses are believed to emerge. The first pathway involves reassortment of genetic material among different sources of influenza viruses including human, avian (bird) and swine (pig) origin, yielding an entirely new virus which has the ability to infect humans. This is considered possible if someone were simultaneously infected with a human influenza A virus and an avian or swine influenza A virus.[52, 56] Scientific evidence suggests that the influenza viruses that caused the 1957 and 1968 pandemics each arose from reassortment between

human and avian influenza viruses.[6] The second pathway involves multiple mutations of an influenza virus of animal origin to gradually adapt to the human host.

Recent work to characterize the 1918 influenza virus has led researchers to believe that the "1918 virus was not a reassortant virus (like those of the 1957 and 1968 pandemics) but more likely an entirely avian-like virus that adapted to humans", supporting findings from previous studies.[57, p. 889] Around the same time as the pandemic of 1918-1919, swine influenza outbreaks had also been detected in the Midwestern United States.[4] The 1918 pandemic influenza virus, H1N1, was later found to be genetically similar to swine influenza viruses of that time[4] and pigs were implicated in the transmission of this virus to people.[58]

1.4.2 The human antibody response following infection with influenza:

As with all viruses, influenza viruses can only replicate within living cells.[59] The specific mechanisms through which influenza viruses infect and replicate within living cells, including those of humans, is thoroughly addressed elsewhere.[5, 60] The incubation of influenza in humans is approximately 2 days and virus levels peak about 3 days after the onset of symptoms.[25] "Virus can be detected in secretions shortly before the onset of illness, usually within 24 hours. The viral load rises to a peak of 10^3-10^7 $TCID_{50}$/ml (tissue culture infective dose) of nasopharyngeal wash, remains high for 24-72 hours, and falls to low values by the fifth day. Virus shedding is longer in young children."[61, p. 1733]

In most viral infections, including influenza, detectable levels of specific antibodies are present about a week after a primary infection, after the virus has been eliminated.[62] This is why serological methods are not clinically useful and can only diagnose influenza infection retrospectively. Antibodies which can be detected include internal type-specific antigens (nucleoprotein and matrix proteins), as well as the strain-specific surface antigens (hemagglutinin and neuraminidase).[25, 63] Circulating antibodies, particularly neutralizing antibodies, are widely accepted as evidence of past infection with the particular virus.[63] To confirm a suspected influenza virus infection serologically generally requires demonstrating a significant rise of specific antibody from the acute phase of illness to that of the convalescent phase, not merely demonstrating presence of antibody to the viral agent.[63] Re-infection or re-activation of a latent infection may boost levels.[63] This poses a challenge in the interpretation of serological test results.

There are situations where a rise in antibody titre cannot be detected in association with an acute viral infection.[63] For instance, individuals with compromised immune systems, those with passively transferred antibody such as newborns having received antibody placentally from their mother, infections which fail to induce a humoral antibody response, such as a superficial infection as sometimes occurs in respiratory infections, and in those situations when the acute serum sample is collected after the serum antibody levels had peaked.[63]

Secondary immune responses often result in the recall of antibody directed against the "hemagglutinin and neuraminidase of the first strain of the same type or subtype to which

an individual is exposed, such that the greatest antibody titre post infection (or post vaccination) may be to a previous strain, not the strain causing the current infection."[25 p. 347] Following vaccination with one strain of influenza, a proportion of individuals may mount an immune response to the immunizing strain and an unrelated strain to which no known exposure has occurred.[25] Most human and many chicken, pig and horse sera contain antibodies to influenza viruses. This is why the antigenic experience of the individual including immunization history is important in human studies relying on serologic methods as evidence of previous infection.

1.4.3 Laboratory diagnosis of influenza infections of humans:

A variety of laboratory methods can be used to aid in the detection of influenza virus infection among both humans and animals, and they can be grouped as viral isolation methods, detection of influenza antigen in an appropriate clinical specimen, and methods to detect a rise in antibody specific for one or more influenza virus antigens.[60] Detection of virus or viral RNA at the time of exposure or illness is considered to be definitive evidence of active infection.[64] While virus detection is superior to antibody detection for the diagnosis of influenza infections, antibody testing is a useful complementary tool for confirming the diagnosis retrospectively.[62] Testing methods are common to both detection of influenza infection among animals and humans[65], however this section will focus on detection of influenza virus infections among humans.

The appropriateness of each and interpretation of results depends upon the context within which the tests are used and consideration of related clinical and epidemiological information.[66] The sensitivity and specificity of any test in the diagnosis of influenza may depend on several factors such as the laboratory performing the test, the type of test used and the type of specimen collected.[66] "The accuracy of an influenza diagnostic test is determined by the sensitivity and specificity of the test to detect an influenza virus infection compared with a "gold" standard (usually culture) and the prevalence of influenza in the community."[67, p. 2]

Isolation of virus from clinical specimens using egg or cell cultures, followed by virus identification using immunologic or genetic techniques (or by electron microscopy) are standard methods used for diagnosis of viral infections, including influenza.[65] Virus isolation is highly sensitive and has the advantage of virus being available for further studies such as genetic and antigenic characterization and drug susceptibility testing.[65]

Only virus isolation can provide specific subtype and strain information about influenza viruses, and is considered by most influenza experts as the gold standard for confirming influenza infection.[62] For influenza virus isolation in humans, swabs of the respiratory tract are collected and cultured. Specimens for culture should optimally be obtained within the first 3 days of illness.[60] For the detection of human infections with human influenza strains, the nasopharyngeal swab is preferred respiratory specimen[66], whereas emerging evidence regarding human infections with A/H5N1 avian influenza suggests that throat swabs are more likely to yield virus than nasopharyngeal swabs.[68]

Once influenza virus has been isolated or detected, the virus must be identified. Non-immunologic methods for virus identification include: electron microscopy, direct antigen detection, DNA probes and hemagglutination. Immunologic methods include: immunofluorescence, ELISA, and neuraminidase and hemagglutination inhibition.[59]

Direct antigen detection tests are available as commercially prepared kits, and while they do not require viable virus to be present in the sample, they only identify the presence of influenza A or B virus matrix protein, not the subtype or strain of the virus.[62] It must be noted that a negative result with a direct antigen detection test does not exclude the possibility that the individual has an influenza infection, but rather means that no virus was detected.[65] Rapid tests are not recommended for the detection of human infections with avian influenza A viruses.[67]

Serological methods detect the presence of antibodies to specific types and subtypes of influenza A virus.[65] Techniques used include: hemagglutination inhibition (HI or HAI), complement fixation (CF), enzyme immunoassay (EIA or ELISA), neutralizing antibody assay, microneutralization, hemolysis in gel or single radial hemolysis (SRH), indirect immunofluorescence assays (IFA) and Western Blot (WB).[62] The World Health Organization (WHO) prefers the HI test in its global influenza surveillance program,[65] however HI testing is not recommended for detection of avian influenza among humans.[69, 70] CF and ELISA methods can only detect type-specific antibodies, whereas the HI test can detect both type and sub-type specific antibodies.[62] Serological diagnosis of influenza requires paired serum samples to be collected. The first sample, the acute sample, should be obtained within 7 days of onset of illness; and the second sample, the convalescent sample, collected between the 14th and 21st days after onset of illness.[60]

1.4.4 Interpretation of Diagnostic results:

Unless the isolate has been tested with hemagglutinin or neuraminidase specific antisera to fully characterize antigens, the results should only be reported as to type (A, B, or C). If hemagglutinin specific antisera were used to identify the virus, the corresponding neuraminidase subtype is typically inferred. If virus isolation has not been performed, caution is advised in the interpretation of serologic tests for diagnosis of influenza infection.[60] Only if an HI antibody rise to a single type or subtype is detected may the test be interpreted to identify the type or subtype of influenza A virus responsible for a recent infection, respectively.[25] Inclusion of antigens that closely resemble currently prevalent strains and antigens of past prevalent strains in HI testing is recommended for maximum diagnostic efficiency.[60] Results from the HI test and other tests which use doubling dilutions of serum showing a four-fold or greater rise in antibody, is considered significant and diagnostically positive,[60, 65] whereas a twofold difference is considered within the technical error of the technique.[63]

In retrospective studies the time lapse from the acute phase to the time of the study makes virus isolation highly unlikely, and often only convalescent serum samples can be obtained. However, the detection of influenza infection using the hemagglutination inhibition (HI) test, based on a single serum sample is generally not considered reliable

and so is not recommended.[60, 65] This is because of the possibility of secondary immune responses and immune responses to strains unrelated to the infecting strain.

Single serum samples can be used for presumptive diagnosis during outbreak investigations provided appropriate research methodology is used. When single serum samples are used in outbreak investigations, 10 or more patients in the acute stage of illness can be matched by age and other demographic factors to a cohort in the convalescent phase (experienced similar symptoms 10 or more days earlier). The sera are tested for antibody titres by HI, CF or neutralization tests, and the geometric mean titres of the 2 cohorts compared. The convalescent cohort's geometric mean titre (GMT) should be significantly higher than the acute cohort's GMT, as tested by the t-test. Statistical testing can be waived if the difference GMT is 4 fold or higher, as these results are considered significant. Optimally, these results are confirmed by virus identification and testing of paired sera.[60] Alternatively, single sera from cases can be paired with matched non-ill controls from the outbreak, or to their own sample collected prior to the outbreak. However, in this latter approach, the analysis must take into consideration the possibility that control sera from either suggested source may have antibody titres high enough to obscure the results, due to asymptomatic infection or undocumented influenza virus circulating in the population at the time the historical samples were collected.[65]

In summary, the gold standard in diagnosing human influenza infection is viral isolation or detection, using traditional viral isolation techniques, or viral detection through techniques such as RT-PCR. Viral isolation has been in use since the 1940s,[60] whereas RT-PCR has only been in practice since approximately 1986.[71] Serologic diagnosis of a recent influenza infection requires the use of paired acute and convalescent sera. As noted above, various serologic techniques can be used for this purpose, and most have been in use since the 1940s (hemagglutination inhibition, neuraminidase inhibition, neutralization, complement fixation), and others, such as single radial hemolysis have been available since the 1970s. It is important to note that the use of hemagglutination inhibition testing for detection of avian influenza virus infections of humans is considered inappropriate due to lack of sensitivity in detecting antibodies to avian influenza viruses in human serum samples. This has been in the published literature dating back to 1982.[69, 70] Western blot testing and enzyme-linked immunoassay techniques were not available until the 1980s. Single serum samples are sufficient only for detecting seroprevalence of antibodies to a particular influenza virus, suggesting that one has met with such a virus at some time in the past.

Laboratory methods used in influenza research studies must be taken into account when evaluating the strength of evidence of human infection with influenza strains of zoonotic origin associated with a particular exposure. Issues such as antigenic experience of the subject(s), possible cross reactions and secondary immune responses and the efforts taken by the researchers to control for these variables, must be considered. Thorough studies identify the immunization history of the subjects where known, and outline the methodology used to control for possible cross reactions. An overview of laboratory methods used in the studies included in this review is provided in Appendix 3, along with further detail regarding the challenges and limitations of each.

1.5 Influenza in Birds:

All hemagglutinin and neuraminidase subtypes of influenza A are found in the aquatic birds of the world,[6, 27] and these birds are considered to be the global reservoir of "all influenza viruses for avian and mammalian species." [5, p. 156; 27, 34] Wild ducks harbour influenza viruses without showing signs of disease, and shed the virus in feces. In ducks, virus can be shed in the feces for up to 30 days, however it is not entirely clear how the virus persists in duck populations from year to year.[29] The mechanism by which avian influenza virus infection causes disease is complex and involves gene products from both the virus and the host.[29] Multiple lineages of avian influenza viruses co-circulate in nature.[29] Transmission of avian influenza viruses between different birds are not fully understood.[29]

It has been estimated that "up to 30% of (wild) birds hatched each year shed influenza viruses in their feces."[29, p. 126] Surveillance studies of influenza viruses in wild waterfowl have yielded prevalence estimates of 15% for ducks and geese and approximately 2% for all other species, of influenza viruses of low pathogenicity to poultry.[72]

Compared to mammalian influenza viruses, avian influenza viruses have lower nucleotide variation rates.[29] The antigenicity of avian influenza viruses is conserved in nature. Ito and Kawaoka [29] outline possible explanations for this:

(i) "the antibody response of ducks to avian influenza viruses is weak and short lived. Ducks appear to be readily reinfected with the same virus within two months of the initial infection;

(ii) even if ducks produce neutralizing antibody, the serum antibodies may not be effective at inhibiting viral replication in the intestinal tract, the site in ducks where these viruses preferentially replicate;[29]

(iii) every year, large numbers of susceptible ducks are added to the population; over 30% of the annual duck population consists of juvenile birds that are hatched that year." [29,p. 122]

Influenza is not considered enzootic in turkeys or chickens. Outbreaks of influenza in domestic poultry sometimes result from the introduction of influenza virus from wild birds into domestic flocks.[72] Once introduced into domestic flocks, infection spreads rapidly within the flock by infected fecal material.[9, 72] "High concentrations of virus are present in the respiratory and digestive tracts of infected birds. Fecal material from infected birds may contain up to 16×10^6 virions/gm of feces and one gram contains enough virus to infect one million birds." [9] Secondary spread from farm to farm can occur by movement of birds, people, contaminated equipment[72] and aerosol spread has been identified as a possible mechanism of transmission when barns are close together.[9] Influenza viruses can persist in the environment, for example in water and ice, remaining infectious in water for up to 200 days, depending on temperature and viral factors.[5]

Veterinary scientists classify avian influenza viruses based on their pathogenicity in birds. This is determined by infecting chicks and evaluating the clinical outcome. Using the intravenous pathogenicity index (IVPI) as a guide, avian influenza viruses are deemed

to be either low pathogenic (LPAI) or high pathogenic (HPAI). According to the Canadian Food Inspection Agency, HPAI viruses "have an intravenous pathogenicity index (IVPI) in 6 week-old chickens greater than 1.2 or cause at least 75% mortality in eight 4- to 8-week-old chickens infected intravenously."[73] HPAI tends to be a systemic infection whereas LPAI tends to remain localized in the respiratory and/or gastrointestinal tracts.[73] "The HPAI virus is more likely to be present within or on the surface of eggs when hens are infected." [73] Influenza infection of domestic poultry can result in clinical outcomes ranging from mild symptoms such as decreased egg production, diarrhea and/or respiratory illness, as is typical of LPAI, to severe disease involving multiple organ systems and death as is more likely with HPAI.[73]

"The number of subtypes that have crossed the species barrier and established stable lineages in mammals is limited." [27, p.3] In general, "avian influenza viruses do not establish themselves in the human population and, vice versa, human viruses do not establish themselves in bird populations."[30, p.137] Therefore, the species barrier between humans and birds is considered tight. In some instances, avian influenza viruses can pass the species barrier to mammals and can do so without reassortment. "The molecular, biological or ecological factors determining the apparent subtype specific ability of viruses to cross species barriers and spread among a range of hosts remain largely unresolved." [27,p.3] There have been documented cases where reassortant viruses involving avian influenza virus genes have been isolated from both pigs and humans and also situations where wholly avian influenza viruses have been isolated from these mammalian hosts.[29]

Among commercial poultry flocks, turkeys warrant special mention. The physiology of turkeys is known to "tolerate a broad host range of productive influenza infections".[74, p. 496] From an animal health perspective, virologists have proposed that infection with swine strains of H1N1 influenza is possibly a greater threat than infection with influenza viruses of avian origin.[74] It is not known exactly when influenza viruses were first transmitted from swine to turkeys, however when Wright and colleagues studied H1N1 influenza viruses from turkeys in the United States in the early 1990s, they found that of the 11 viruses isolated, 8 of them (73%) contained swine influenza virus genes. Another of the viruses was a reassortant virus.[74] In contrast to domestic chickens, domestic turkey flocks are often let outdoors in North America during the months when weather is favourable.[74, 75] This gives the birds opportunity to come into direct or indirect contact with wild waterfowl. In Manitoba, mixed farms including turkeys are common. Up to half of turkey farms in Manitoba also have chicken flocks (meat or egg), and about 20% of egg producing chicken farms also have turkeys. Approximately 25% of turkey farms also have pigs.[75] Turkeys are therefore a domestic flock of concern with respect to opportunity for influenza viruses to co-mingle.

A 2005 Canadian wild bird survey involving a sample of Manitoba birds identified that 92 of 548 samples tested positive for influenza A virus. Of these 92 samples, 5 tested were positive for avian influenza A/H5, and further testing revealed that these 5 were positive for the low pathogenic North American strain of H5N1 avian influenza virus.[16, 76] This 2005 North American H5N1 strain was different from the H5N1 virus circulating in Asia and Europe at that time.[42, 58]

1.6 Influenza in Swine

Influenza disease in swine is a respiratory tract infection and is the most prevalent cause of respiratory disease in swine.[58] In swine, receptor sites for influenza viruses are located in the trachea and viral replication takes place in the respiratory tract.[10] Pigs are considered permissive to both avian and human influenza viruses[77] and have been implicated in transmission of influenza to turkeys[78] and humans.[79, 80]

Among swine, transmission of influenza is primarily through the nasopharyngeal route, with the virus being shed in nasal secretions and spread by droplets or aerosols.[58] Factors contributing to spread of influenza among swine include close contact, stress, and also meteorological and environmental factors.[58] Swine influenza is a herd disease and once the herd is infected, influenza is typically maintained with annual episodes of acute disease. Influenza disease in swine is generally characterized by high morbidity (approaching 100%) and low mortality (<1%) rates.[10, p. 3204] The severity of disease in pigs, however, depends on a variety of factors, e.g., host age, virus strain, and secondary infections.[10] The only measure to completely eliminate the disease from a herd is depopulation.[58]

H1N1, H3N2 and H1N2 are the three main types of influenza virus which circulate among different pig populations of the world. Of currently circulating swine variants, H1N1 is most commonly isolated[7] and H1N1 and H3N2 influenza viruses are considered endemic in pig populations worldwide.[58] Historically, there have been two main lineages of H1N1 viruses in swine: classical swine H1N1 viruses in North America and the European H1N1 swine viruses.[30,49]

In North America:
In North America, classical swine H1N1 viruses were the exclusive cause of swine influenza from 1930 to 1998.[81] Seroprevalence studies of influenza H1N1 infection among swine populations in the United States have found various seroprevalence rates of different influenza viruses among North American swine populations, and over time have demonstrated the evolution of these viruses in this swine population.

Studies conducted in North America during the late 1970s and early 1980s indicated that approximately 25% of fattening pigs had evidence of infection as did up to 45% of breeding pigs, thought to be due to their longer lifespan.[58] A different publication states that in 1976-77, seropositivity to classical swine H1N1 was in the range of 20-47%, and in 1988-89, was 51%.[82] During the time period between 1991 and 1998, H1N1 viruses drifted and novel swine influenza viruses emerged in the North America.[81] Serologic testing of 2,375 pigs from September 1997 to August 1998 found that 27.7% had antibodies against classical H1 swine influenza viruses, 7.6% had antibodies to H1 avian influenza viruses, and 8.0% to human H3 influenza viruses. Seroprevalence rates among pigs of antibodies to avian and human influenza viruses were substantially higher than that of previous studies.[82]

Serologic studies of H3N2 influenza A infection among swine herds in the U.S. conducted in the late 1970s to late 1980s, identified seroprevalence rates of less than 1.5%.[58] Later studies of H3N2 seroprevalence, conducted in 1997-98, indicated an increase in the prevalence rate to 8% and subsequently in a large 1998 seroprevalence study, found to be 20.5% of a triple reassortant H3N2 virus (Sw/TX/98) containing human, swine and avian genes; 8.3% to a double reassortant (Sw/NC/98) containing human and swine genes and 28.3% to H1N1 (Sw/IA/90).[7] The researchers concluded that H3N2 triple reassortant viruses containing avian genes had spread throughout a large proportion of swine herds in the U.S, and that such viruses with avian genes have a selective advantage in swine. Furthermore, the triple-reassortant viruses are believed to have undergone reassortment with human H3N2 viruses at least three times.[7] In North America, both double and triple reassortant H3N2 swine viruses have been detected circulating among the swine populations, [7, 10, 49, 50] with triple reassortant H3N2 virus being recently identified in Manitoba.[38] It is generally accepted that since 1998, H3N2 viruses among pigs are triple-reassortant viruses, containing genes from influenza viruses of swine, avian, and human origin.[83] Wholly human H3N2 viruses have been isolated from pigs, most recently in a single baby pig in Ontario in 1997,[50] and earlier in Colorado, in 1977.[83]

In November 1999, H1N2 was isolated from swine on a farm in Indiana. Further sequencing and phylogenetic analyses revealed that the isolated virus was a reassortant virus, involving genes from the triple-reassortant H3N2 viruses and classical H1N1 swine influenza viruses. Since that time, these "second generation reassortant" H1N2 swine influenza viruses have been isolated from pigs in at least 6 states.[81]

In 1999, as part of the discovery of emerging novel swine influenza viruses in North America, an H4N6 influenza virus was isolated in pigs with pneumonia in Ontario, Canada. This virus was found to be a wholly avian virus (not mutated), of North American lineage.[34] This was described as the first report of H4 avian influenza virus being transmitted to pigs under natural conditions.[34] The suspected source of avian influenza virus in the Ontario outbreak was a nearby lake with waterfowl, from which water for the barn was sometimes drawn.[34] These viruses have not spread beyond the Ontario farm of origin.[81]

A study of influenza virus among swine in the U.S. published in the spring of 2006 reported the emergence of a novel swine influenza virus subtype, H3N1.[56] Characterization of this novel virus suggest that the "hemagglutinin gene may have been acquired from an H3N2 turkey isolate, the neuraminidase gene from a human H1N1 isolate, and the remaining genes from currently circulating swine influenza viruses." [56, p. 787] It is not known whether or to what extent this novel subtype will spread among swine populations in North America.[56]

Beyond North America:

In a serosurvey of pigs in southeastern China to detect evidence of influenza virus infections, Ninomiya et al [84] found neutralizing antibodies to H1, H3, H4, and H5 viruses in swine serum samples collected in 1977-82 and 1998. Antibodies were also found to

H9 virus, but only in the sera collected in 1998, not in the earlier samples.[84] In 1994, a novel H1N7 influenza virus was isolated from two pigs on a farm in England. Eight pigs were subsequently experimentally infected with the H1N7 viruses, however in spite of the fact that they excreted virus, only 1 of 8 seroconverted.[85]

Between 1998 and 2000, H9N2 was found to be co-circulating with human H3N2 influenza virus in pigs in China.[77] It is believed that H9N2 was transmitted from poultry to pigs, but the ability of this virus to spread among pigs is not known.[58] These pigs were also found to be infected with human H3N2 virus. This raised concern that the two viruses could mix in pigs. This was the first time influenza virus had been detected in pigs that was not H1 or H3.

Pigs have been recently found to be capable of being infected with the Asian H5N1 avian influenza viruses, but under experimental conditions these viruses were not readily transmitted between the pigs.[86] Nevertheless, this is concerning due to the possibility of reassortment of viruses in co-infected pigs.[27]

In contrast to the species barrier between birds and humans, the species barriers between pigs and birds or humans are much lower. Pigs are readily infected with avian influenza viruses whereas humans are not.[34] Most avian influenza viruses can replicate in swine.[34] Pigs have been implicated as the 'mixing vessels' for reassortment of influenza A viruses, because they can be infected with influenza viruses of both avian and human origin.[5, 10, 30, 33, 34, 56, 58, 77, 84, 87] H1N1 viruses have been transmitted from humans to pigs[58] as have H3N2 viruses.[58, 88] "There is good evidence that pigs are more frequently involved in interspecies transmission of influenza A viruses than are other animals."[49, p. 48]

1.7 Human infection with zoonotic influenza viruses associated with exposure to poultry:

The first reported instance of an avian influenza virus causing clinical respiratory disease in humans associated with exposure to poultry, occurred in Hong Kong in 1997. In total, 18 people were identified as cases of H5N1 avian influenza virus and six of them died. Further investigation into the source of the virus resulted in poultry markets being identified as the source. During the investigation of the second wave of infection, it was suggested that the virus was a result of direct avian to human transmission and that the virus had emerged from the wild bird markets.[1, 43] Prior to this event, H5N1 avian influenza virus had only been detected in avian species.[1] In 1999, two human cases of H9N2 avian influenza were detected in Hong Kong, however the actual source of these infections has not been determined.[8, 43]

H7 avian influenza viruses have been noted to cause mostly mild illness in humans, with predominantly conjunctivitis and / or mild influenza like symptoms. H7N7 avian influenza caused human illness in association with exposure to infected poultry in large poultry outbreak response in 2003 in the Netherlands.[20] In that outbreak, exposure to highly pathogenic avian A/H7N7 infected poultry caused 82 primary human cases including one death.[20]

H7N3 has been identified in a man with conjunctivitis, in association with a poultry outbreak in England in 2006. This investigation is ongoing at the time of writing so additional cases may be still found.[89] In the spring of 2004, two poultry workers in the province of B.C. were confirmed to have been infected with avian influenza H7N3 during culling operations in response to poultry outbreaks in the lower mainland area of that province.[19] These individuals were mildly ill and there were others also mildly ill who were suspected but not confirmed as cases (n=55). At the same time as the avian outbreak was ongoing in B.C., human influenza A outbreaks were detected, increasing concerns about the possibility of mixture of avian and human influenza viruses.[19]

Although our documented experience with A/H7 influenza virus infections in humans have thus far only been found to cause mild illness among humans, experts believe that the threat they pose should not be underestimated. In the B.C. outbreak, investigators concluded that the pandemic potential of H7 viruses should not be minimized due to their lower virulence than H5, as this may provide them with greater opportunity to spread and reassort, mutating into a more virulent virus.[19] Investigators in the outbreak in the Netherlands also concluded that "because H7N7 viruses have caused disease in mammals, including horses, seals, and humans, on several occasions in the past, they may be unusual in their zoonotic potential and, thus, form a pandemic threat to humans."[90, p.1356]

The Asian A/H5N1 virus re-emerged in late 2003, and outbreaks in poultry with sporadic cases in humans are ongoing in a small but increasing number of countries.[91] The H5N1 virus has been identified in birds that migrate to other parts of the world. From December 2003 to June 1, 2006, there were over 200 human cases of avian influenza resulting in just over 100 deaths in a small but growing number of countries combined. Many but not all of these cases have been linked to contact with infected chickens.[91] Although there have been a small number of instances where human to human transmission was hypothesized to have occurred with this virus, the evidence collected to date has been inconclusive.[91] It is important to note that some of the same mutations that were traced in the evolution of the 1918 virus have also recently been detected in the H5N1 avian influenza virus that has caused human illness and death in Southeast Asia.[57]

In a 1991 publication, Beare and Webster describe experiments involving human inoculation with avian influenza virus subtypes H4N8, H6N1 and H10N7. Eleven out of 40 volunteer human subjects shed virus and experienced mild clinical symptoms, however they did not produce a detectable antibody response. The authors concluded that this was due to limited virus reproduction which was not sufficient to stimulate a primary antibody response in the subjects.[92]

1.8 Human infection with zoonotic influenza viruses associated with exposure to swine:

The question of swine influenza virus transmission from swine to humans received attention in the 1960s due to a small number of reports of serological diagnosis of swine influenza in humans, however results were not conclusive due to cross-reacting antibody and other confounding factors, and no virus was isolated.[93] The first instance of swine virus isolation from humans occurred in the United States in 1974.[93] Since that time, the transmission of influenza viruses from swine to humans has been well documented.[4, 34, 42, 58, 94]

From 1976 to 2006, single cases of human infection with swine influenza A/H1N1 in association with exposure to swine have sporadically been reported in the literature. Most of these cases were found as a result of people seeking medical treatment for respiratory illnesses of varying severity.[32, 33, 42, 58, 79, 80, 87, 88, 95-99] A small number of these cases were fatal.[8, 58, 79, 97, 99] There have been other documented instances of human infection with swine influenza viruses, however data regarding exposure to swine were lacking,[87, 100-102] or secondary transmission from human to human was hypothesized.[103, 104]

Influenza H1N1 [42, 58, 79, 80, 88, 95-98] and H1N2 [33] have been transmitted from pigs to humans; and evidence exists which suggests that H3N2 viruses have also been transmitted from pigs to humans, however it is not conclusive. Isolation of a triple reassortant H3N2 (human/classical swine/avian) has been documented in one swine farmer, however a systemic antibody response was not detected.[38] In another instance, H3N2 viruses isolated from children in The Netherlands were found to be human-avian reassortants, which were currently also circulating in swine, however no clear exposure to swine was documented.[87] In a third study, cross-reactivity between swine H3N2 and human H3N2 was shown by the detection of elevated titres in humans against swine H3N2 virus in association with elevated titres against human H3N2.[33] The researchers were not surprised by this finding, as the hemagglutinin gene of the swine H3N2 virus is of human origin and cross-reactivity is found between swine and human strains of H3N2.[33]

Serological surveys of swine workers have also produced evidence of human infection with swine strains of influenza virus.[42, 95, 96] It has been estimated that "up to 10% of persons with occupational exposure to pigs develop antibodies to swine influenza virus."[105, p.440] In a serosurvey of swine farm residents and employees in rural Wisconsin to explore human infection with H1 swine flu, results indicated that "swine virus seropositivity was significantly ($p<0.05$) associated with being a farm owner or a farm family member, living on a farm, or entering the swine barn $\geq$ 4 days per week."[42, p.814] In 2006, a controlled, cross-sectional seroprevalence study conducted in Iowa, detected human seropositivity to swine H1N2 among swine farm workers that had not been previously detected.[33] These researchers concluded that occupational exposure to pigs greatly increases workers' risk of swine influenza virus infection, and that swine influenza virus infections frequently occur among swine workers.[33]

As the above examples demonstrate, avian influenza virus detection among swine and swine influenza itself is of concern to public health in addition to that of veterinary health.[7, 10, 34]

1.9 Gaps in Knowledge:

Human infection with zoonotic influenza viruses and, rarely, serious illness and death, associated with direct contact with infected animals have been documented,[8, 19, 20, 42, 58, 68] and therefore, it is possible for this to occur again. It is not known what the probability is of a new influenza strain emerging from infected animals and infecting humans, nor is it known what the probability is of such a virus developing the ability to spread among people in a limited or widespread fashion. Despite a growing number of human cases of H5N1 avian influenza virus and documented instances of possible limited human to human transmission, knowledge of the epidemiology and natural history of related disease in humans is incomplete. The frequency of human infection has not been determined.[68] So while it is generally accepted that humans may become infected with zoonotic influenza viruses associated with exposures to animals during influenza outbreaks among animals, an evaluation of the likelihood of human infection has not been well articulated in the literature.

An evidence-based evaluation of the probability of human infection with zoonotic influenza viruses would help to inform public health policy regarding investment of resources in developing surveillance measures for zoonotic influenza infections of humans and in preparing for a response to human health issues in relation to an influenza outbreak among poultry and / or swine. The first step in developing such a public health risk assessment is to identify evidence of an association between exposure to domestic swine or poultry in agricultural settings and human infection with influenza of zoonotic origin, and if found, to evaluate the strength of such an association. It would be important to note and describe any factors which may modify the association. The next step would be to determine the probability of human infection with a zoonotic strain of influenza in settings where humans may have direct contact with infected animals and their immediate environment, such as in agricultural settings.

1.10 Conclusion:

As was noted during the B.C. outbreak of H7N3 avian influenza in 2004, human infections with avian influenza can happen in Canada, despite personal protective measures and antiviral prophylaxis used during outbreak response operations involving culling of poultry.[9, 73, 106] The B.C. experience demonstrated that recombination events can occur in commercial poultry operations in Canada. The experiences in North America with reassortant viruses being transmitted from swine to humans[7, 19, 34, 42] suggest this can occur again. Therefore, it is possible for novel influenza strains to emerge in Canada including Manitoba, as a result of a recombination or reassortment event.[2, 3] It is plausible that humans could become infected with resulting novel influenza virus strains, however it is not known how likely this is to occur and if it did occur, what the potential impact would be for human health.

One of the key challenges for the 21st century is "to accumulate the necessary epidemiological data on animal influenza viruses", to inform the further development of international, national and local influenza surveillance systems." [1, p.S16] Developing an integrated human and animal network of influenza surveillance which "considers sources of new emergent influenza virus strains, intermediate hosts, inter-species transmission, and mild and severe human cases, is important to public health." [107, p. 116] Establishing such surveillance systems will contribute to our understanding of influenza viruses and assist us in developing strategies for future pandemics of influenza A in humans. "Influenza will continue to be a re-emerging zoonotic infectious disease, requiring attention from researchers in veterinary and human infectious diseases." [6, p. 461]

In order to determine the value of investing in a comprehensive, integrated surveillance system for any zoonotic disease, including influenza, a public health risk assessment must be completed. Such a risk assessment would assess the likelihood of human infection with the infectious agent of interest occurring and, subsequently, to assess the possible consequences of such an infection, e.g., the risks to human health.

As a first step of contributing to the overall public health literature, the goal of this research is to examine the first part of the equation, that is, to identify and evaluate documented evidence of an association between exposure to swine or poultry in agricultural settings and human infection with zoonotic influenza viruses. A systematic review of the literature will be performed to identify and evaluate evidence of human infection with zoonotic influenza viruses associated with exposure to poultry and swine in agricultural settings. If sufficient evidence exists and if appropriate, the probability of human infection will be quantified using meta-analysis methodology.

2. OBJECTIVES:

2.1. Summary of purpose of research:

- The purpose of this review is to identify and evaluate available evidence from the literature to determine if a relationship exists between exposure to domestic swine or poultry in agricultural settings and human infection with influenza viruses of zoonotic origin; and if so, to describe the relationship; and, to assess the likelihood of Manitoba poultry and swine farmers becoming infected with zoonotic influenza viruses through their work.[6, 8, 48, 73, 106] (It is beyond the scope of this review to address the impact of human infection with zoonotic influenza viruses, e.g., clinical outcomes.)

2.2. Objectives:

- To identify and evaluate published evidence regarding the association between exposure to domestic swine or poultry in agricultural settings and human infection with influenza viruses of zoonotic origin;
- If an association is found between exposure and infection:
    - describe the strength of that association;
    - identify and describe any factors which may modify (increase or decrease) the odds of becoming infected;
    - identify and describe the prevalence of zoonotic influenza infections among people exposed to swine or poultry in agricultural settings;
    - quantify the probability of human infection with zoonotic influenza virus associated with exposure to domestic poultry or swine in agricultural settings, if sufficient data are found and if appropriate. The aim of this step is to produce confidence intervals describing the probability of human infection among those exposed to the animals in question; and,
- To describe the population at potential risk in Manitoba.

2.3. Research Question:

Is there evidence of an association between exposure to domestic swine or poultry in an agricultural setting and human infection with an influenza virus of zoonotic origin? If so, what is the relationship, and given an exposure, what are the factors that modify (increase or decrease) the odds of becoming infected?

2.4. Definitions:

Agricultural setting
Commercial or small flock / herd. Small includes backyard flocks/herds. Reference numbers of animals for commercial and "small" operations will be defined by typical agriculture industry averages in North America or Manitoba. Poultry market settings are not of interest in this review as they are not relevant to Manitoba.

Animals of interest
For the purposes of this review, swine and poultry (chickens and turkeys) will be included. Horses, ducks, geese and other birds will be excluded.

Confounding variable
According to Last's Dictionary of Epidemiology[108], a confounding variable, or confounder, is: "A variable that can cause or prevent the outcome of interest, is not an intermediate variable, and is associated with the factor under investigation. Unless it is possible to adjust for confounding variables, their effects cannot be distinguished from those of factor(s) being studied. Bias can occur when adjustment is made for any factor that is caused in part by the exposure and is also correlated with the outcome."[108, p. 38]

As noted, confounding can contribute to bias. Bias, then, is defined as: "Deviation of results or inferences from the truth, or processes leading to such deviation. Any trend in the collection, analysis, interpretation, publication, or review of data that can lead to conclusions that are systematically different from the truth."[108, p.14]

Confounding bias:
"Distortion of the estimated effect of an exposure on an outcome, caused by the presence of an extraneous factor associated both with the exposure and the outcome, i.e., confounding caused by a variable that is a risk factor for the outcome among non-exposed persons, and is associated with the exposure of interest, but is not an intermediate step in the causal pathway between exposure and outcome."[108, p. 38.]

Exposure
Direct contact with a live, sick or dead animal of interest or their immediate environment. Environmental sources of influenza virus include contact with those surfaces which may have been contaminated by an infected animal shedding virus or their waste products or carcass which may contain virus. As this review is limited to transmission from animals to humans, studies relating to possible instances of human to human transmission of influenza viruses of zoonotic origin, or where evidence of infection is not associated with documented exposure to an animal of interest or their immediate environment, will be excluded, unless the subjects are part of a comparison group.

Exposure to animals of interest in the following specific agricultural settings will be included in the review:

- Commercial swine operations
- Small or backyard swine herds
- Commercial poultry operations
- Small or backyard poultry flocks

Human infection
Defined as laboratory evidence of infection (either recent or past) e.g., presence of antibodies to reference strains of influenza virus in human serum, or isolation of an influenza strain of zoonotic origin from a human specimen, e.g., conjunctival, nasal,

throat or nasopharyngeal swab. Different lab methods and criteria for assessing evidence of infection are expected to be found in studies of different subtypes of influenza and in older studies as compared to recent studies due to advances in laboratory testing methods. Laboratory diagnosis of influenza will be addressed in the review, to inform the critical appraisal of relevant studies.

Incidence:
"The rate of development of a disease in a group over a certain time period; this period of time is included in the denominator."[109, p.94]

Odds:
"The ratio of probability of occurrence of an event to that of nonoccurrence, or the ratio of the probability that something is so to the probability that it is not so"[108, p. 128]

Odds Ratio[108]:
"The *exposure-odds ratio* for a set of case control data is the ratio of the odds in favour of exposure among the cases (a/b) to the odds in favour of exposure among noncases (c/d). This reduces to ad/bc. The *disease-odds ratio* for a cohort or cross-sectional study is the ratio of the odds in favour of disease among the exposed (a/c) to the odds in favour of disease among the unexposed (b/d). This reduces to ad/bc and hence is equal to the exposure-odds ratio for the cohort or cross-section. The *prevalence-odds ratio* refers to an odds ratio derived cross-sectionally, as for example, an odds ratio derived from studies of prevalent (rather than incident) cases. The *risk-odds ratio* is the ratio for the odds in favour of getting the disease, if exposed, to the odds in favour of getting disease if not exposed."[p.128]

	Infected	Not infected	
Exposed	A	B	A+B
Not exposed	C	D	C+D
	A+C	B+D	Total

Disease-odds ratio, where the outcome is infection as opposed to disease:
Odds for being infected if exposed (cases)

$$= \frac{\text{A: Number of exposed individuals who were infected}}{\text{C: Number of unexposed individuals who were infected}}$$

Odds for being infected if not exposed (controls)

$$= \frac{\text{B: Number of those exposed who were not infected}}{\text{D: Number of those unexposed who were not infected}}$$

$$\text{OR} = \frac{\text{odds for cases (infected individuals)}}{\text{Odds for controls (not infected)}} = \frac{(A/C)}{(B/D)} = \frac{AD}{BC}$$

Population at potential risk:
The population potentially at risk and of interest in this study is Manitoba swine and poultry workers and closely related groups and occupational groups. Farm owners,

operators, and workers, including poultry catchers, are included in the term "farm workers" for the purposes of this review. Closely related groups may include any group of people who may have exposure to the animals of interest and their immediate environment in an agricultural setting, such as farm family members or household members in a backyard flock setting. Closely related occupational groups may include veterinarians, slaughterhouse / abattoir workers. This list of closely related occupational groups will be informed by the review and amended as required.

The Manitoba population potentially at risk will be described based on available statistics on Manitoba swine and poultry workers. If information is available, a description of closely related groups and occupational groups will also be included.

Prevalence:
"A measure of the existing number of cases of disease in a population at a point in time or over a specified period of time."[109, p. 121]

Probability:
"1. The limit of the relative frequency of an event in a sequence of N random trials as N approaches infinity, i.e., the limit of:

$$\frac{\text{Number of occurrences of the event}}{N}$$

2. A measure, ranging from zero to 1, of the degree of belief in a hypothesis or statement."[108, p. 143]

Record:
In the context of the search process for this review, a record of a publication is considered to be the reference information retrieved upon application of the search strategy to a literature search engine. Records generally consist of a title, author, and year of publication, journal title, volume, issue number and page numbers. Records reviewed to assess study eligibility were abstracts if available and titles if abstracts were not available.

Relative Risk: $\frac{\text{Risk of infection among those exposed}}{\text{Risk of infection among those not exposed}}$[110]

Risk:
"A statement of the probability or chance that an individual will develop a disease over a specified period, conditioned on that individual's not dying from any other disease during the period... Statements of risk also require a specific reference period.... Risk can be estimated as the cumulative incidence of a particular disease."[109, p. 97]

Zoonotic diseases:
Those which are transmitted from animals to humans. Therefore, exposure to an agent known to cause a zoonotic disease, e.g., bacterium, virus, is a necessary cause of human infection with a zoonotic agent and disease may follow.[111]

Zoonotic origin

Transmission from animal to human; influenza virus of any evolutionary origin, e.g., wholly avian virus, recombinant swine/human; recombinant avian/swine, etc.[111]

3. METHODOLOGY:

3.1. Overview of the Review:

As this review was completed as a Master's thesis, the vast majority of steps were completed by a single reviewer, with direction and input provided by a committee comprised of experts in epidemiology, public health and veterinary medicine.

The following process for meeting the objectives of this review was examined and accepted by the review committee:

3.1.1 Using published guidelines and tools for best practices in systematic review of the literature, a systematic review of the literature will be constructed and implemented, to accomplish the following aims:

- Identify, evaluate and describe published evidence of an association between an exposure to animals of interest in an agricultural setting and human infection with a zoonotic strain of influenza virus;
- Describe the prevalence of zoonotic influenza virus infections among people exposed;
- Identify, evaluate and describe those factors which may modify (increase or decrease) the odds of becoming infected with a zoonotic influenza virus.

3.1.2 If sufficient data are found as a result of the systematic review, and if appropriate for the data collected, a meta-analysis will be performed to quantify the probability of human infection with zoonotic influenza virus associated with exposure to domestic poultry and swine in agricultural settings.

- The main objective of the meta-analysis is to produce confidence intervals which describe the probability of transmission of influenza from animals (e.g., pigs and birds) to humans among those exposed to the animals of interest.

- Results from the meta-analysis will be used to develop and test a theoretical probability model of human infection with zoonotic influenza virus in an agricultural setting.

3.1.3 Key Facts and Assumptions supporting the research question:

1. In order to become infected with an influenza virus of any type, including zoonotic strains, a person must be exposed to the virus. Therefore, it is assumed that exposure to swine influenza virus (SIV) or avian influenza virus (AIV) is a necessary cause of human infection with such a virus.
2. It is assumed that potential sources of avian and swine strains of influenza virus are the animals themselves, e.g., birds and pigs respectively, or their immediate environment, subject to virus survival in the environment.[112]

3. It is assumed that in North America, exposure to swine and /or poultry is relatively rare among the general population. However, exposure to the animals of interest would be common among the population of interest in this review. Nonetheless, exposure to a source of the virus (AIV or SIV), e.g., exposure to an infected animal or its immediate environment which has been contaminated with virus would be considered rare among the small proportion of the general population who works directly with swine and / or poultry.
4. It is assumed that rates of human infection with avian and swine influenza viruses do not exist for the general population and that human infections with zoonotic influenza viruses are rare occurrences in the general (non agricultural) population.
5. Given that these infections are assumed to be rare, it is also assumed that the expected proportion of these infections among the general unexposed population is extremely low, if not negligible. We can expect this to be true because:
   - If such infections were not rare, we would pick this up in routine global influenza surveillance;
   - If a novel strain of influenza were to be spread from person to person, we would have a pandemic, and this would be detected by global influenza surveillance.
6. In case reports, "no comparisons are made between study groups (e.g., exposed versus non-exposed) and consequently, no conclusions about associations between exposures and outcomes can be made."[113, p. 140] Therefore, case reports will not be accepted as studies capable of answering the research question.
7. Sampling in cohort designs is based on exposure; in case-control studies on outcome; and in cross-sectional studies on neither exposure nor outcome. It is known that cross-sectional studies are not suited to ascertainment of exposure-disease temporality; case-control studies are not useful for rare exposures and cohort studies are difficult to use with rare diseases. An exposure-based cohort would be appropriate in such instances where it may be desirable to quantify levels of exposure and determine incidence rates of a specific outcome.[109]
8. Prevalence rates are limited to describing the magnitude of the problem and one cannot infer temporality of exposure and outcome.[110]
9. It is generally accepted that for rare diseases, the relative risk of getting the disease (or infection) approximates the odds of getting the disease (or infection) compared to the odds of not getting the disease (or infection).[109, 110]

3.1.4. Implications for Methodology:

- Assessing the odds of becoming infected if exposed requires evaluation of infection in an exposed group, for which the denominator is known. Cross-sectional and single-cohort studies will be included to achieve this aim.

- The odds of becoming infected among exposed will be compared to the odds of becoming infected among the unexposed, if available, allowing calculation of an odds ratio, which in turn may be used as an approximation of the relative risk. Case-control studies are traditionally used to calculate the odds ratio. Cross sectional studies

wherein the comparison group is completely unexposed will allow calculation of a prevalence-odds ratio.[108]

- Prevalence data, if available, will be used to describe the magnitude of the "problem", e.g., the prevalence of human infection with zoonotic strains of influenza viruses among those exposed to domestic poultry or swine in agricultural settings. Cross-sectional seroprevalence studies will be included to achieve this aim.

- Studies which explore gradients of exposure associated with different types of work, e.g., intense, moderate, minimal exposure, will be included to assess the odds of becoming infected given a specific type of exposure. The study population with the least amount of exposure may be considered as the comparison group. Given the facts and assumptions supporting the notion that human infections with zoonotic influenza viruses are rare, this type of study design may ultimately yield data of more practical relevance to the populations potentially at risk.

- Those studies which identify a single exposed cohort and subsequently explore the intensity, duration and nature of exposure among those infected vs. those not infected, e.g., case-control studies, will be used to inform the identification of factors positively or negatively associated with infection.

- Cohort studies are known to be of limited usefulness for rare diseases, and so are unlikely to be identified in the available literature on this topic. However, if double cohort studies are found, they will be used to identify risk of infection associated with the exposure of interest, i.e., the relative risk.

It is important to note that the articulation of these key assumptions and implication for the methodology of the review led to further refinement of the study eligibility criteria to exclude single case reports. This process is described in further detail in the sections that follow.

3.2. Search strategy for identification of studies

The literature search for this review took place from March to October 2006, inclusive. The primary search involved a thorough search for potentially relevant articles, which was conducted from March to August 2006. The secondary search involved reviewing bibliographies of full text articles selected for review during the primary search, and ongoing review of a small number of publications via list serves. The secondary search was conducted between August and October 2006.

3.2.1. Primary search:

Studies for consideration in this review were identified primarily using the PubMed database. The search strategy was built using Pub Med's MeSH controlled vocabulary index to develop search terms relevant to the review. Search terms were informed by those listed on published articles previously collected. No date restrictions were placed on the searches. The Google Scholar search engine was also used.

Sources of grey literature accessed for this review included sources known to the reviewer; governmental (Canada and United States) and non-governmental websites (World Health Organization). Epidemiological reports of human cases of avian influenza A/H5N1 were found on the World Health Organization (WHO) website. Reports accessed included the WHO's situation update reports, case count charts, and an outbreak summary epidemiological report. Single case reports of human infection with avian A/H5N1, dated January 14th, 2004 to June 1st, 2006 were reviewed and assessed according to inclusion criteria.

Results from a previous literature search conducted by a committee member (TW) were utilized in the primary search. This collection of articles included both published peer-reviewed articles and sources of grey literature such as powerpoint presentations and conference reports, pertaining to avian and swine influenza, primarily focused on the health of animals.

Search terms used:

- Avian influenza and humans;
- Avian flu and human infection;
- Avian influenza and humans;
- Human infection and avian influenza;
- Flu in birds and human and infection;
- Flu A and birds and humans;
- Flu A and birds or flu in birds and humans;
- Avian influenza and human infection swine influenza and humans,
- Influenza in birds;
- Influenza in birds and human infection;
- Swine influenza and humans.
- Flu A and swine;
- Human infection and swine influenza;
- Influenza A virus and swine
- H1N1 swine influenza and humans;
- Influenza A virus and swine and humans and infection;
- Zoonotic flu infections and humans;
- Zoonotic influenza infections and humans;
- Zoonotic diseases;
- Public health risk and avian influenza or swine influenza
- Serologic evidence swine or avian influenza and humans

The search for relevant studies was an iterative process, with modification of search terminology as required, as recommended by the Cochrane Systematic Reviewer's Handbook.[114, p. 71] Records reviewed to assess study eligibility were abstracts if available and titles if abstracts were not available. In the context of the search process for this review, a record of a publication is considered to be the reference information retrieved upon application of the search strategy to a literature search engine. Records generally

consist of a title, author, and year of publication, journal title, volume, issue number and page numbers.

3.2.2. Secondary Search:

The secondary search involved reviewing the reference lists of full text articles reviewed (included and excluded articles, n=69), arising out of the primary search. These records were cross-referenced to primary search results to exclude duplicate records. Any records of articles not previously identified in the primary search were assessed for relevance by title, and full text articles retrieved and reviewed accordingly. Titles of articles published in Euro Surveillance Weekly, Emerging Infectious Diseases Journal, and The New England Journal of Medicine were assessed weekly during the search period (March to October 2006) via list serve notification. Two relevant articles were obtained from these sources.

3.3. Inclusion criteria: Assessing study eligibility for inclusion in this review:

Records of publications retrieved during the literature search for this review yielded a variety of articles, including those describing research studies and those providing background information and / or editorial commentary.

Records of articles containing the words "zoonotic" or "swine" or "pigs" or "avian" or "birds" and "influenza" and "humans" were assessed for eligibility using the study eligibility process outlined below. Those records meeting all eligibility criteria were retrieved in full, for further assessment of eligibility. Records of articles which met some of the eligibility criteria and were unclear for other criteria were considered to be unclear overall, and full text was retrieved and assessed.

Records of articles which clearly did not meet all of the eligibility criteria were considered to be unlikely to be relevant and the corresponding full text articles were not retrieved. Records of articles which were editorial, such as those which referred to the need for and importance of pandemic influenza planning but not to the epidemiology of avian or swine influenza infection in humans, were considered unlikely to be relevant and the corresponding full text articles were not retrieved.

Participants considered relevant to the review included: Farm owners, operators, and workers ("farmers"), farm family members residing on the farm (commercial or small flock) setting; closely related occupational groups, which may include veterinarians, slaughterhouse / abattoir workers, or other identified relevant occupational groups with a relevant exposure. Participants of any age, gender, and residing in any country were considered relevant. Comparison subjects may include those who have not had any exposure to the animal(s) of interest in the study, or who have had an exposure to a different population of the same animal, e.g., on a different farm or in a separate barn.

Exposures of interest included: Direct contact with live or dead swine or poultry, or their immediate environment, e.g., within the barn, or on the property; such as feeding the animals, cleaning the barn or barnyard, catching or slaughtering them.

Market settings were not considered relevant to this review, due to the inherent differences between market and agricultural settings, and so studies taking place in market settings were excluded. Markets typically sell a variety of live birds such as ducks, quail, chickens, and geese, including both wild-caught and farm-raised birds, as well as red-meat animals, though these tend to be separated from birds.[115] The mixture of species and origin of the various birds and other animals kept in close proximity to one another, along with daily introduction of new birds and other animals, is believed to contribute to spread and amplification of viruses such as influenza, providing ample opportunity for viral reassortment. In addition, these are busy public settings, with new people visiting daily. Furthermore, a key objective of this review is to identify the potential implications for Manitoba poultry and swine workers of being exposed to zoonotic influenza viruses in agricultural settings, and market settings are not relevant in Manitoba, as with most North American settings.

By contrast, agricultural settings are more controlled in terms of biosecurity, with only staff allowed into the barns and efforts taken to keep wild birds away from domestic birds. The stringency and consistency with which these measures are applied likely varies from country to country.

Outcomes of interest included assessment of the presence or absence of laboratory evidence of human infection with a swine or avian strain of influenza following known exposure to swine or poultry in agricultural settings. The inclusion criteria were reviewed by the thesis committee.

3.3.1. Study eligibility screening tool:

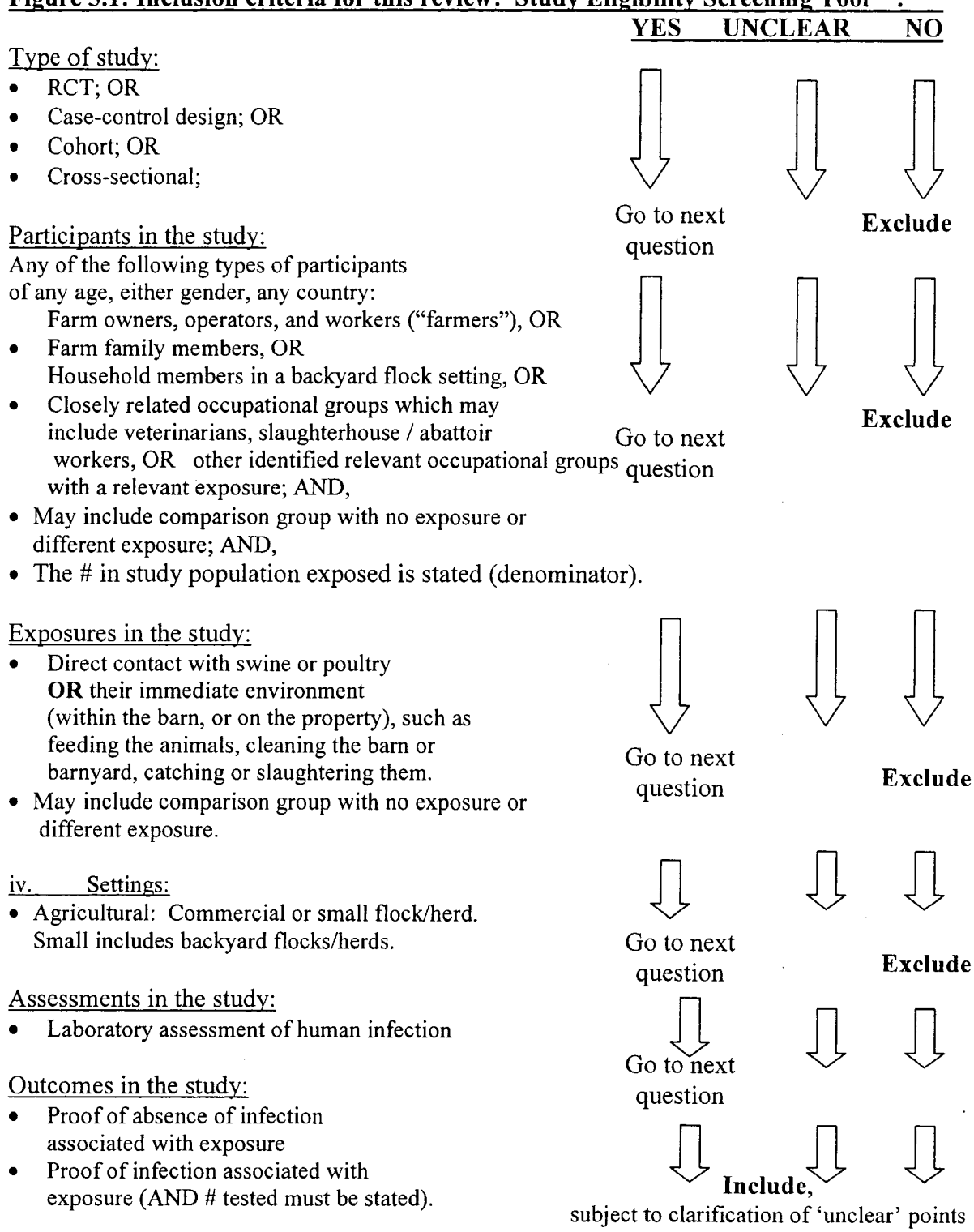
A screening tool was developed to reflect the study eligibility criteria. This tool, shown below, was used to screen records of articles. If the record was screened in, i.e., it passed the eligibility criteria, the corresponding full text article was retrieved and also screened for eligibility with this same tool outlining the study eligibility criteria. This process determined which studies were eligible for inclusion in this review.

The study eligibility screening tool was piloted prior to assessing the full results of search. Three articles which were expected to meet the inclusion criteria were assessed,[42, 52, 116] two which were not expected to meet the inclusion criteria,[1, 8] and one or which the outcome was less certain.[117] The abstracts of each of the six articles were assessed using the study eligibility form. The articles expected to meet the criteria did so, those expected to not meet the criteria did not, and the record for which the outcome was doubtful remained unclear. Accordingly, the full texts were retrieved for all but the two articles which did not meet the criteria upon initial screening. Those two articles were excluded at the initial assessment and full texts not reviewed.

Initially accepted study designs included: Randomized controlled trials, case-control, cohort (prospective and retrospective), and cross-sectional and single case investigation reports. Any sample size was considered acceptable, if other criteria were met. As described in the sections that follow, the original criteria were refined after the literature

search had been completed. This was as a result of the identification that single case studies were not capable of answering the research question. The tool shown below in figure 3.1 is the final tool used to assess study eligibility for inclusion in this review.

Figure 3.1: Inclusion criteria for this review: Study Eligibility Screening Tool[118]:



Decisions: **Include** **Unclear** **Exclude**

- **Decision to review full text: review "include" and "unclear"; those clearly excluded will not be reviewed. Assessment of full texts—if still unclear, discuss with committee prior to excluding. Only those full texts clearly meeting inclusion criteria will be included.**

3.4 Data collection process and tool:

The data collection form used, provided in Appendix 2, section 2.1, "Data Collection Form", was developed based on the contents of the chapter entitled "Critical Review of Epidemiologic Studies" in the 2003 text "Essentials of Epidemiology in Public Health" by Aschengrau and Seague,[119] with consideration to categories of information recommended by both the Cochrane Collaboration's Handbook for Systematic Reviews of Interventions and Guidelines for Systematic Reviews of Health Promotion and Public Health Interventions.[114, 120] Using this tool, data was collected from included studies and some of this work was checked by the committee, using the set of studies assigned for the quality assessment of the inclusion criteria. As a result of this process, edits to the form were made, resulting in a refinement of the data categories. This tool was not tested for validity or reliability.

3.5 Assessment of methodological quality of included studies: process and tool:

The quality assessment tool and associated guide to component ratings used for this review has been adapted from that published in the Cochrane Collaboration's Guidelines for Systematic Reviews of Health Promotion and Public Health Interventions.[120] The tool published by the Cochrane Collaboration was evaluated for both construct and content validity and assesses both internal and external validity.

The original tool developed by the Cochrane Collaboration was modified to suit the specific nature of the subject matter in this review and the nature of available studies in the literature on this topic, i.e., observational studies. The quality assessment tool developed for this review was piloted, circulated for feedback by the committee, and further refined to suit the specific needs of this review. This process took place after the study eligibility process had been completed. Once consensus had been reached on the confounders and the laboratory methodology criteria, the quality tool was updated and circulated for review by the committee and work to evaluate the evidence in the included studies began. The quality assessment tool used in this review has not been tested for validity or reliability.

Methodological components assessed in the quality appraisal process included: selection bias, allocation bias, confounders, evidence of human infection, evidence of animal infection, data collection methods, withdrawals and drop-outs. Studies were rated as weak, moderate, or strong on each criterion. An overall quality rating for included studies was not done. The Cochrane Collaboration's Handbook for Systematic Reviews of Interventions[114] discourages weighting quality criteria and the calculation of quality scores due to the fact that this approach cannot be validated.

The quality assessment tool used to evaluate the methodological quality of studies included in this review is appended at Appendix 2, along with the guide to component ratings, i.e., how the ratings on individual questions combined to yield overall ratings for each criterion. Components of the quality assessment tool and a description of the rating system are summarized below.

3.5.1. Selection bias:

Consistent with the original Cochrane tool, selection bias was assessed by two questions, with the overall rating for this criterion taking into consideration the score for each question. The first question was "Are the individuals selected to participate in the study likely to be representative of the target population?" Assessment of this was based on subject recruitment methods used in the study. Those studies in which subjects were more likely to be representative of the target population yielded a stronger overall rating for the selection bias criterion. The second question was "What percentage of selected individuals agreed to participate in the study, e.g., to answer questions, have a lab specimen collected from them?" A higher percentage of participants who agreed to participate contributed to a stronger overall rating for selection bias, meaning the study was unlikely to be affected by selection bias.

Studies which rely on volunteer subjects or subjects recruited from a specific place at a particular time may introduce selection bias into their results. Another anticipated source of selection bias in the literature on this subject is selection of ill individuals for evaluation of evidence of infection, rather than evaluating all exposed persons.

This review is not concerned with clinical outcomes resulting from infection with influenza, in studies of human infection of zoonotic influenza viruses. However, a potential source of bias is the selection of those subjects who present with symptoms of influenza disease, particularly those with severe illness. Such a study design may miss mildly symptomatic subjects and would miss asymptomatic infected subjects.

Persons with asymptomatic infection or those with mild illness are less likely to present for medical assessment and treatment and therefore in normal circumstances, are less likely to be tested and identified as infected. Thus, persons with co-morbid conditions lending themselves to complications of influenza, such as diabetes and asthma,[22] regardless of the source of the virus, may be over-represented in single case studies or those cross-sectional studies in which subjects are selected on the basis of symptomatic illness. While single case studies and cross-sectional studies with no denominator are excluded from this review, cross-sectional studies with a denominator are included even if they focused diagnostic testing on subjects presenting with symptoms. Such studies should be interpreted with caution.

3.5.2. Allocation bias:

The original tool adapted from the Cochrane Collaboration describes allocation bias as "the extent that assessments of exposure and outcomes are likely to be independent."[120, p.62] Following the example of the Cochrane Quality Assessment Tool, study design was used as an indication of the degree of allocation bias present. Modifications made to this segment of the Cochrane tool for use in this review included the removal of three sub-questions relating to the management of random allocation, as random allocation was not relevant to the observational studies included in this review.

Randomized controlled trials (RCTs) are the gold standard in study design because they ensure that assessments of exposure and outcome are independent, due to random

allocation of eligible subjects to either an exposure or control group. Although RCTs were neither expected nor found in the literature on this subject, this category was left in the quality assessment tool for the purpose of ranking the methodological quality of available studies according to a gold standard.

Cross-sectional studies are recognized as weak in terms of allocation bias, because the population is assessed for both exposure and outcome at a single point in time, indicating that the assessments are not independent. Descriptive and observational studies were expected to be the most likely study designs found in the published literature on this subject. Non-probability sampling methods such as convenience sampling were expected to be used by researchers of this topic. This is important to note as it can introduce sampling bias.[108]

3.5.3. Confounders:

To accompany the quality assessment tool developed for this review, a description of possible confounders in the included studies was developed. This list and associated descriptions were circulated for comment by committee members so a consensus list of confounders could be established and subsequently applied to the quality assessment (QA) tool and its associated guide to component ratings.

The following list of variables was agreed to by the review committee as potential confounders in included studies:

i. Antigenic experience (age, immunization history, knowledge of circulating strains in the community, history of previous exposures to animals of interest);
ii. Smoking;
iii. Use of antivirals as pre-exposure prophylaxis (relevant for commercial poultry farm outbreaks);
iv. Nature of exposure:
    - Use of personal protective equipment and related infection prevention and control practices (PPE relevant to commercial farm settings only).
    - Duration
    - Intensity
    - Frequency
    - Direct / indirect
    - Setting, e.g., confined space or outdoor interaction with animals

Rationale and supporting evidence for these variables as potential confounders are outlined in Appendix 2, section 2.3, potential confounders in studies of human infection with zoonotic influenza viruses, identified to facilitate quality assessment of included studies. Quality assessment of included studies included listing those confounders identified in the study and an evaluation of how they were managed. Four questions were asked, concerning: identification of between group differences in confounders prior to exposure; management of such between group differences in confounders through data analysis; consideration of confounders in the data analysis for the exposed group; and, any important confounders not reported. Those studies which only included one single exposed group of subjects were assessed on how they managed the confounders in the

data collection and analysis. If confounders were adequately identified and managed, a study was rated stronger. Conversely, those studies which failed to identify key confounders and/or did not manage confounders in the analysis, scored weaker.

Modifications to this component of the original Cochrane tool included the addition of a "not applicable" category for the question "Prior to the exposure were there between group differences for confounders reported in the paper?" The rationale for this change is to address those studies in which only one group of subjects was used, i.e., an exposed group. Due to this type of study being included in the review, and the nature of the subjects included in these studies, an additional question was added to allow for the assessment of the management of confounders within the exposed group: "Within the exposed group, did the analysis take confounders into consideration?" Examples of possible sources of confounding within an exposed group of subjects include: having been exposed in settings other than the study setting, previous antigenic experience with avian or swine influenza viruses among some subjects through cumulative exposure to animals of interest, previous vaccination with the swine influenza virus vaccine in 1976, or previous infection.

3.5.4. Evidence of infection:

The criteria related to evaluating evidence of infection, both human and animal, were added to the quality assessment tool used for this review to assess detection and measurement bias specific to the subject matter of the review. These criteria are critical in the assessment of the methodological quality of studies evaluating human infection with zoonotic influenza viruses. This section of the tool was added in the place of that which addressed blinding in the original Cochrane tool.

Included studies were assessed for quality with respect to evidence of infection of both human subjects and animals of interest. The methods used by individual studies to assess human infection with zoonotic influenza viruses were rated on a gradient from strongest to weakest, as described in the quality assessment tool and associated guide to component ratings, provided in Appendix 2. To evaluate evidence of human infection, the stated outcome of interest for each study, e.g., evidence of antibodies / past infection or evidence of recent infection, was compared against the laboratory methods used.

Isolation and identification of the specific virus of interest from the human subjects in the study should ideally be described. Techniques used to detect infection in humans (and in animals, if performed) should be noted so the quality of laboratory methods can be assessed. The quality of laboratory testing is of critical importance, so the use of appropriate test methodology and laboratory controls and techniques to detect possible cross-reacting antibodies should be specifically noted in the study. Antigens closely resembling currently prevalent strains and antigens of past prevalent strains should be used in hemagglutination inhibition testing.

Human:
Laboratory diagnosis methodology was deemed to be a critical determinant of establishing evidence of infection among human subjects. To inform this component of the quality assessment (QA) tool, two public health laboratory experts were consulted (GH and PVC) [121, 122] to ensure the accuracy of background information on accepted laboratory practices for laboratory diagnosis of influenza, particularly serological diagnosis. As a result of this consultation, a gradient of strength of evidence of infection was established, ranging from the gold standard of virus isolation or nucleic acid testing, to indirect evidence of infection as determined by a four-fold or greater rise in specific antibody. Evidence of seroprevalence was also included in the scale, to accommodate assessment of those studies which only sought to assess antibody prevalence, and a category for insufficient evidence was included to address those studies using inappropriate testing methods, e.g., the use of hemagglutination inhibition for assessment of avian influenza virus infection among humans. All studies were evaluated for the quality of laboratory controls, e.g., for possible cross-reactions and non-specific inhibition. These were rated as appropriate, not appropriate, or not reported.

Animal:
Also included in the QA tool was the strength of evidence of animal infection, e.g., were the animals to which human subjects were exposed, infected? Three questions were used to answer this broad question: 1) "Were attempts made to detect influenza infection in the animals?" 2) "Was there laboratory evidence of influenza infection in the animals?" and 3) "Were the animals found to have illness compatible with influenza at the time of the study?"

3.5.5. Data Collection Methods:
It is expected that studies of human infection with zoonotic influenza viruses associated with exposure to animals in agricultural settings would involve use of a questionnaire to collect data on a number of important variables such as those described above. In this case, the data collected would have been self-reported by the subject or their proxy. Self-reported exposure data is subject to recall bias, and such a bias may be more likely to impact results if longer time periods occur between the exposure of interest and data collection.

The data collection methods reported in included studies were assessed by the following questions: Did the authors report using a questionnaire? Was the questionnaire self-administered or administered by study personnel? Were the data collection tools known or shown to be valid and reliable? The first and second questions were added to the tool used for this review, to address those studies which may not have used or reported using a specific questionnaire, and those studies in which participants completed data collection forms as compared to a questionnaire delivered by study personnel. A "not applicable" category was added to the third and fourth questions on data collection tool reliability and validity, to address those studies in which a data collection tool was either not used or not reported.

Those studies rating strongest for this criterion overall used a data collection tool which had been known or shown to be valid and reliable. Consideration was given to questionnaire administration in the narrative analysis.

3.5.6. Withdrawals and Drop-Outs:
The percentage of participants who completed the study, e.g., those that had both completed a questionnaire and had specimens collected from them for laboratory analysis, was assessed as an indication of study quality. Those studies reporting a higher percentage of participants who completed the study were rated as stronger than those who reported a lower completion rate or which did not report the number of participants completing the study.

3.5.7. Analysis:
Five questions were used to assess the quality of the analysis of data in the studies, and these were structured with the review objectives in mind. Specifically, questions addressed if an association was identified between exposure and infection or seroprevalence; whether statistics were reported, the appropriateness of the statistical methods and measures of statistical stability and whether a statistically significant difference was identified between exposed and unexposed groups, if appropriate. The cumulative results of these questions were considered in the narrative analysis of each study. The questions within the analysis section of the quality assessment tool used for this review were tailored to the nature of the available studies. In keeping with the original Cochrane Quality Assessment Tool, strength ratings were not applied to this criterion.

3.5.8. Intervention Integrity (Exposure):
Intervention in this review refers to the exposure of interest. The types of comparison subjects among the included studies varied from those subjects who were unexposed to those with an exposure to a different population of animals, such as in an uninfected barn or on another farm altogether. An exposed group of subjects may be exposed to other sources of animals of interest through their daily life and work life. In the context of this review, a comparison group could refer to being completely unexposed to the animals of interest, or it could refer to being unexposed to a specific population of animals, e.g., a population of animals known to be infected with influenza, but perhaps being exposed to a different population of the same animal.

There is a potential for bias to be introduced into a study through misclassification, if "unexposed" subjects have received an exposure outside of the context of the study. This contaminating exposure, referred to as an unintended exposure in the context of this review, could bias study results. Furthermore, selection of a comparison group which is exposed, but exposed to a different animal population may also be misleading. This is because even if the comparison group of animals may not have been infected at the time of the study, they may have previously been infected, leading to an over-estimation of controls with evidence of infection. This would be especially important to note in seroprevalence studies.

The questions used in this section of the quality assessment tool used in this review were tailored to the subject area, e.g., the questions focused on the exposure of interest and not an "intervention". Three questions were used to assess intervention integrity: "What percentage of participants received the exposure of interest?"; "Was the exposure appropriately measured?" and, "Is it likely that subjects received an unintended exposure that may influence the results?" This latter question was asked of both the "exposed" and "comparison" groups, to address the possibility of unintended exposures among these groups of subjects, i.e., exposures occurring outside of the study setting such as on another farm. In keeping with the original Cochrane Quality Assessment Tool, strength ratings were not applied to this criterion. The cumulative results of these questions were considered in the narrative analysis.

3.5.9. Interpretation of Data:

Six questions pertaining to the interpretation of data were added to aid in overall assessment of study quality and applicability to the research question and associated objectives. These questions were adapted from a chapter on critical appraisal of epidemiological studies in a public health epidemiology text.[119] Such a section was not included in the original Cochrane Collaboration tool used as the basis for the QA tool used in this review. This section of the quality assessment tool was used to synthesize data collected using the data collection forms, so as to inform the descriptive analysis of included studies.

4. RESULTS:

4.1. Results of the Search:

Primary:

Using PubMed, a total of 878 records were reviewed. Of these, 602 records pertained to avian influenza, 239 to swine influenza and 37 to the generic term zoonotic influenza. There was considerable overlap in the records resulting from each search due to the overlap and similarities of the search terminology used, and duplicate records were excluded. Records were assessed against inclusion criteria and full text articles were retrieved for those which potentially fulfilled the inclusion criteria or could not clearly be excluded. In total, 730 records, including 42 records in languages other than English, were excluded. Due to significant overlap in search results for English articles, it is reasonable to expect that the actual number of potentially relevant articles in languages other than English is likely much smaller than 42.

One hundred and six full text articles were retrieved and assessed for possible inclusion and, of these, 37 were considered to be purely background information and 69 relevant to the review. Of those 69 potentially relevant studies, 31 were subsequently included and 38 excluded. Included and excluded studies are described below.

Results from a literature review completed by a local veterinary scientist (committee member, TW) yielding a collection of 190 articles on avian influenza, were shared with the reviewer. This collection included both peer reviewed journal articles and sources of

grey literature, e.g., conference proceedings, presentations, and reports. The vast majority of these publications did not pertain to avian influenza in humans but rather, avian influenza in birds. So while these publications were not eligible for inclusion in this review, they were extremely helpful in preparing the background section of this review. See figure 4.1 below for search results.

Secondary:
The secondary search was ongoing from August to October 2006. The titles of articles referenced within the bibliographies of full text articles reviewed (both included, n=31; and excluded, n=38) were assessed for additional articles. Once previously retrieved articles were accounted for, 48 additional full text articles were retrieved for assessment. Of these, 22 were found to be potentially relevant and 26 provided additional background information. Of the 22 potentially relevant articles, further assessment yielded four articles for inclusion and 18 were found to not meet inclusion criteria so were excluded. At this point in the study assessment process, 35 studies were considered to be eligible for inclusion in the review.

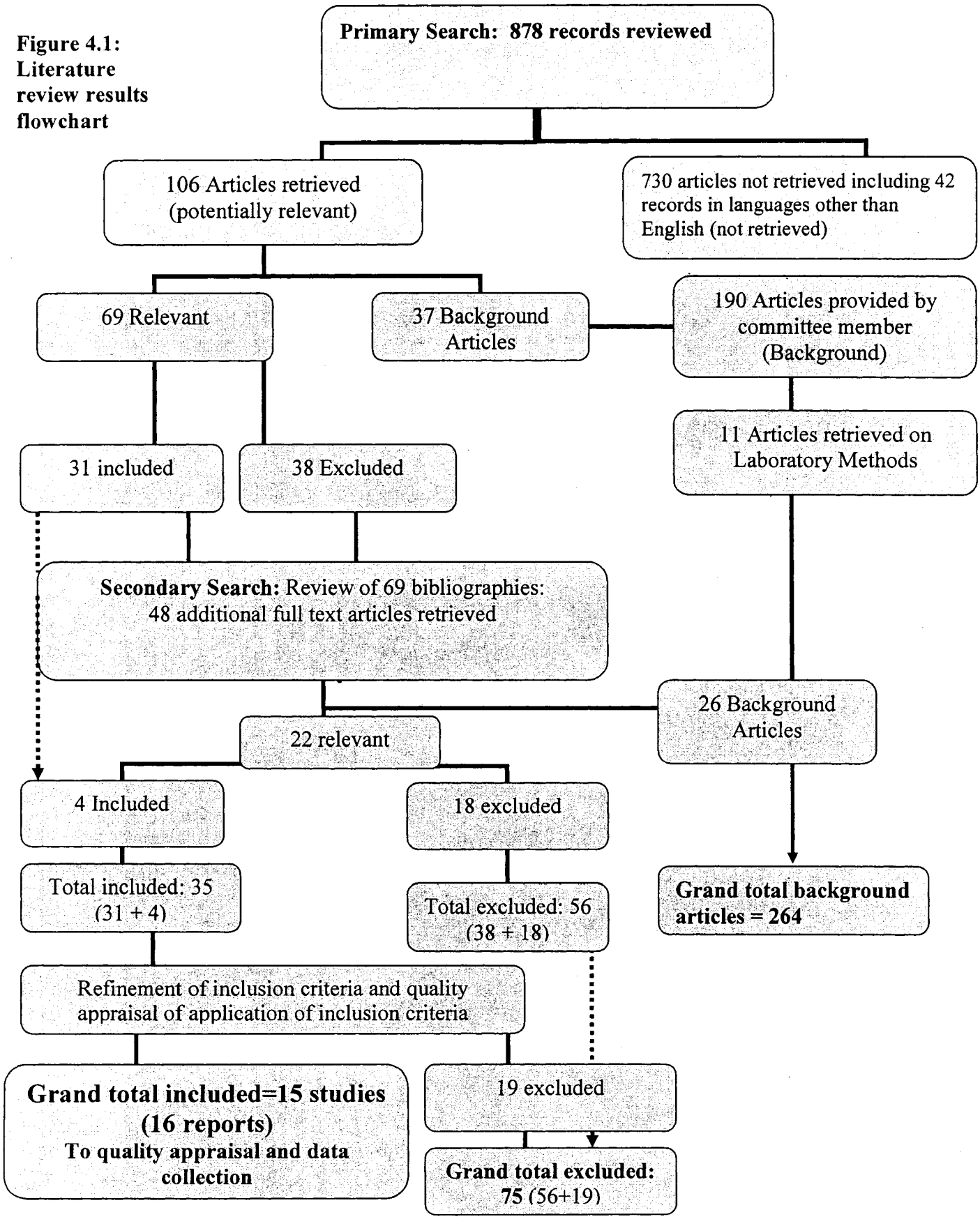
Quality assessment of application of inclusion criteria:
To assess the reviewer's application of the original inclusion criteria, six different studies from the preliminary set of included studies were provided to each of four committee members (EW, CB, WL, and TW) and an overlapping set of those studies (6) provided to the lead advisor (WM). Committee members assessed their sample of studies against the inclusion criteria. Eight studies which had previously been included were excluded as a result of this process, leaving 27 studies eligible for inclusion.

Refinement of the research question and impact on inclusion criteria:
The wording of the research question was further refined after initially screening relevant retrieved studies, however the intent did not change. It was identified that the word "risk of infection" would be more appropriately replaced with "odds of becoming infected". It was also identified that single case studies are not capable of answering the research question pertaining to likelihood of becoming infected, due to the lack of a denominator, and single case reports were excluded.

The inclusion criteria were modified to require eligible studies to state the number of subjects who were exposed to the animals of interest and the number tested for infection. The search strategy, including search terms were still relevant and broad enough that repeating the search was not deemed necessary as a result of tightening inclusion criteria. Previously excluded studies were not re-screened as a result of this tightening of the inclusion criteria, as they had already failed to meet other inclusion criteria. All initially included studies, less those found to be ineligible through the quality assessment process involving the committee, (27) were re-screened. As a consequence of this change in inclusion criteria, 11 single case studies, including one case finding study were subsequently excluded. Sixteen reports remain included, representing a total of 15 studies (one was a duplicate report of the same study yielding further detail); nine related to swine influenza and six relating to avian influenza.

Having initially included single case studies allowed for the identification of published cases of human infection with zoonotic strains of influenza associated with exposure to swine or poultry. These studies are described in the description of excluded studies, in section 4.2.2.

Figure 4.1: Literature review results flowchart



4.2. Description of Studies:

4.2.1. Included studies:

All studies which met the inclusion criteria for this review were cross-sectional. For the purposes of analysis, included studies have been grouped into the following two categories: Cross-sectional studies of influenza-infected humans associated with exposure to poultry and to swine, respectively. Characteristics of included studies, 6 avian studies and 9 swine studies, are described below in tables 4.2-a and 4.2-b, respectively, and the text that follows. A small number of authors had reported their studies as cohort design; however, the assessment of infection with zoonotic strains of influenza associated with exposure to animals of interest occurred at single points in time so these studies were classified in this review as cross-sectional.

The studies included in this review involve subjects with a known exposure to the animals of interest, sometimes in the context of a known or suspected outbreak among animals and sometimes not. Included in these studies are people who may have been exposed and not infected as well as those who may have been exposed and subsequently infected. Some or none of the participants in these studies may have experienced symptoms of varying severity, though this was not the focus of this review. The use and definition of comparison subjects among included studies varied from unexposed subjects to subjects exposed to another population of the same animal, on a different farm, e.g., an uninfected farm.

Table 4.2-a.: Characteristics of included avian studies: Influenza associated with exposure to poultry: (7 reports of 6 studies; 5 events)

Study:	Country and Date:	Outcome of interest in human subjects:
Bosman 2005 *Summary report*	The Netherlands 2003	Human infection with A/H7N7 influenza.
Bosman 2004 *Detailed report (same study)*	The Netherlands 2003	Human infection with A/H7N7 influenza.
Buxton-Bridges 2002	Hong Kong 1997-1998	Rates and risk factors of A/H5N1 infection among those exposed to poultry. Note: Poultry workers excluded from this review due to market exposure.
Chen 2001	China 1998	Infection with avian influenza viruses.
Koopmans 2004	The Netherlands 2003	Human infection with A/H7N7 influenza; Cases = illness + confirmation of infection.
Puzelli 2005	Italy 1999-2003	Evidence of anti-H7 antibodies in serum samples among those exposed to poultry.
Vong 2006	Cambodia 2005	Presence of antibodies to A/H5N1 influenza virus.

Table 4.2.-b Characteristics of included swine studies:
Influenza associated with exposure to swine: (9 reports of 9 studies; 9 events).

Study:	Country and Date:	Outcome of interest in human subjects:
Ayora-Talavera 2005	Mexico 2000	Prevalence of antibodies to swine influenza virus
Myers 2006	Iowa 2002-2004	Detection of antibodies to swine influenza virus.
Olsen 2002	Wisconsin 1996-1997	Detection of antibodies to swine influenza virus.
Olson 1977	Taiwan 1975	Prevalence of antibodies to swine influenza virus
Ramirez 2006	Iowa 2004-2005	Presence of antibodies to swine influenza virus
Schnurrenberger 1970	Illinois 1966	Prevalence of antibodies to swine influenza virus
Shu 1996	China 1992-1993	Human infection with influenza viruses including those of zoonotic origin.
Wells 1991	Wisconsin 1988	Infection or illness following exposure to swine
Zhou 1996	China 1994	Presence of antibodies to swine influenza viruses and duck viruses.

4.2.1.1 Cross-sectional studies—exposure to poultry:

a) Overview of studies:
There were seven published reports of six cross-sectional studies representing five events (two with a comparison group) meeting the inclusion criteria for this review, addressing human infections with avian influenza associated with exposure to poultry. Viruses represented in these studies were: H5N1, H9N2, H7N1, H7N3, and H7N7, during the time period from 1997-2006. Among the studies involving avian influenza, five studies were initiated in response to known or suspected poultry outbreaks and one was not.[123] Of those studies associated with poultry outbreaks, four of five were associated with commercial poultry outbreaks. Four countries are represented among the six included studies, none of which are in North America (two in Europe; two in Asia). Of the six studies, three described an 'unexposed' group. The Bosman study[124, 125] referred to 100 'controls', however no data were provided on these subjects. Vong[126] described as "unexposed" those subjects which had poultry in their household which were not suspected of being infected. Chen[123] described an investigation of an 'unexposed' group, for which no information is outlined.

b) Studies of commercial poultry farm outbreaks:
Two studies[124,125,20] described the human health implications of the outbreak of A/H7N7 among poultry on 261 commercial poultry farms in the Netherlands in 2003. The Koopmans[20] study was a case-finding study, in which symptomatic exposed persons were

investigated for avian influenza infection. The Bosman[124, 125] study described the above case-finding study,[20] and also conducted a population study to identify infections among those exposed to poultry, including asymptomatic infections. Research by Puzelli et al.[127] evaluated 983 poultry workers involved in six domestic poultry outbreaks in Italy from 1999-2003. Buxton-Bridges[128] studied 293 government workers who worked to cull poultry on farms associated with the 1997-1998 Hong Kong outbreaks, and 1525 poultry workers who worked in the poultry markets. The latter group of subjects was excluded from this review as their exposure and work setting did not meet the inclusion criteria.

c) Studies not associated with commercial outbreaks:
Chen[123] evaluated evidence of infection among 1512 persons belonging to an occupational group of raising, selling and slaughtering chickens and 885 unexposed persons. Vong[126] evaluated seroprevalence of influenza A/H5N1 antibodies among 351 people residing in a rural area of Cambodia where a confirmed human case of influenza A/H5N1 had been found.

d) Study populations:
Study populations among these studies ranged from commercial poultry farm workers, veterinarians, and people raising and selling poultry, to both urban and rural families with backyard poultry flocks. A total of 4,880 subjects were included in these studies which were exposed to poultry and found to be relevant to this review. Bosman[124, 125] studied 1300 of the total of 4500 people who were involved in the commercial poultry farm outbreaks in the Netherlands in 2003. Koopmans[20] investigated 453 symptomatic people, of the 4500. Of these, 441 were exposed to poultry: farmers, family, cullers, veterinarians and the remaining 12 were medical personnel, exposed to symptomatic people.

e) Outcome of interest:
Of the six included studies pertaining to exposures to poultry, four of the six described their outcome of interest as evidence of infection with avian influenza virus(es): Bosman,[124, 125] Buxton-Bridges,[128] Chen,[123] and Koopmans.[20] The remaining 2 examined seroprevalence of antibodies to avian influenza virus(es): Puzelli[127] and Vong.[126]

4.2.1.2 Cross-sectional studies—swine influenza:

a) Overview of studies:
In total, nine cross-sectional studies on human infection with swine influenza viruses were collected; one conducted in each of the 1960s, 1970s, and 1980s; three in the 1990s, and another three since 2000. Two studies took place in southern China, one in Taiwan, one in the Yucatan peninsula of Mexico and the remaining five took place on swine farms in the American Mid-West (Iowa, Illinois, and Wisconsin). Viruses represented in these studies included influenza A/H1N1, H3N2, and H1N2. A total of three studies[129,130,42] involved exposure to pigs suspected of being infected with influenza (influenza-compatible illness or lab confirmation). In one of these studies[130] an outbreak of influenza was noted among swine, however the study of humans was conducted one

year later. Seven of the nine studies on swine influenza infection of humans included a comparison group; six of the seven were comparison groups which were unexposed to swine, and one of seven included a comparison group exposed to healthy swine in a neighboring county, as compared to exposure to ill swine in the setting of interest.

b) Studies in commercial swine farm settings:

The studies by Ramirez,[94] Myers,[33] Olsen[42] and Schnurrenberger[96] took place in commercial swine farm settings in the American Mid-West. Schnurrenberger,[96] Myers[33] and Olsen[42] also involved other related occupational groups: veterinarians, packing plant workers and all of the above studies included an unexposed group of subjects. The Ramirez[94] study specifically focused on risk factors associated with human infection with swine influenza viruses and so gathered information on the type of personal protective equipment worn by swine confinement workers and the consistency with which they used the equipment.

c) Studies not associated with commercial swine farm settings:

Olson[130] took place at the Taiwan Sugar Corporation, where employees apparently also raise pigs. Studies by Shu[131] and Zhou[132] took place in rural and urban backyard herd settings in China, however Zhou[132] also included women who raised pigs and slaughterhouse workers as subjects. Wells[129] studied human infection or illness among junior swine exhibitors following exposure to ill swine at a rural Wisconsin agricultural fair in 1989. A comparison group of junior swine exhibitors was also included in the study, and these subjects resided in and exhibited their pigs in another county.

d) Study populations:

The population groups investigated in these studies included the following occupational groups: commercial swine producers, veterinarians and students, packing plant employees, hog buyers, producers' wives or daughters (family members), small herd farmers, junior swine exhibitors, and rural residents in countries where swine live amongst humans. A total of 2035 subjects who were exposed to swine were included in these studies. In addition, Shu[131] studied 156 serum samples and 1175 throat swabs, however it is not known how many individuals on the farms this captured, as the authors indicate the same person may or may not have been tested twice.

e) Outcome of interest:

Seven of the nine studies pertaining to exposure to swine sought to assess the presence of antibodies to swine influenza virus in humans exposed to swine.[133,94,33,132,42,96,130] The remaining two studies,[129,131] stated human infection with swine influenza viruses as their outcome of interest. All of these studies used serological methods, specifically the hemagglutination inhibition test to diagnose human infection, and the Zhou study[132] also used ELISA testing. Studies conducted prior to 2000 used lower cut points in HI titres as evidence of human infection with swine influenza viruses than those conducted after 2000. The two studies from the 1960s and 1970s used HI titre cut-points of $\geq$ 1:20[96] and $\geq$1:10[130], respectively. Studies conducted in the 1980s and1990s used titre cut-points of >1:20[129] and $\geq$1:10; [131]one study[94] grouped HI titres as being <10, 10, or >10; and one study[132] reported all HI test results without stating a specific cut-point, though they

acknowledge that titres of 20 are at the lower limit of specificity. The three studies conducted post-2000 all used ≥1:40.[33, 42, 133]

4.2.2. Excluded studies:

Of the full text articles thought to be relevant (n=69), 38 were excluded upon application of the inclusion criteria. Excluded studies can be described as falling into the following categories: Studies which failed to meet the study design criterion, and studies which failed to meet criteria related to exposure, setting, participants, +/- design. Some of these studies described lab confirmed human cases of zoonotic influenza virus infections; cases which have been recognized in the literature, however such studies were excluded if they failed to meet the inclusion criteria for this review. The single case studies which failed to meet the study design criterion warrant mention in this review, to confirm their identification by the literature search process and assessment against inclusion criteria. A complete listing of excluded studies, along with the reason for exclusion, is provided at Appendix 5, along with tables summarizing the noteworthy single case studies.

4.2.2.1 Excluded studies which failed to meet the study design criterion:

a) Studies reporting human infection associated with exposure to poultry

Among the studies of single human cases of avian influenza infection, two reports of two events described three confirmed human cases of avian influenza infection, all symptomatic and all recovered. These are described below.

i) Single human cases of influenza A infection associated with exposure to poultry:

One study from England reported a single human case of avian influenza in 2006.[89] This case was a male poultry worker on a commercial poultry farm in which an outbreak of avian A/H7N3 had been identified. As a single case report, this study failed to meet the study design criterion and so was excluded.

ii) Cross-sectional studies of of influenza A infection associated with exposure to poultry:

A cross-sectional case finding study was performed in response to an outbreak of A/H7N3 among commercial poultry in the Fraser Valley area of British Columbia in 2004, in which 42 of roughly 600 commercial poultry farms in the region and 11 backyard flocks were confirmed infected.[19] A total of approximately 1.3 million birds populated these infected farms. Approximately 2000 poultry workers and 650 federal workers were involved in the outbreak and outbreak response however, not all were exposed to the poultry. Following the same methodology as the Netherlands (Koopmans, 2004) study, the Tweed study selected out only symptomatic exposed persons for testing. However, unlike the Koopmans study, the Tweed study did not document the number of exposed persons, so this study was excluded.

In the B.C. outbreak, 57 suspected human cases were identified. Forty-nine people were tested for avian influenza infection and two cases were confirmed. Both of the case patients were mildly ill. In this outbreak, the identified cases described either not wearing appropriate protective gear (one case) or having had debris breach their protective gear (1 case). Neither of these cases seroconverted, however AI virus was isolated from their conjunctival swabs. Both of these cases were male, aged 40 and 45 years.[19]

b) Studies reporting human cases of influenza A infection associated with exposure to swine:

A total of nine reports described 11 cases of human infection with zoonotic influenza viruses associated with known exposure to swine in agricultural settings have been documented in the past 30 years. All of these cases involved symptomatic individuals presenting for medical assessment and treatment following exposure. In total, three of 11 cases were fatal. The settings in which these cases were exposed varied from farms to livestock fairs. All but one case involved H1N1 swine influenza virus; the most recently reported case in 2006 involved a triple-reassortant H3N2 virus, containing genes from swine, avian and human influenza viruses.

Nine of 11 cases had virus isolated from their respiratory specimens. Hemagglutination inhibition testing was also used for detecting antibodies to swine influenza viruses and laboratory cut-points for defining evidence of infection ranged from $\geq$1:20 in a single serum sample to a four-fold rise in antibody titre in paired serum samples.

4.2.2.2. Excluded reports which failed to meet criteria related to exposure, setting, participants, +/- design:

A number of full text articles were reviewed and subsequently excluded. Key reasons for exclusion were lack of exposure data or a focus on human to human transmission of a zoonotic influenza virus, not on the primary infection itself. A small number of studies provided background information on avian influenza, however no information on specific human cases of avian influenza. Single case studies involving subjects who experienced symptoms and were found to have been infected with avian or swine influenza virus, but for whom a clear exposure history was not established or documented, were excluded. A small number of studies were also excluded which documented human cases of zoonotic influenza viruses in settings other than agricultural settings, such as poultry market settings. Human infections of influenza A viruses in association with an exposure to ducks were also excluded.

a) Excluded reports related to avian influenza:

Single human cases of avian influenza A/H5N1 were assessed for inclusion largely from data posted on the World Health Organization (WHO) website, e.g., situation update reports. These single reports were numerous and were not counted in the number of excluded reports. A number of prominent articles on the A/H5N1 outbreaks were

reviewed; however most of these were excluded, as either the exposure data was not clear, the setting involved poultry markets, or they explored the evidence of possible human to human transmission, not primary infections (transmission from poultry to humans). Examples of such studies not meeting inclusion criteria for this review include Ungchusak et al.[52] and Katz et al.[134]

Other excluded studies pertaining to avian influenza included: editorial articles, articles on human infections with A/H9N2 in which exposure data were lacking, and studies outlining mostly clinical data for human cases associated with A/H5N1 outbreaks, including the 1997/98 Hong Kong outbreak and the outbreaks post-2004. Reports were identified which duplicated data reported in the WHO reports,[135] which were also excluded.

Single cases of human infection with A/H5N1 avian influenza, both associated with the 1997-1998 poultry outbreaks in Hong Kong, and those associated with poultry outbreaks since 2003, were described as having been identified through encounters with the health care system, e.g., they developed symptoms consistent with avian influenza virus disease in humans, severe enough to seek treatment, and were subsequently confirmed to have been infected with A/H5N1 virus. In some cases, but not all, such individuals were also found to have had an exposure of interest (to poultry). Therefore, these studies only represent ill or severely ill cases and cannot describe the risk of infection associated with an exposure to poultry. Asymptomatic influenza cases or those cases with mild symptoms are rarely found in routine surveillance efforts, so these are likely under-represented in the literature.

b) Excluded reports relating to swine influenza:

Three studies pertaining to the 1976 outbreak of swine influenza among military recruits at Fort Dix, New Jersey[101,102, 104] were excluded due to lack of information reported on exposure histories and the fact that these were single case studies. In this event, the patients had no direct contact to swine revealed through investigation. In fact, there were no hog barns within the vicinity of Fort Dix.[136] Speculation on the source of the outbreak is that the virus was introduced onto the base by an infected recruit, though no details are provided about the exposure history of the index case.[102] Although limited human to human spread was noted on the army base, no spread was detected into the surrounding community.[93] Other single case reports of human infection with swine influenza viruses were identified and subsequently excluded.

4.3. Methodological quality of included studies:

The quality assessment tool was adapted from its original purpose, which was to evaluate the quality of studies in a subject area where it is reasonable to expect randomized controlled trials (RCT) studies. The current review is focused on a topic where observational studies and not RCTs were expected to be identified in the literature. Therefore, certain aspects of the original tool were not as meaningful to the assessment of studies included in this review.

4.3.1. Overview of issues identified in the quality appraisal of the methodology of included studies:

a) Selection bias:

All included studies were cross-sectional, so consequently subjects are not likely to be representative of the target population. Subjects were assessed for exposure retrospectively, so the percentage of individuals who agreed to participate tended to be high, since those approached for participation in the study were included after exposure had occurred. Sometimes this was not reported, however, which resulted in a weak rating for the criterion "selection bias", meaning that selection bias is likely to be present. It is possible that this wasn't reported often as it is not as relevant in a cross-sectional study as compared to an RCT.

b) Allocation bias:

Cross-sectional designs are known to be weak in the extent to which assessments of exposure and outcome are likely to be independent (allocation bias), because they evaluate both exposure and outcome at a single point in time, after the exposure has already occurred. It is not practical for the study of natural human infections with zoonotic influenza viruses associated with exposure to swine and/ or poultry, to randomly allocate participants to exposed and unexposed groups. Practical study designs for such a topic would be: case-control, before/after study, cross-sectional designs, and possibly prospective or retrospective cohort studies. Of the observational studies, cross-sectional studies are considered to be the weakest, as they cannot temporally associate an exposure with an outcome.[109]

Studies of human infection with zoonotic influenza viruses associated with outbreaks of influenza among the animal population need to be opportunistic, and given the rare occurrence of human infections with zoonotic influenza viruses, there remain gaps in our knowledge of the mechanisms by which humans are infected. In spite of their methodological weaknesses with respect to etiologic inference, e.g., exposure-disease temporality, cross-sectional study designs are useful for hypothesis-generating, and so they are an appropriate choice for this topic. Therefore, cross-sectional studies can be expected to dominate the literature on this topic. Not surprisingly, all included studies rated weak for allocation bias, due to their cross-sectional design. Evaluated in the context of observational studies, cross-sectional studies are weaker than cohort and case-control studies for etiologic inference.[109] Nevertheless, it is still reasonable to expect that attention is paid to recommended best practices in study design, implementation and reporting.

c) Withdrawals and Drop-outs:

The percentage of withdrawals and drop-outs is not as relevant in a cross-sectional study as it would be in a cohort study or RCT. This section was included in the QA tool, to acknowledge the degree to which missing data, e.g., missing questionnaires or refusals, may have impacted on the results and conclusions of the study. Approximately half of all included studies rated strong for this criterion.

d) Intervention Integrity (Exposure)
The percentage of participants among the exposed group who were actually exposed is not highly relevant to the included cross-sectional studies, because subjects were exposed prior to the study being conducted, so to be included in an exposed group, one would already have been exposed. The most important questions in this section were the appropriateness of the measurement of the exposure, and the likelihood that subjects received an unintended exposure. An unintended exposure is an exposure in a setting outside of the specific study setting, e.g., at a neighbor's farm.

Given the nature of cross-sectional studies, and the industry of interest, it is common to find subjects among an "exposed" group that have had an exposure outside of the study setting, particularly if they manage more than one barn or farm, or live on a different farm from that which they work on. Mixed exposures are also possible, if poultry and swine are raised on the same farm. Study populations in included studies can be expected to have exposures over a number of years, in addition to the specific exposure of interest in the study. These issues can be expected to impact on the interpretation of results if not taken into account by data collection and analysis.

e) Data collection tools:
This component of the quality assessment tool assesses whether the data collection tools used in included studies were known or shown to be valid and reliable. Given that studies of human infections with zoonotic influenza viruses are not common in the literature, it might be unreasonable to expect to find published questionnaires which have been demonstrated to be valid and reliable. However, it is still reasonable to expect a study to outline whether or not a questionnaire was used, what topics it addressed and whether or not trained interviewers were used or if the questionnaire was self-administered, e.g., that the methodology and data collection procedures were outlined. Any validity and/or reliability testing should be reported, either if it was done within the context of the current study or if it had been done in association with a previous, similar study.

f) Potential confounders:
No single study reported data on all confounding variables noted in the list agreed upon by the committee. Two swine studies came close, identifying most confounders.[42, 94] The most striking gap among included studies, was the lack of detail gathered on exposure history, e.g., the number of years subjects worked with swine and the nature of their exposure in terms of frequency, duration, intensity and use of any personal protective equipment (PPE). Swine studies were more likely to collect demographic data and immunization status information than were poultry studies. It is reasonable to expect cross-sectional studies to collect detailed exposure histories to enable analysis of the effects of exposure over time and other variables.

g) Evidence of infection:
While it would be challenging to implement a cross-sectional study using viral isolation to detect evidence of human infection associated with exposure to animals in agricultural settings, it is possible. This approach would be most feasible among poultry studies,

since identification of an infected flock is possible through mortality surveillance. Swine studies, on the other hand, favour serological methods of detecting evidence of human infection, since pigs may be asymptomatic or mildly symptomatic with influenza and are generally less prone to mortality than are poultry. It remains reasonable to expect, however, that studies using serological methods evaluate paired sera, particularly when influenza-like-illness is known to be present among swine. Those studies choosing a single serum sample should identify themselves as seroprevalence studies, rather than studies evaluating evidence of human infection, since the latter leads one to expect an evaluation of recent infection rather than past infection.

4.3.2 Overview of methodological quality of individual included studies:

The following discussion is a description of the findings of the quality assessment process for included studies in this review. A summary of the quality assessment results for each study included in this review is outlined in table 4.3-a.

4.3.2.1 Studies of human infection associated with exposure to poultry:

All six studies of human infection with or evidence of antibodies to, avian influenza viruses associated with exposure to poultry scored weak for selection bias, allocation bias (study design), and management of confounders.

All but one study[126] scored weak on data collection methods. The Vong study[126] scored strong on this criterion. The majority (5/6) studies scored weak. This was due to the fact that they did not state if the data collection tools were shown or known to be valid and reliable. However, five of six studies did declare the use of a specific questionnaire (one not reported)[123] and of the five that used questionnaires, two were administered by study personnel;[126,20] two were self administered;[128,127] and the Bosman study[124, 125] did not state how the questionnaire was administered. Those studies in which data collection was done without a consistent tool and those in which questionnaires were self-administered can be expected to have the most problems related to validity and reliability of the resulting data.

For the group of confounding variables contributing to the antigenic experience of subjects, four of the six studies reported collecting data on age,[20, 126-128] and only one of six reported collecting data on immunization history,[20] however this study identified only the current year's influenza vaccine as a requirement in the outbreak response, and no mention was made of having collected data on previous vaccination history of subjects. Vong[126] described exposure histories during the past 12 months, and no studies noted historical exposure data beyond the context of the relevant poultry outbreak prompting the study in the first place. One of six studies[20] collected data on community influenza activity. As for confounders, one[128] collected data on the smoking behaviour of subjects.

Adequacy of data on the nature of exposure was defined as having outlined: The use of personal protective equipment and related infection prevention and control practices, duration, intensity, frequency, direct / indirect contact, and the setting, e.g., confined

space or outdoor interaction with animals. Only one study[126] described collected data on all details of exposure history, including intensity of exposure; and four[124, 125,128,20,127] describe having collected data on the work performed by subjects during the poultry outbreaks, however none of these studies reported details on the exposure history of its subjects. The Buxton-Bridges study[128] also collected data on exposures to humans with H5N1 illness. Only two of the avian studies described the use of personal protective equipment, however details were not reported.[128,124, 125] None of the six studies noted exposures occurring at the study setting as well as the subject's home environment. Vong[126] did take place in the home / neighborhood setting. Two studies did not describe having recorded any exposure details.[123,127]

The use of antivirals as pre-exposure prophylaxis was noted in two studies.[20,124,125] In the Koopmans[20] study, it was reported that antivirals were started after 19 people had been diagnosed as being infected with an avian influenza virus, and that over half (56%) of the infections reported in total occurred before the prophylactic program began. Bosman[124, 125] described the percentage of participants who used antivirals and the percentage of those whose therapy was interrupted. This variable was considered to be only relevant for commercial poultry farm outbreaks in developed countries, so it is not considered a weakness that this was not noted in any of the studies pertaining to backyard flocks and those studies taking place in developing countries where the resources are likely inadequate to consider the use of antiviral drugs for poultry workers.

Four of the six studies[128,123,20,126] scored strong on withdrawals and dropouts and the remaining two studies[124,125,127] scored weak.

a) Evidence of Infection:

Human:

Four of the six studies had human infection with an avian influenza virus as their outcome of interest,[20, 123-125, 128] while the remaining two were interested in finding evidence of antibodies to AI viruses in humans,[126, 127] which equates to evidence of past infection. Those looking for evidence of human infection did not state whether or not they were looking for recent infection or just infection at any time in the past. Of these studies, only two[20, 128] of four performed an appropriate laboratory test to enable evaluation of recent infection, but as noted previously, it cannot be determined if this implies methodological weakness in the remaining two studies, or lack of clarity in their stated objectives. Koopmans[20] used viral isolation and PCR and so was able to describe direct evidence of infection among subjects. Buxton-Bridges[128] used paired sera among the government worker subjects (the cohort of interest in this review), and so was able to establish indirect evidence of recent human infection. Bosman[124, 125] and Chen[123] used hemagglutination inhibition testing to test single serum samples from subjects, so this was insufficient to evaluate the presence or absence of both recent infection and also insufficient for evaluating seroprevalence. Of those studies looking for seroprevalence, Puzelli's[127] methodology was strong, e.g., acceptable techniques were used as well as laboratory controls, and Vong's[126] methodology was weak, due to the lack of laboratory controls or reporting thereof.

Animal:
All but one study scored strong for evidence of animal infection. Buxton-Bridges[128] scored weak. This study evaluated infection with avian influenza viruses in 2 cohorts, poultry workers and government workers. The poultry workers worked to cull chickens in the poultry markets. These chickens were known to be infected with A/H5N1 influenza. The government workers, on the other hand, worked to cull chickens on farms and the authors noted that they were less likely than poultry workers to be exposed to infected chickens. This could either mean that the chickens themselves were less likely to be infected than their market counterparts, or it could mean that the workers were less likely to come in contact with avian influenza virus due to the fact that they wore personal protective equipment, whereas the poultry workers did not. This leaves much to speculation. The background information provided in this study indicates that 20% of chickens in the markets were infected with H5N1, and no similar data is provided for poultry on farms. Furthermore, the impetus for the Hong Kong-wide cull of 1.5 million chickens and several hundred thousand other domestic fowl was to reduce the potential for further spread of this virus from poultry to humans in the poultry markets. The rationale for this measure arose from the findings of a case-control study by Mounts et al.[116] which identified "exposure to poultry in retail markets as the primary risk factor for human H5N1 illness".[128, p. 1004] Due to the fact that the study did not state that the chickens on the farms were known to be infected, a weak rating was given.

The key reason for the generally high quality of evidence of animal infection among these studies appears to be the settings and context in which the studies took place. Three of the six studies took place in domestic commercial poultry barns where outbreaks of avian influenza were known to be occurring and the human health response and study of exposed individuals was secondary to the animal health response, so it was known that people working in the infected barns had been in an environment along with infected poultry.

4.3.2.2 Studies of human infection associated with exposure to swine:

Of the nine swine studies, two scored moderate for selection bias.[42,129] The remaining seven scored weak. All nine studies scored weak for allocation bias (study design).

All but one study scored weak on data collection methods. Wells[129] scored strong because they reported that they used a standardized questionnaire. The majority, eight of nine studies, did not report their data collection methodology, so it is possible that validated questionnaires were used, but not reported. One study, Ayora-Talavera,[133] tested samples accessed through a clinical laboratory bank of individuals seeking medical care and thus no information was known about the subjects. Two other studies[131,132] appear to not have collected any data on their subjects, as evidenced by lack of descriptive data in the results and no mention of having done so in the respective publications. Four of nine studies did declare the use of a specific questionnaire;[42,129,33,94] four did not report whether a questionnaire was used;[131,132,130,96] and one did not gather any data on participants.[133] Of the four studies that declared the use of a questionnaire,[33,

[42, 94, 129, 131] two were administered by study personnel; [42, 129]one was self administered; [94]and one did not state how the questionnaire was administered.[33]

Three of the nine studies scored moderate for their management of confounders,[33,94,129] with the remaining six studies scoring weak. Regarding the antigenic experience of subjects, eight of the nine studies collected data on the age (all except Ayora-Talavera[133]), and five collected data on immunization history including 1 that only described having ever been vaccinated.[33,42,94,96,132] Only three studies collected data on history of previous exposures to animals of interest[42,129,130] and 1 of these only noted this for controls.[130] Also, only one of nine studies collected data on community influenza activity.[129] Olsen[42] did not state that data on community influenza activity were collected, however it was noted that the study took place during influenza season, so this remains a possible source of confounding. One study collected data on the smoking behaviour of subjects[94] and smoking was considered irrelevant for 1 study due to the young age of the subjects.[129]

Adequacy of data on the nature of exposure was defined as having outlined: The use of personal protective equipment and related infection prevention and control practices, duration, intensity, frequency, direct / indirect contact, and the setting, e.g., confined space or outdoor interaction with animals. Olsen[42] collected data on all details of exposure history. Myers[33] addressed exposure history but did not provide details or information on historical exposures. Ramirez[94] was the only swine-related study to describe the use of personal protective equipment, and only gloves were described. Schnurrenberger[96] described exposures as maximal, moderate and minimal, depending on the participant's occupation, but did not assess individual exposures or report on past exposures. Wells[129] noted exposures occurring at the study setting (agricultural fair) as well as the subject's home environment, but they did not note details such as frequency, duration or intensity of exposure. Four studies did not describe any exposure details.[133,130,131, 132] The use of antivirals as pre-exposure prophylaxis was considered to be only relevant for commercial poultry farm outbreaks in developed countries, so it is not considered a weakness that this was not noted in any of the swine-related studies.

Ayora-Talavera[133] did not report on withdrawals and drop-outs; however this is consistent with the fact that they did not have true "participants", since they used stored blood samples from a clinical laboratory. Of the remaining eight studies, half scored strong[33,42,130,129] and the other half scored weak.[94,96,131,132]

a) Evidence of Infection:

Human:

Whereas the majority of avian studies sought to evaluate evidence of recent human infection with avian influenza viruses, the majority of swine studies did the opposite, looking for presence or prevalence of antibodies (past infection) to swine influenza viruses in humans. Only Shu[131] and Wells[129] sought to evaluate human infection with swine influenza viruses. Studies which used appropriate laboratory methodology to enable description of recent human infection were Olsen,[42] Shu,[131] and Zhou,[132] the latter

two only doing so for some of the study subjects. The remaining six studies used laboratory methodology sufficient to determine the presence or absence of antibodies to swine influenza viruses. Only three of these used appropriate laboratory controls, garnering a rating of "strong evidence of seroprevalence".

According to a laboratory expert consulted on this aspect of the quality assessment tool, genetic analysis (sequencing) is required to definitively differentiate H1 and H3 influenza viruses of animal origin from those of human origin.[121] All nine swine-related studies used hemagglutination inhibition testing on human serum samples, with the specific viruses used varying by study, e.g., specific swine, human and / or swine-human recombinant viruses. While they were tested against specific swine H1 and H3 virus antisera, this is not gold-standard evidence of zoonotic transmission. Four studies reported the use of laboratory controls to control for cross-reactions and non-specific inhibition: Ayora-Talavera,[133, 132] Myers,[33] and Olsen.[42] Schnurrenberger[96] mentions the use of controls for non-specific inhibition but not for cross-reaction. The remaining four studies did not report the use of laboratory controls: Wells,[129] Olson,[130] Ramirez,[94] and Shu.[131]

Animal:
In contrast to the avian studies, only two of the nine swine studies scored greater than weak for the criterion of "evidence of animal infection". Olsen[42] and Wells[129] scored moderate. Olsen[42] tested the animals, and the testing was inconclusive, but it was reported that animals on one farm were ill with influenza compatible illness. Wells[129] did not test the pigs in their study; however the animals were reported to have influenza-compatible symptoms.

4.3.2.3 Summary of quality assessment results:

Overall, the methodology of included studies appears to be weak. This is partially due to the fact that these are cross-sectional studies, and as such are not designed to draw causal inferences, but rather, to describe the magnitude of a particular problem.[109] Poor reporting quality appears to be an important issue among included studies, leaving it impossible to determine to what extent the studies were indeed weak and that to which the reporting was weak. Among included studies, those describing both evidence of recent human infection (direct, or indirect) or strong evidence of seroprevalence of antibodies to zoonotic influenza viruses among human subjects, and strong evidence of animal influenza infection were scarce. Koopmans[129] described direct evidence of human infection and strong evidence of poultry infection. Olsen[42] described indirect evidence of human infection and moderate evidence of swine infection. Puzelli[127] described strong evidence of seroprevalence of human antibodies to avian strains of influenza and strong evidence of poultry infection, however it should be noted that a significant time lag (> 1 year) between the epizootic among the poultry in that study and human testing occurred for one of the study sites. The remaining studies demonstrated lesser evidence on the human side or the animal side of the equation.

Table 4.3.-a: Methodological quality of the included studies:

Legend:

AIV= Avian influenza virus; SIV= Swine influenza virus

W = Weak; M= Moderate; S= Strong; NA = Not applicable

SS= Strong evidence of seroprevalence; WS= Weak evidence of seroprevalence; DE= Direct evidence of recent infection; IE= Indirect evidence of recent infection; ISE = insufficient evidence.

		Studies re: Exposure to poultry						
Study	**Outcome of interest**	**Selection Bias**	**Study Design**	**Confounders**	**Evidence of Human Infection**	**Evidence of Animal Infection**	**Data Collection Methods**	**Withdrawals and Drop-Outs**
Bosman 2004 & 2005	Human infection with A/H7N7	W	W	W	ISE	S	W	W
Buxton-Bridges 2002	Rates and risk factors for infection with A/H5N1	W	W	W	IE	W	W	S
Chen 2001	Infection with AIV	W	W	W	ISE	S	N/A	S
Koopmans 2004	Infection with A/H7N7	W	W	W	DE	S	W	S
Puzelli 2005	Evidence of anti-H7 antibodies	W	W	W	SS	S	W	W
Vong 2006	Presence of antibodies to A/H5N1	W	W	W	WS	S	S	S

		Studies re: Exposure to swine						
Study	**Outcome of interest**	**Selection Bias**	**Study Design**	**Confounders**	**Evidence of Human Infection**	**Evidence of Animal Infection**	**Data Collection Methods**	**Withdrawals and Drop-Outs**
Ayora-Talavera 2005	Prevalence of antibodies to SIV	W	W	W	SS	W	N/A	NA
Myers 2006	Detection of antibodies to SIV	W	W	M	SS	W	W	S
Olsen 2002	Detection of antibodies to SIV	M	W	W	IE	M	W	S
Olson 1977	Prevalence of antibodies to SIV	W	W	W	WS	W	N/A	S
Ramirez 2006	Presence of antibodies to swine influenza virus	W	W	M	WS	W	W	W
Schnurren berger 1970	Prevalence of antibodies to SIV	W	W	W	WS	W	W	W
Shu 1996	Infection with influenza viruses	W	W	W	Some--DE Some--WS	W	N/A	W
Wells 1991	Infection or illness following exposure to swine	M	W	M	WS	M	S	S
Zhou 1996	Presence of antibodies to SIV and AIV	W	W	W	Some—IE Some—SS	W	N/A	W

4.4 Data Tables: Data from Included Studies:

Data tables 4.4 (a), (b) and (c) below provide an overview of key findings among included studies.

4.4: Data Tables: Table 4.4-a: Data from studies re: Avian influenza infections of humans, 1999-2006:

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Bosman, 2004 & 2005 H7N7 2003 Netherlands	• ~4500 exposed people (estimated total associated with the outbreak)[90]; • 1300 targeted for inclusion in this study. • 500 asymptomatic people included in this study (excluded from Koopmans study of symptomatic exposed).	• Modified HI assay used; "measurable antibodies" not defined.	• The percentage of poultry farmers with eye complaints: about 5 times higher on infected vs. non-infected farms (14% vs. 2.4%); RR=5.2; 95% CI= 2.35-11.59. • A/H7N7 antibodies frequent in poultry farmers (63%) and workers exposed to infected poultry (50.6%). • Oseltamivir protects against conjunctivitis (OR=0.14; 95%CI=0.08-0.27) and infection without specific symptoms (OR=0.47; 95% CI=0.25-0.88). Drug was taken by 85 (48%) 185 of farmers on infected farms and by 456 of 604 (75.5%) outbreak control personnel. Prophylaxis interrupted by 324 (71%)-- forgetfulness and reduced drug availability. • No protective effect was demonstrated for safety goggles or mouth-nose masks. • Contact with chicken manure-- only factor with an elevated risk for conjunctivitis (OR=1.99; 95%CI=1.00-3.93), after correction for other factors. • Persons screening poultry on infected farms-- increased probability of H7 antibodies (OR=2.12; 95% CI=1.10-4.07) after correction for other risk factors. • Symptomatic infected people shed virus for more than 3 days. • The majority of the AI infections in examined groups were asymptomatic. • Estimated that A/H7N7 infection occurred in at least 1000 people and perhaps up to 2000.	• Not clearly stated what proportion of the 500 persons tested came from the poultry farmer, family or other worker groups. • Laboratory cut point not stated • Results data not shown—no information on the seropositive 50%, lab results, demographics, exposures. For example, some of the poultry farmers and their family members may have been from uninfected farms. • Details on contents of questionnaire not stated.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Buxton-Bridges 2002 1997-1998 Hong Kong	• 293 government workers; 1525 poultry workers (the latter not of interest in this review). GWs often wore protective clothing (e.g., gowns, gloves, masks) • All government workers were under 60 years; • median age 41 (range=22-58). • 85% of government. workers were male. • 22.5% of GWs smoked.	• Microneutralization followed by western blot test on paired serum samples. Considered positive by MN if anti-H5 titres of ≥80 were obtained. If positive by MN, confirmed by WB. Positives by both tests considered positive.	• Among GWs, 9 (3%) were both MN and WB positive on ≥1 sample. 78% of GWs (229/293) had paired samples. • Of 229 GWs with paired serum samples, 1 seroconverted. This person had respiratory illness. • Positives by age group: 0% (0 of 30) ages 22-29; 4% (6/166) among 30-44 yr olds and 3% (3/97) among 45-58 yr olds. • H5 seroprevalence rates of 3% (GWs) and 10% (PWs) suggest that a substantial number of mild or asymptomatic infections occurred in these occasionally exposed populations. • Smoking was found to be a risk factor for H5 antibody among GWs; being a current smoker was associated with seropositivity for H5 (5/66 vs. 4/223); P=0.03, Fischer's exact test. Smoking appeared to increase the risk only among those without preexisting antibody titres.	It is not known how many GWs worked on the farms (states "most" did); and if any had exposure on farms; also not stated what proportion of GWs wore PPE consistently or if they took antiviral drugs.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Chen 2001; China, 1998	• 1512 people in an occupational group exposed to poultry	• HI titres and viral isolation. • H5N1 tested by HI; titre of 1:20 or more was diagnosed to be positive; • The blood collection time was in intervals of about 2-3 months after their onset of disease.	• No positives among the cohort of interest (0/1512).	• Lab testing—HAI not recommended for avian influenza; also low cut point (≥1:20) • No demographic or other data described. • Not clear if tested for human strains. • "General exposure group" --no exposure history stated; but H9 isolated in 9. One of the 9 had HI titres 1:120-160 and others had 1:20. • Details of exposure of occupational group not outlined.
Koopmans (2004)	• ~4500 exposed (estimated)[90] • Recruited symptomatic exposed; n=453 suspected cases investigated. • 441 exposed to poultry; 12 exposed to symptomatic humans.	• RT-PCR and/or viral isolation;	• 82 primary cases found confirmed by RT-PCR and/or viral isolation; • Mean age=30.4 yrs; gender not specified. • Mostly mild illness; however one death occurred. • Most experienced conjunctivitis (n=75); conjunctivitis + ILI (n=5); only 2 had solely ILI.	• Case-finding study; investigated exposed symptomatic persons. Thus, total number infected could be grossly underestimated; asymptomatic and mildly ill cases may have been missed. • Viral load data not reported.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Puzelli 2005 Italy 1999-2003; H7N3 and H7N7	• 983 farm workers from several categories of labour.	• Microneutralization used on each sample and each tested twice. • Considered positive if had s >20 twice. • Positives tested by western blot. • HI and SRH also used.	• Unequivocal serological evidence of exposure to or infection with H7 viruses in 7 subjects. • 7/185 (outbreaks 5 (n=43) and 6 (n=142) reactive to both viruses by microneutralization • All seropositive subjects had close direct physical contact with either turkeys or chickens in (dusty) poultry housing. • 7 positive results 3 male; all were 35-62 years old; 3 female; data missing for 1.	• Samples collected from workers >1 year post-outbreak (outbreak #4). • No specific analysis of risk factors. • Nature of exposure within each occupational group not described.
Vong 2006 H5N1 2005 Cambodia	• 351 persons in the affected village; • 166 persons from households where no chickens died; and, • 96 from households with a high probability of an outbreak.	• Serologic evidence of infection defined as H5N1 neutralizing antibody titre ≥80 with a confirmatory western blot.	• No positives; None (0/351) • Transmission of H5N1 viruses from infected poultry to humans appears to have been low in rural Cambodian population with confirmed and suspected poultry outbreaks and where a human case occurred in 2005. • None of the villagers interviewed reported having a febrile or respiratory illness in the past year. • Households purchasing live poultry in past year were almost 4 times more likely to have had H5N1 in their flock than households that did not buy live chickens. •	• 12 months recall period. • Time lag from exposure to testing may have impacted on serology. • Did not state if possible contact with the case patient was identified or controlled for. • Temporal association between behavioural risk factors and poultry infection was difficult to establish. • Unconfirmed poultry infection status may have contributed to misclassification of exposures.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant; Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Table 4.4-b: Data from studies re: Swine influenza infections of humans, 1970-2006:

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Ayora-Talavera, 2005	115 presumed exposed **# unexposed** None	• HI titre cut point ≥1:40 • Serum treated; controls used to rule out induction of non-specific hemagglutination.	• The 15-24 year old age group were most commonly seropositive (H1 or H3 not specified). • Overall, 31 (26.9%) of 115 samples were positive to H1 and 93 (80.8%) were seropositive to H3; however applying the cutoff values in this study, seropositivity to swine H1 virus was only detected in 2 samples from persons 43 and 59 years of age. Weaker reactions were noted in 4 other persons 33-55 years of age, which could indicate previous exposure to viruses of swine origin, according to authors, a situation that has not occurred in persons >30. • The weak reactivity to H1 virus could suggest a past exposure of adult persons to viruses of swine origin. • The RR of being seropositive for H1 or H3 viruses from exposure to pigs was 1.93 with human H1 (95%CI, 1.2-3.0); 0.88 with human H3 (0.55-1.4); 0.6 with swine H1 (0.08-4.2) and 1.0 with swine H3 (0.62-1.6). • The highest seropositivity rates across all age groups were detected with the A/Sw/Minnesota (H3N2 reassortant) virus as antigen, taken from American pigs. However, the NA, HA and PB1 genes are of human origin.	• It cannot be concluded that the seropositivity noted in this study is a result of exposure to swine; due to lack of detailed exposure histories or any information about study participants. • Assumed the study population exposed to swine, but also noted that mixed exposures: pigs, chickens, ducks likely, based on knowledge of animal husbandry practices in this region of Mexico.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Myers, 2006	273: 111 farmers; 97 meat processing workers; 65 vets **# unexposed** 79; volunteers: not randomly selected; no information gathered	• HI titre cut-point ≥1:40	In dichotomous comparisons: • Farmers had much greater odds than did control subjects of being seropositive (titre >40) against both the swine H1N1 virus (17.4% vs. 0%; OR, 22.9; 95%CI, 3.9-∞) and the swine H1N2 virus (20.7% vs. 1.3%; OR, 20.7; 95%CI, 2.5-172.1). • Veterinarians also had increased odds of being seropositive for the swine H1N1 virus (10.9% vs. 0%; OR, 12.8; 95%CI, 1.9-∞). And the swine H1N2 virus (19.1% vs. 1.3% OR, 18.1; 95% CI, 2.3-138.8). • Meat processing workers had no increased odds of seropositivity against any swine virus (data not shown). • All 3 exposure groups had a high prevalence of antibodies against the swine H3N2 isolate, but none of these prevalence values were significantly different than the controls' (data not shown). • Elevated HI titres against sw H3N2 isolate were associated with having received a 2003-2004 influenza vaccination as well as with presence of others in the household. • Among all groups, elevated titres against swine H3N2 were associated with having elevated titres against human H3N2 strains, suggesting cross reactivity.	• Lack of detailed exposure information for the farmer group. • Study design did not allow researchers to determine whether individuals developed clinical symptoms with seroconversion; • It is possible that the elevated titres compared by proportional odds modeling do not correlate with infection.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Olsen, 2002	74 farm owners, employees, their family members and veterinarians **# unexposed** 114 urban controls; not randomly selected; no info gathered on controls; no serum tested.	• HI titres ≥40 considered positive. • The farm cohort had pre and post season titres evaluated (4-fold rise).	• Seropositivity to SIV significantly ($p<0.05$) associated with being a farm owner / farm family member, living on a farm, or entering the swine barn >4 days a week, being >50. Also associated with: having received swine flu vaccine in 1976-77 (n=4) or other influenza virus vaccine. • 17/74 swine farm participants had significantly higher ($p<0.001$) positive HI antibody titres >40 to SIV than 1/114 urban controls. GMTs significantly higher $p<0.001$ among farm participants.	• Multivariate analysis was not done because of the small number of participants with elevated preseason titres to swine influenza viruses.
Olson, 1977	• Exposed (61) and un-exposed (56) farm employees at the Taiwan Sugar Corporation and their family members. • Blood specimens also obtained from unexposed outpatients in urban Taipei with complaints other than upper respiratory illnesses.	• HI tests performed at the CDC Atlanta. • Cut point was ≥1:10.	• Higher prevalence (29%) among exposed 20-29 year-olds than TSC (7%) and Taipei (2%) controls; ($p<0.12$ and $p<0.07$ levels, respectively, n/s). GMT was higher than in either control group $p<0.04$.; • Higher antibody prevalence (19%) among exposed 30-39 year-olds than TSC (6%) or Taipei (6%) controls ($p<0.08$ and $p<0.06$, respectively, n/s). GMT higher among exposed than in either control group ($p<0.08$ [n/s], $p<0.008$). • Antibody prevalence among 40-49 year olds: not greater in exp (8%) vs. unexp, TSC (10%); Taipei (31%). GMT--slightly greater in exposed (5.62) vs. unexposed TSC workers (5.37); $p<.39$ (n/s--may be due to chance). • Antibody prevalence in exposed ≥50 yrs not statistically significantly different than unexposed, $p>0.3$. GMT lower among exposed than either control group.	• Definition of positive titre ≥1:10; substantially lower than current day studies • No virus isolation among humans • Single specimens were collected a year after the epizootic in swine.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p\leq0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Ramirez 2006	49 confinement workers **# unexposed** 79 controls enrolled in a concurrent study at the U of Iowa Not matched but age distribution similar	• HI titre levels grouped <10; 10; and >10 • Not stated if lab controls used.	• Persons who received the 2003-04 flu vaccine were significantly more likely to have elevated titres (≥10) against swine H1N1 virus as well as swine H1N2. • A cross-reaction with 1 of the viruses in the vaccine or a circulating flu virus may explain this; higher titres would have been expected for all vaccinated persons (including controls),but this was not observed. • Suggest this represents other behavioural or health-related confounders not included in the questionnaire for this study. • Workers who sometimes or never used gloves were significantly more likely (OR 30.3, 95%CI 3.8-243.5) to have elevated titres to H1N1 than the nonexposed controls. These workers were also significantly more likely to have elevated titres than the other confinement workers who used gloves most of the time or always. (OR 12.7, CI 1.1-151.1) • Workers who reported smoking also had high OR (data not shown) for elevated titres to H1N1. Those who smoked (OR 18.7) most frequently had evidence of previous H1N1 swine virus.	• Not stated if controls were matched, so assumed not; however, age distribution was similar for the two groups. • Small sample size • Lab data on how/ if cross reactions were controlled for was not outlined. • Lab results were grouped but data (titre levels) not shown. • Language barriers were cited as an issue in communicating with swine confinement workers.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Schnurrenberger1970	• 168 pork producers, 248 veterinarians or veterinary students, 551 packing plant employees, 24 hog buyers or vocational agriculture teachers, and 13 pork producers' wives or daughters. • Unexposed: 816—298 general population; 518 premarital blood samples.	• HI testing used to test treated serum samples. 307 of 332 samples were tested against 2 swine viruses agreed within a twofold dilution (92.5%); and 328/332 (98.8%) within a fourfold dilution. • A titre of $\geq$1:20 was considered reactive.	• Reaction rates varied by occupational group; 15% among producers to 45% among abattoir workers; age adjustment decreased the rates slightly. • Prevalence of titres against swine influenza virus increased directly with age; antibody detected in fewer than 3% of persons born after 1935 in contrast to 73 % of those born before 1920. • No difference was noted in the reactor rates for persons who were veterans of the armed forces and those who were not. No serum from the producers' wives and daughters was reactive. • Among persons who had been vaccinated in the year before sampling, the crude reactor rate was 41%, in contrast to 20% among unvaccinated persons. Age adjusting reduced but did not eliminate the difference by vaccination history. • Reactor rate for veterinarians was 34.4% in 1966 and 35.3% in 1968. A small difference in reactor rates of vets with moderate or greater exposure and those with minimal exposure. • Marked difference in reactor rates within group 3 of the general population when examined by collection date: 1.2% for those collected before July 1965 and 41.8% after Oct 1966; age adjusted rates= 4.9% and 45.2% respectively—no reason could be identified for this finding.	• Lack of random selection—cannot generalize results to the occupations represented. • Did not control for cross-reactions (did control for non-specific inhibition) • Veterinarians: Less detailed personal information collected with second serum sample than first. • General population group: Assessed record of community influenza outbreak with second serum sample. No outbreak of respiratory disease reported; acknowledged that it could have been sub clinical or unreported.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p$\leq$0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
	•	•	• No significant difference found between the abattoir workers in departments having close swine contact and those having no direct contact with animals or product. Rates unchanged by age adjustment; could have been due to vaccination or spread within the abattoir. • Abattoir workers and general population: Both sexes were adequately represented to permit calculation of valid sex-specific rates: 196/451 males (43.5%) and 50/100 female abattoir workers; 21.1% (79/375) for men and 14.7% (61/416) for women in the general population. Age adjusting the data reduced the sex difference to 4% in both populations.	•
Shu, 1996	• 20 Farm families who raised pigs and ducks in their homes	• Virus isolation studies of human throat swabs, duck fecal samples and nasal swabs from pigs. • Isolates were identified by HI testing with a panel of monospecific antisera. • Human serum samples tested. • Sera tested with HI and NI assays for antibodies against human, pig and duck influenza viruses. • An HI or NI titre of $\geq$1:20 considered positive.	• RR of one or more family members being seropositive for H4N4 or H7N4 viruses for exposure to ducks testing positive for one of these viruses was 1.1 (95% CI; 0.3-3.9; n/s). • While no evidence was found for genetic reassortment of viruses, findings do support the concept that intermingling of humans, pigs and ducks on Chinese farms is favorable to the generation of new, potentially hazardous strains of influenza virus. • 8/156 human serum samples inhibited the neuraminidase activity of two of the duck isolates, raising the possibility of interspecies transmission of these avian viruses.	• The same person in each household was not tested at each visit. Therefore, each individual may have been tested more than once and some may not have been tested. • Detailed exposure histories not collected. • Sampling focused on symptomatic exposed individuals.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p$\leq$0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Wells, 1991		• HI titres ≥20 considered positive.	• More unexposed exhibitors had lived on a farm where pigs were raised than had exposed exhibitors (P=0.05) and the mean number of years of exhibiting pigs was greater for those who were unexposed (P>0.05). • Significantly more (31 of 50) exposed exhibitors than unexposed exhibitors (3 of 50) reported having exhibited a pig with ILI either at the time of their county fair or afterward (P<0.0001). • Among the 25 exposed exhibitors providing a serum specimen, 19 (76%) including 5 of 6 who were first-time exhibitors, had an SIV Hi titre of 20 or more, while none of the unexposed exhibitors had levels detectable at a dilution of 10 and therefore were reported as less than 10 (P<0.0001, chi square, Yates corrected) • Significantly more exposed (7/50) than unexposed (1/50) exhibitors had ILI in September RR, 7.0; 95%CI, 1.3-3.5; P=.03. Five of these 7 exposed who were ill reported onsets within 5 days of exposure to the pigs who were ill at the fair and also had an SIV HI titre of 20 or greater.	• All 156 exhibitors were considered exposed due to the swine being kept in the same barn, however, information on illness among the specific swine of those exhibitors tested, and the movement of exhibitors and swine within the barn is not provided, nor is information about the swine on the exhibitors' home farms. • Single serum sample testing prevents linking the fair with the antibody response among the exhibitors.

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Subjects	Laboratory methodology	Summary of Key Findings:	Limitations
Zhou, 1996	• 268 slaughterhouse workers and 200 women who raised pigs--also raised ducks and worked in rice fields; ages ranged 18-50, mean=31.3 yrs. • people who had little or no contact with pigs (200 university students at local medical school; 19-22 yrs; mean= 20.7 yrs; • people living and working in the US (32 employees at St. Jude Children's research Hospital);	• Virus isolation and serologic testing (twice at 6 month intervals) for slaughterhouse workers and once for other subjects. • Serology used HI and NI assays to test for antibodies against human, swine and avian influenza viruses. • In the swine virus testing, NI testing was not performed on the 205 student controls, but was done on the exposed populations and the 32 Memphis controls. • A modified ELISA method was employed for detection of influenza virus antibodies of low titre, especially avian virus antibodies in humans and pigs. • Serum from Nanchang and Memphis controls also tested by ELISA.	• Since results for the human-like swine virus may reflect the triggering of antibody memory generated in response to exposure to recent H3N2 strains, the serologic data do not indicate transmission of swine viruses to humans in Nanchang. • Rates of reactivity and antibody titres with characteristic swine viruses were essentially the same in slaughterhouse workers and pig farmers as in students who were not exposed to pigs. • H7 duck virus-- highest reactivity rates found in women raising pigs and ducks in houses and who worked in rice fields (25% with maximum titre of 800); remaining groups had low or negligible rates. H11N2 virus: Nanchang slaughterhouse workers and Memphis controls: 26% positivity rate with H11N2 virus and the NI assay in Nanchang slaughterhouse workers; may reflect cross reactivity with human N2 strains. • N8 antigen of Nanchang/1681/93 detected in 2 slaughterhouse workers (H3N8). Since virus used was H3N8, assay was repeated with HA of H7N8 (A/equine/Prague/1/56and N8 NA (H7N8) as the antigen, to confirm reactivity in human sera to N8 antibodies.	• Methodology not thoroughly outlined; • Mixed exposures possible; not detailed, • Duck feces sampled may have represented visiting wild birds and/or resident domestic ducks

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Table 4.4-c: Summary of key results of included studies: Exposure to poultry

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)	Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Bosman 2004 & 2005 n=500 tested	• Human infection with A/H7N7; • Tested for seroprevalence but did not use a recommended test for avian influenza viruses (insufficient evidence).	• Contact with manure associated with conjunctivitis.	• OR=1.99; 95%CI, 1.00-3.93; • n/s	• A/H7N7 antibodies: farmers=63%; • Workers exposed to infected poultry =50.6%	• n/a	• Taking antivirals protected against conjunctivitis; OR=0.14; 95% CI, 0.25-0.88. • No protective effect noted for goggles or masks.
		• Screening infected poultry increased probability of H7 antibodies.	• OR=2.12; 95% CI, 1.10-4.07.			
Buxton-Bridges 2002 n=293 government workers	• Rates and risk factors for infection with A/H5N1; • Indirect evidence of infection (paired sera with controls).	See prevalence.	n/a	• 3% seroprevalence among government workers (9/293)	• Smoking associated with seropositivity; 5/66 vs. 4/223; P=0.03.	• No data on the effect of wearing PPE.
Chen 2001 n=1512	• Infection with AIV • Insufficient evidence (used inappropriate test).	n/a	n/a	0/1512 positive	n/a	n/a
Koopmans 2004 n=453	• Infection with A/H7N7 • Direct evidence—viral isolation and/or RT-PCR.	n/a	n/a	• Seroprevalence not tested. 82 cases confirmed.	n/a	n/a

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)	Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Puzelli 2005 n=983	• Evidence of anti-H7 antibodies • Strong evidence of seroprevalence (single sample, appropriate testing + controls).	n/a	n/a	7/185 tested positive (outbreaks 5 & 6); 3.7% seropositivity.	n/a	n/a
Vong 2006 n=351	• Presence of antibodies to A/H5N1 • Weak evidence of (lack of) seroprevalence; appropriate test used no mention of controls.	n/a	n/a	0/351 positives.	n/a	n/a

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Summary of key results of included studies: Exposure to swine

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)	Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Ayora-Talavera 2005 n=115	• Prevalence of antibodies to SIV • Strong evidence of seroprevalence (appropriate test plus controls used).	Not significant (n/s)	• RR being seropositive for swine H1 (0.6; 95%CI, 0.08-4.2) (n/s); H3 (1.0; CI=0.62-1.6) (n/s).	• Swine H1N1: 4% (35-44 year olds); 8% (45-53 year olds); • Swine H3N2 (reassortant): range 66% (35-44 year olds) to 88% (5-24 year olds).	n/a	n/a
Myers 2006 n=273; 111 farmers; 97 meat processing workers; 65 veterinarians; 79 controls.	• Detection of antibodies to SIV. • Strong evidence of seroprevalence.	Compared with controls: Farmers and Veterinarians had increased odds of seropositivity; Meat processing workers had no increased odds of seropositivity	Farmers vs. controls: H1N1: OR=22.9; 95%CI, 3.9-∞; H1N2: OR=20.7; 95%CI, 2.5-172.1	• Farmers: 17.4% to H1N1; 20.7% to H1N2	Type of work appears to increase odds of seropositivity	n/a
			Veterinarians vs. controls: H1N1: OR=12.8; 95%CI, 1.9-∞; H1N2: OR=18.1; 95%CI, 2.3-138.8	• Veterinarians: 10.9% to H1N1; 19.1% to H1N2;		
			Meat processing workers: data not shown.	• Meat processing workers: data not shown.		

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)		Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Olsen 2002 n=74 farmers; 114 controls	• Detection of antibodies to SIV • Indirect evidence of infection (paired sera with controls).	Seropositivity associated with being in the exposed group	17/74 significantly higher titres(>40) than controls (1/114); p<0.001 Geometric mean titres significantly higher among farm participants (p<0.001).		GMTs to A/Nebraska/01/92 H1N1: farm participants=13.2*; controls=5.1 GMTs to A/Swine/Indiana/1726/88 H1N1: farm participants =15.7*; controls =5.4 **p>0.0001 using Wilcoxon rank sum analysis with normal approximation.*	Being a farm owner, family member, living on a farm; entering swine barn >4 days / week, being >50 years old, and having received swine flu vaccine or any flu vaccine.	n/a
		Being a farm owner	HI≥40 p=0.04	HI≥80 p=0.02			
		Farm owner or family member	p=0.03	p=0.02			
		living on a farm	(p=0.07)	p=0.04			
		entering barn >4 days a week	(p=0.12)	p=0.04			
		Age >50 years	p=0.02	p=0.03			
		Receipt of swine flu vaccine 1976-77	p=0.02	(p=0.44)			
		Receipt of other influenza vaccine	p=0.03	(p=0.19)			

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)	Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Olson 1977 n=61 exposed; 56 unexposed	• Prevalence of antibodies to SIV • Weak evidence of seroprevalence; single serum sample tested; no controls noted.	Higher prevalence of antibodies to swine virus among exposed vs. controls	20-29 year olds: exposed=29% (2/7) prevalence controls: TSC=7% (1/15) and Taipei=2% (1/46); p<0.12 and p<0.07, respectively.		Being exposed and under 40 years of age.	n/a
			30-39 year olds: exposed =19% (6/32) prevalence; controls: TSC=6% (1/17); Taipei=6% (2/34); p<0.08 and p<0.06, respectively.			
			40-49 year olds: exposed =8% (1/13) prevalence; controls: TSC=10% (1/10); Taipei=31% (8/26);			
			50 + year olds: no statistical difference in seroprevalence; p>0.3. GMT lower in exposed than either control group. Exposed=67% (6/9); controls--TSC=57% (8/14); Taipei=80% (49/61).			
Ramirez 2006 n= 49 swine workers; 79 controls	• Presence of antibodies to swine influenza virus • Weak evidence of seroprevalence.	Sometimes or never wore gloves	OR for having elevated titres to H1N1compared to controls: 30.3; 95% CI, 3.8-243.5	n/a; data not shown.	Smoking	Wearing gloves
		Most of the time or always wore gloves	OR 12.7; CI, 1.1-151.1			
		Smoking and seropositivity	OR 18.7; CI data not shown			

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)	Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Schnurrenberger 1970 n= 168 pork producers; 248 veterinarians; 551 packing plant workers; 24 hog buyers or vocational teachers; 13 producers' wives or daughters; 816 unexposed.	• Prevalence of antibodies to SIV • Weak evidence of seroprevalence	n/a	n/a	Age adjusted rates: Producers: 19.9% Veterinarians: 39.7% Packing plant workers: 46.0% Hog buyers: 22.2% Producers' wives and daughters 0% Unexposed: 38.8%	n/a; query cross-reaction with circulating strain of human flu as evidenced by high prevalence among unexposed group (38.8%).	n/a
Shu 1996 n=20 farm families (174 people).	• Infection with influenza viruses • Some participants: viral isolation used (strong evidence of infection); others—weak evidence of seroprevalence.	n/a for exposure of interest in this review	n/a for exposure of interest in this review	n/a	n/a	n/a
Wells 1991 n= 50 exposed; 50 controls	• Infection or illness following exposure to swine • Weak evidence of seroprevalence.	Exposure associated with ILI	7/50 exposed vs. 1/50 unexposed had ILI in the month of interest; RR, 7.0; 95% CI, 1.3-3.5 p=0.03.	19/25 (76%) had an SIV HI titre of 20 or more; none of the unexposed had detectable levels (HI titre of 10); p<0.0001.	Exposure to ill pigs	n/a

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=p≤0.05; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

Study	Outcome of interest and quality ranking	Association between exposure and outcome of interest	Strength of association (measures of association)	Prevalence of antibodies to zoonotic strains of influenza	Factors more strongly associated with infection or seroprevalence	Factors which have a protective effect
Zhou 1996 n= 232 controls; 268 slaughterhouse worker; 200 women raising pigs;	• Presence of antibodies to SIV and AIV • Some: strong evidence of infection (viral isolation); others: strong evidence of seroprevalence	n/a for exposure of interest in this review	n/a for exposure of interest in this review	n/a	n/a	n/a

OR=Odds Ratio; RR= Relative Risk; GMT= Geometric Mean Titre; n/a= not applicable; n/s= not significant;
Cut point for significance=$p \leq 0.05$; SIV= Swine Influenza Virus; AIV= Avian Influenza Virus;

4.5. Description of the Manitoba population at potential risk.

An objective of this review was to identify and describe the Manitoba population at potential risk of exposure to and possibly, infection with, influenza viruses of zoonotic origin in agricultural settings. The following section describes Manitoba's poultry and swine industry and those who work in this segment of Manitoba's agriculture sector. However, it must be emphasized that the results of this review must be applied to the population described here, such that if sufficient evidence is found to describe the likelihood of becoming infected with zoonotic strains of influenza in either poultry or swine farm settings, then this may have implications for this portion of the population. On the other hand, if insufficient evidence is found, it does not necessarily mean that there is no risk for these workers, but rather that insufficient evidence was identified in the literature.

4.5.1. Overview of available Statistics on Manitoba's Agriculture Sector as a whole, and the Swine and Poultry Industries specifically:

a) Statistics Canada Definitions:

Census Farm: An agriculture operation that produced at least one of the following products for sale: crops, livestock (includes pigs), poultry (hens, chickens, turkeys, chicks, other poultry), animal products or other agriculture products.
Farm Operator: A person responsible for the day-to-day management decisions made in operating a census farm. In 1991, 1996, and 2001, >1 operator could be reported for each farm.

b) 2001 Statistics for Manitoba's Agriculture Industry as a whole:

Farm operators by sex, age, education and country of origin are described, but not specific to type of farming. Note that the statistics described here reflect Manitoba's Agriculture sector generally, and available statistics are only broken down by commodity type for the purposes of outlining production volumes and other economic indicators, and do not describe the population who works in the specific segment of the agriculture sector.[15] The 2006 Census of Agriculture, not yet released by Statistics Canada at the time of writing, promises to provide comprehensive data on farms and the people who manage them.[137]

Statistics on Manitoba's agricultural industry indicate that in 2001, there were 19,818 farms in Manitoba; 4.9% were hog farms; 1.4% were poultry and egg farms; and 1.8% had a combination of livestock.[15]

Total number of census farm families:	16,425
Average size of a census farm family:	3.3 persons

Actual number of hours worked per week by industry, seasonally adjusted (monthly)

- Agriculture in July 2006 = 15,138.6 hours/week

Employment by major industry groups, seasonally adjusted (monthly)
- Agriculture in July 2006 = 28.9 (thousands)

Distribution of employed people, by industry, by province;
- Agriculture, Manitoba, 2005= 30,000.

c) Swine production in Manitoba, 2nd quarter 2006 (reported quarterly):

Total:	3,024.0 thousand head	
Breeding stock:	381.2	
Boars ≥6months		5.2
Sows ≥ 6months		376.0
All other pigs	2.642.8	
<20 kg		934.4
20-60		915.9
>60		792.5

d) Poultry production in Manitoba:

Statistics Canada Poultry Production Statistics, Manitoba, 2005[15]:
- Chickens=29,125 (thousand birds)
- Turkeys= 1,432 (thousand birds)
- Eggs (thousand dozens) = 85,135

Description of the industry:
There are approximately 400 to 450 commercial poultry operations in Manitoba. This includes approximately 124 meat chicken operations, approximately 172 egg producing operations, approximately 65 hatching egg operations, and 66 turkey operations.[138]

Manitoba's $180 million industry employs more than 1500 people. Manitoba poultry farms are typically family run operations and most are located in the southeastern portion of the province. The average number of birds per flock is 10,000 to 30,000. Three processors package poultry products for sale to local and international markets.[18]

Chicken and Egg:
The chicken industry is encompasses both chickens for meat and egg production. Each has its own parent breeding stock of chickens.[75] Egg producing chickens are kept longer than meat chickens, with the latter being kept for about 45 days. Both live in confinement barns under strict biosecurity. Agriculture experts indicate that unless avian influenza were to be introduced, there is little opportunity for disease to be maintained among these flocks.[75]

The average flock size of laying hens is 13,500 birds (range=603-125,000). A small flock of poultry is generally considered to have less than 1,000 birds.[75] In 2006, there were 2200 backyard flocks registered in Manitoba.[139] Many poultry farms in Manitoba are mixed farms, meaning they have more than one type of bird.[75]

Turkeys:
Turkeys are commonly let outside, and so have increased opportunity for infection with avian influenza through contact (direct or indirect) with wild waterfowl which may carry influenza viruses. If turkeys do become infected with influenza, they generally tend to show more mild clinical symptoms than do chickens, so infection may go un-noticed. A swine-adapted H3N2 influenza virus has recently been detected among Manitoba turkey breeding flocks, in spite of widespread vaccination of the birds and adherence to recommended biosecurity protocols by the farmers.[75]

For a detailed risk assessment of the likelihood and potential impact of an avian influenza outbreak occurring among commercial poultry in Manitoba, refer to the Manitoba Agriculture, Food and Rural Initiatives' Vulnerability Assessment: Avian Influenza Introduction into Manitoba Domestic Poultry and Swine.[75]

4.5.2 Description of the people who work in the Manitoba swine and poultry industries:

a) Statistics Canada: 2001 Statistics for Manitoba's Swine and Poultry Industry Workers:

- Total farm operators in Manitoba: 28,795
- Hog operators: 1,360
- Poultry and egg operators: 475
- Livestock combination: 555

- Farm census families (represents all farms, including crops, livestock and other products)
- Note: only unincorporated farms are included because incorporated farms are legal entities.

Statistics are not available from Statistics Canada for the number of workers in Manitoba's swine and poultry industries, aside from those defined as farm operators. More than one farm operator can be involved in a single farm. However, based on the total number of persons working in agriculture in Manitoba cited above (30,000 people), this comprises a maximum of 2.5% of the provincial population that might have some degree of contact with poultry and/or pigs. This is expected to be a gross overestimate of the number of Manitobans who have contact with pigs and/or poultry at any given time, as these statistics include crops and other types of livestock, and not all agricultural jobs require contact with livestock and particularly with poultry and / or pigs.

However, the 2001 Census of Agriculture does describe Manitoba's farm population. The following highlights provide some insight into the population which would be affected by an avian influenza outbreak. These highlights pertain to the total agricultural population of Manitoba, and are therefore not specific to swine and poultry farmers:

- 6.2% of Manitoba's population lived on farms in 2001 as compared to 7.2% five years earlier. The 2001 Manitoba farm population was 68,135, which is a 14.7% decrease from 1996.
- In 2001, 28,795 farm operators managed 21,071 farms. 77% (22,230) of these operators were male and 22.8% (6,565) were female.
- Approximately 98.6% of the farm population resided on the farm in 2001, which is slightly more than in 1996.
- Approximately 14% of operators were under 35 years of age; approximately 54% were 35-54 and 32.5% were 55 years and older
- About 83.4% of Manitoba farm operators were married in 2001.
- The top four languages reported as mother tongue by operators in Manitoba were: English (72.5%); German, (12.9%); Ukrainian (5.2%); and French (5.0%).
- The average farm family income for unincorporated farms in 2000 was $49,826.
- 18,695 or 65% of Manitoba farm operators were involved in only in farming and other agricultural operations, whereas the rest also had a non-agricultural occupation.
- In 2001, on average, Manitoba farmers had 11.6 years of schooling, up slightly from 1996.[15]

These statistics are useful for informing preparations for an outbreak on a Manitoba farm, for example to target educational materials to the most appropriate education level, and languages most commonly used. Furthermore, it must be recognized by planners that with ~35% of farm operators holding down a non-agricultural job in addition to their farm responsibilities, paired with a relatively low average farm family income (average of 3.3 people per census farm); recommended public health measures such as self-isolation following exposure to avian influenza virus may not be followed by farmers who need to work at their non-agricultural job.

b) Swine Industry:

According to the Manitoba Pork Council,[140] there are approximately 2244 Manitobans exposed to live pigs in the province at any given time. This is based on the following estimated calculations:

- A total of 1874 farmers who work directly in the barn:
    - 360,000 sows, requiring one employee per 300 sows, for a total of 1200 employees.
    - 3 million nursery pigs, 6.5 turns, 2700 pigs per employee; 2 employers, for a total of 340 people.
    - 2 million finishers, 3 turns per year, 2000 pigs per employee, 333 employees.
- 200 truckers, not counting farmers who also truck;
- 100 slaughter plant workers who work in the barn; all others work with dead pigs;
- 70 Veterinarians.

Official statistics on the number of workers who work directly with swine are not available from the industry or Statistics Canada.

c) Poultry Industry:

Chickens and Eggs:
For flocks of less than 5000 birds, approximately two people are required to care for the birds. An average of approximately eight people work on each average size farm, including the owner, family members, weekend egg collectors and transport workers.[141] Official statistics on the number of Manitobans in contact with chickens on either commercial farms or associated with backyard flocks are not available from the industry or from Statistics Canada.[141] Backyard flocks are those small flocks raised by families for personal consumption, hobby, or show birds, i.e., those not for commercial production and sale.

Turkeys:
There are six turkey breeder farms and 58 commodity turkey farms in Manitoba. Approximately 20-25 of the 58 commodity farms are Hutterite colonies, with approximately 100 people living on an average size colony. Many turkey producers also have swine and some turkey producers operate two locations. Approximately 80% of turkey producers have more than one commodity on their farm, e.g., swine, crops, other livestock. Therefore, it is very difficult to estimate the number of Manitobans in contact with turkeys at any one point in time, and specific statistics, even estimates, are not available.[139]

While formal statistics are not available for the number of workers in Manitoba's poultry industry, information from the Manitoba government website suggests that 400 poultry farms (400 operators) employ over 1500 people, so at a minimum, there could be approximately 1900 people exposed to poultry in Manitoba. This is a rough estimate and it is not known how many of the people employed in the industry actually have direct contact with poultry.

4.6. Analysis of results:

The following section provides the results of the analysis of included studies, including:

Descriptive analysis of these findings with respect to the objectives of the review:

- To identify and evaluate published evidence regarding the association between exposure to domestic swine or poultry in agricultural settings and human infection with influenza viruses of zoonotic origin;
- If an association is found between exposure and infection:
  - describe the strength of that association;
  - identify and describe factors which are either more strongly associated with infection or have a protective effect;
  - identify and describe the prevalence of zoonotic influenza infections among people exposed to swine or poultry in agricultural settings.

4.6.1. Studies reporting direct evidence of recent human infection with zoonotic influenza viruses associated with exposure to animals of interest in an agricultural setting (n=1).

During the A/H7N7 avian influenza outbreaks among commercial poultry in the Netherlands in 2003, Koopmans[20] and colleagues performed an active case finding investigation among poultry workers, recruiting symptomatic exposed workers for testing using a combination of viral isolation and RT-PCR testing. Of the 453 workers presenting with health complaints, 441 of these people were exposed to poultry, 349 people experienced conjunctivitis, 90 had influenza-like-illness and 67 had other complaints. Eighty-two primary cases were confirmed by RT-PCR, virus isolation or both. Questionnaire data was missing for two subjects and two workers with positive viral isolation did not meet the case definition. The researchers concluded that veterinarians and people who cull infected poultry have the highest risk of A/H7 influenza virus infection. Since this was a case-finding study, the results can be expected to underestimate the number of infections, as asymptomatic or mild infections would have been missed. However, among all included studies, this study represents the strongest evidence of human infection with zoonotic influenza viruses associated with exposure to infected animals of interest. The laboratory evidence of infection was strong for both humans and the poultry they were exposed to in this study.

4.6.2. Studies reporting a measured and / or statistically significant association between exposure to animals of interest and human infection with zoonotic influenza viruses (n=5).

The studies discussed below are mixed in their definition of human infection with zoonotic influenza viruses. Some studies equate infection with seropositivity, e.g., past infection; and others have looked for evidence of recent infection associated with an exposure to a group of animals of interest. The serological methods therefore vary, with some studies testing single human serum samples for presence of antibodies, and one study using paired samples. Among the studies using hemagglutination inhibition (HI)

testing, antibody titre cut-points used to define seropositivity varied, ranging from ≥1:10 to ≥ 1:40. Two studies[128,94] reported statistically significant associations for specific factors modifying the association between exposure and human infection, but did not state an overall association between exposure and infection, so these are discussed in the section below on modifying factors.

a) Exposure to poultry (n=1):

The two reports by Bosman[124, 125] on their population-based study of poultry workers and their families in the H7N7 influenza outbreak among commercial poultry in The Netherlands in 2003 reported a statistically significant association between exposure to infected poultry and having antibodies against H7 avian influenza virus, OR=2.12; 95% CI=1.10-4.07 after correction for other risk factors. Thus the odds of having H7 antibodies were found to be twice as high for those exposed to poultry than the odds of having H7 antibodies among those not exposed.

These results need to be interpreted with caution, within the context of several limitations. Of the study population tested for antibodies, it is not clear how many people had what type of exposure, e.g., infected vs. uninfected farms, protected exposure vs. unprotected, so misclassification may have occurred. The laboratory test used was one not recommended by laboratory experts for the detection of antibodies to avian influenza viruses (hemagglutination inhibition testing). Furthermore, the cut-point for establishing seropositivity (or not) was not stated. Several potentially confounding variables were not reported for this study population, and so it is not clear if these variables were taken into account in the analysis. Data were not shown for the OR calculation given above.

b) Exposure to swine (n=4)

Myers[33], Olsen[42], Olson[130], and Wells[129] all reported an association between exposure to swine and evidence of human infection. All of these studies except Olsen[42] used laboratory methods sufficient to determine seropositivity to swine influenza viruses. Olsen[42] used paired serum samples in their farm cohort to enable comparison of pre-and post-season titres.

Myers[33] compared the odds of seropositivity (HI ≥40) among various occupational groups to the odds of seropositivity among unexposed controls, for both H1N1 and H1N2 swine influenza viruses. The greatest odds of being seropositive for these viruses were found among the farmer cohort, followed by the veterinarian cohort.

In dichotomous comparisons, the odds ratio for farmers vs. controls for H1N1 was 22.9 (95%CI, 3.9-∞); and, for H1N2 was 20.7 (95%CI, 2.5-172.1). The odds ratio for veterinarians vs. controls for H1N1 was 12.8 (95%CI, 1.9-∞); and, for H1N2 was 18.1 (95%CI, 2.3-138.8). Age, sex and homologous human influenza strains were adjusted through unconditional logistic regression. This process yielded the following ORs for elevated H1N1 and H1N2 titres: for farmers, 30.6 (95%CI, 4.3-∞), and 16.0 (95%CI, 1.9-776.4), respectively. For veterinarians, elevated H1N2 titres, OR= 13.4 (95% CI, 1.5-

670.5). Meat processing workers were found to have had elevated titres against the swine H3N2 virus, OR=5.8 (95% CI, 1.7-23.0). In the unadjusted proportional odds model, the odds ratio for antibodies against both swine H1N1 and H1N2 viruses were elevated for all three occupational groups when compared against controls. Age and sex were used in the model as confounders, and when confounders were controlled, the OR for each group to H1N1 and H1N2 swine viruses were as follows: Farmers: 35.3 (CI, 7.7-161.8) and 13.8 (5.4-35.4), respectively; veterinarians, 17.8 (CI, 3.8-82.7) and 9.5 (CI, 3.6-24.6), respectively; meat processing workers, 6.5 (CI, 1.4-29.5) and 2.7 (1.1-6.7), respectively.

Among the study groups, prevalence of antibody to the swine H3N2 isolate was not significantly different when comparing exposed to unexposed groups. Increased HI titres to swine H3N2 was associated with having received the 2003-2004 influenza vaccine. Also, elevated antibody titres against swine H3N2 was associated with elevated antibody titres against human H3N2 viruses, suggesting cross-reactivity.

The interpretation of these results is limited by several factors. Data collected from the occupational risk factor questionnaire used were not reported in any detail and analysis of specific risk factors is also not reported. Specific exposure data are not described for the various occupational groups studied. Data on the likelihood of the pigs being infected at the time of the study were not reported. Human testing did not allow for a temporal relationship to be described (single serum samples used) and any concurrent influenza-like-illness among the human population was not described. The confidence intervals are extremely wide, so these results are not very precise, however the evaluation of the data using dichotomous comparisons, logistic regression and proportional odds modeling to control for confounders yields convincing serological evidence of human infection among these workers exposed to swine, albeit not a temporal association.

Olsen[42] evaluated the data collected on a farm cohort (n=74) using an occupational questionnaire, and found that swine virus seropositivity was significantly associated with being a farm owner or a farm family member, living on a farm, or entering the swine barn >4 days a week, being >50 years of age and having received the swine influenza virus vaccine in 1976-77 (n=4) or other influenza virus vaccine. HI antibody titres $\geq$40 to swine influenza virus was found in more subjects in the farm cohort (17/74) as compared to the urban control cohort (1/114); p=0.001. Geometric mean titres were also significantly higher among farm cohorts than controls, p=0.001. The authors concluded that the overall frequency of contact with pigs was more important than the length of contact at any one time, which they describe as being consistent with the fact that influenza infections in pigs occur sporadically and pigs only shed virus for approximately seven days after infection.

The small number of participants with elevated pre-season titres to swine influenza viruses precluded multivariate analysis. An example of the impact of this limitation is that the effects of age and exposure to swine over time could not be separated. No data were gathered on the urban controls; however the authors state an assumption that the vaccination history of these individuals would not have been any different than the farm group.

Olson[130] evaluated the prevalence of antibodies to swine influenza viruses in serum samples from a small sample of workers at the Taiwan Sugar Corporation (TSC) who were exposed (n=61) and those unexposed (n=56) to swine. Hemagglutination inhibition testing with a cut point of $\geq$1:10 was used, which is considered very low (at the level of detection) by laboratory experts. The pigs at the TSC were ill with influenza-like symptoms approximately a year prior to the human seroprevalence study.

Antibody prevalence among the exposed was found to be higher for those aged 20-39 years than among unexposed controls and this finding was reported as statistically significant. Prevalence among exposed 20-29 year olds was 29% vs. 7% among TSC controls and 2% among Taipei controls. The study authors deemed these results significant at the p=0.12 and p=0.07 levels, although not significant at the 0.05 level. Among 30-39 year olds, prevalence was 19% among exposed, compared to 6% among both the TSC and Taipei control groups. Again, while the study authors described these results as significant at the p<0.08 and p=0.06 levels, respectively, the results were not significant at the 0.05 level. Antibody prevalence among 40-49 year olds was not greater in the exposed (8%) vs. unexposed, TSC (10%); Taipei (31%). The geometric mean titres were slightly greater for exposed workers (5.62) compared to unexposed TSC workers (5.37) but the p value of 0.39 indicates this could have happened by chance. Prevalence of antibodies in exposed 50 years and older was not statistically significantly different than the unexposed (p>0.3). The geometric mean titres were lower among the exposed than either control group for this age bracket.

The number of individuals in each age group was very small, so analyzing by separate age group may have impacted on the results reported in the study. Using chi-square statistics to compare the exposed TSC group to the unexposed TSC group on the outcome of having HI titres $\geq$1:10, the chi square observed score was 0.41, which is less than the chi square expected score of 3.84, so these distributions are equal. When compared to the unexposed Taipei controls, the chi square observed score was 2.6, which again is less than the chi square expected score of 3.84, indicating these distributions are equal.

The significant time lag between the illness among pigs and human antibody testing (approximately 1 year) can be expected to have had a negative impact on antibody titres at the time of assessment. This time lag, use of single serum samples, and the low titre level cut-point used prevent the association in time of the antibody response in people exposed to pigs and the illness among pigs at the TSC. Furthermore, lack of description of unintended exposures (outside the TSC setting, e.g., at home) among the three study populations limits the meaningfulness of these results.

Wells[129] evaluated evidence of infection among junior swine exhibitors exposed to ill swine at an agricultural fair in rural Wisconsin. This study reported that significantly more exposed exhibitors (n=17/50) had a swine influenza virus HI titre of 20 or more as compared to unexposed exhibitors (n=0/50). None of the unexposed exhibitors had detectable antibody levels (HI titres =10), so these were reported as <10 (p=0.0001). Significantly more exposed (7/50) than unexposed (1/50) had influenza like illness in the month surrounding the fair, RR=7.0, 95% CI, 1.3-3.5; p=0.03. Five of the seven

exhibitors who were ill experienced symptoms within five days of the fair and had titres of 20 or more.

While there was evidence presented of influenza infection among exhibited pigs, the lack of reporting on laboratory controls used for the evaluation of human serum samples and the use of single serum samples, yielded weak evidence of seroprevalence among human subjects as assessed in this review. As a result of using single serum samples to test human subjects, the exposure to pigs at the fair cannot be definitively associated temporally with the seroprevalence of antibodies in the exhibitors. However, the evidence is strengthened by the timing of the appearance of influenza-like-illness symptoms among the small number of seropositive exhibitors. It should also be noted that details about the exhibitors' exposure to pigs at their home farms or at previous agricultural fairs was not reported, a potential source of confounding.

4.6.3. Studies reporting antibody prevalence to zoonotic influenza viruses among human subjects exposed to animals of interest without a measure of association or evaluation of statistical significance (n=3).

Buxton-Bridges[128] explored serological evidence of antibodies to avian influenza virus among workers exposed to poultry during the A/H5N1 avian influenza virus outbreaks among poultry in Hong Kong in 1997-1998. The cohort of interest in this review is the government worker cohort, as this group worked to cull chickens mostly on farms, not in the markets, and studies of market settings are excluded from this review. The government workers commonly wore personal protective equipment (PPE), however details on the proportion of workers who wore PPE consistently is not described. Nine (3%) of the 293 workers were seropositive on at least one sample, by two different recommended assays. Of the 229 workers who had paired samples, one person seroconverted. This person was also ill with respiratory illness. The authors concluded that the seropositivity rate of 3% indicates that a substantial number of mild or asymptomatic infections occurred in these workers. The proportion of the farms which were infected with H5 avian influenza virus was not reported, whereas it is known that poultry workers culling in the markets were definitely exposed to an infected environment.

Puzelli[127] found unequivocal serological evidence of exposure to or infection with H7 influenza viruses in seven subjects exposed to known infected poultry. All seven seropositive subjects had close direct contact with either turkeys or chickens in poultry housing, described as a dusty environment. Of these seven subjects, three were male and three were female, all between the ages of 35-62 years old, with data missing for one. In the quality assessment of this study, the evidence of human infection yielded a rating of "strong evidence of seroprevalence", and the evidence of animal infection was rated as "strong". These results were from outbreaks #5 and #6. The authors indicate that their results may underestimate the true seroprevalence among humans associated with the outbreaks studied, as serum samples from workers in outbreak #4 were collected > 1 year after the outbreak and laboratory methods were stringent, which may explain why no positives were found in that outbreak.

In 1966, Schnurrenberger[96] tested 168 pork producers, 248 veterinarians or veterinary students, 551 packing plant employees, 24 hog buyers or vocational agriculture teachers, 13 pork producers' wives or daughters, as well as 816 unexposed persons, for antibodies to swine influenza virus (H1N1). Age-adjusted antibody reaction rates varied by occupational group: 0% among producers' families; 19.9% among producers, 22.2% among hog buyers, 39.7% among veterinarians, and 46% among abattoir workers. Prevalence of titres against swine influenza virus (H1N1) was found to increase directly proportional to age, which is not a surprising finding, given our knowledge of the effect of age on general antigenic experience.[142] Age adjustment decreased the rates slightly but did not remove the trend. Among veterinarians tested in 1966 and 1968, reactor rates were 34.4% and 35.3%, respectively. A small difference in reactor rates was noted among those with moderate or greater exposure and those with minimal exposure. Overall, the prevalence of antibody was found to increase in association with the degree of swine contact. This was found to be true among the various occupations, but not within them. This also applied to the general population group, whose reactor rate was 38.8%. This is possibly explained by a community-wide outbreak of a related virus which occurred after July 1965.

The Schnurrenberger[96] study used only two viruses, both swine influenza, in the HI test panels. Human strains of influenza were not used. The authors acknowledged the possibility of cross-reaction with a closely related strain not incorporated into testing. Also, they conclude that infection with a swine influenza virus or a closely related agent occurred commonly in Illinois before 1920 and rarely after 1935, yet there is no acknowledgement by the authors of the human influenza pandemic of H1N1 influenza virus which occurred in 1918-1919 as a possible explanation of these results.

4.6.4. Studies of factors modifying the association between exposure to animals of interest and human infection with zoonotic influenza viruses (n=3).

Three studies examined factors which may modify the association between exposure and infection, also referred to in the literature as 'risk factors'. Bosman [125] evaluated the use of oseltamivir, an antiviral drug, as well as the use of goggles and masks among workers exposed to infected poultry. Buxton-Bridges[128] noted the impact smoking had on seropositivity among government workers exposed to poultry. Ramirez[94], the only swine-related study to look at risk factors, examined the use of gloves and the impact of smoking on antibody titres.

Bosman[125] found a statistically significant protective effect of oseltamivir use against conjunctivitis (OR=0.14; 95% CI, 0.08-0.27), and against infection without specific symptoms (OR= 0.47; 95% CI, 0.25-0.88). Almost half (48%) of the poultry farmers were vaccinated against human influenza and 90% of the persons involved in controlling the crisis were also vaccinated. No protective effect was noted for safety goggles or masks; however the overall compliance of using recommended protective measures was low.

Oseltamivir was taken by 85 (48%) of 185 farmers on infected farms and by 456 of 604 (75.5%) persons brought in to control the outbreak. Prophylaxis was interrupted by 324 (71%) due to forgetfulness and reduced availability of the drug.

The use of masks was reported as follows: Of 124 poultry farmers working on infected farms, 22 (17.7%) used the masks, but only eight (6%) of these workers used the masks consistently. Among 495 workers on infected farms in another area affected by the outbreak, 366 (74%) used masks, but only 124 (25%) of them used the masks consistently.

For goggles, four of the 124 poultry farmers on infected farms used this type of equipment while working and only one person (0.8%) reported always wearing the goggles while working. Among the 495 workers from the other outbreak area, 224 (45%) wore goggles while working but only 13% (62) wore them consistently.

Only 109 of 428 (24%) persons brought in to control the outbreaks on infected farms reported that they thought the preventive measures were feasible. Sixty-one workers regularly cited problems in the use of the protective equipment, including 42 workers who reported misting up and poor fit of the goggles.

Buxton-Bridges[128] noted that smoking appeared to be a risk factor for H5 seropositivity among the government workers in that study (5/66 vs. 4/223); P=0.03. Smoking was found to increase the risk only among those without pre-existing antibody titres, as evidenced by the comparison to findings for poultry workers, a number of who had pre-existing antibody titres. It is not known how many, or if any of these government workers took antiviral drugs while they were exposed. Further analysis of the use of personal protective equipment (PPE) among these workers would be informative for other poultry culling operations where workers know in advance that they will be entering an infected space. It may be the case that the use of PPE among these workers had an impact on the rate of seroprevalence of 3%, which is lower than that of poultry workers in the markets (10%). Such comparisons would not be considered valid however, as the settings of the two groups of workers were different (farms vs. markets) and their individual characteristics may also have varied.

Ramirez[94] found that among swine confinement workers, those who sometimes or never wore gloves were found to be significantly more likely (OR=30.3; 95% CI, 3.8-243.5) than unexposed controls to have elevated antibody titres to swine influenza viruses (H1N1). In a comparison against workers who wore gloves most of the time or always, workers who sometimes or never wore gloves were significantly more likely to have elevated antibody titres against swine H1N1 virus (OR=12.7; 95% CI, 1.1-151.1). Having smoked five or more packages of cigarettes in the past year was associated with an increased odds of having elevated antibody titres, OR for those who reported smoking having higher titres = 18.7; 95% CI, 2.5-141.3 than those who did not report smoking.

An association was also found between having received the 2003-2004 influenza vaccine and having elevated titres ($\geq$10) against both swine H1N1 and H1N2 viruses; OR= 16.3;

95% CI, 2.5-107.4). This may be explained by a possible cross-reaction with one of the viruses in the vaccine or a circulating influenza virus, however if this was the case, higher titres would also have been expected for controls and it was not. The authors concluded that another confounding variable not identified in the study, e.g., a behavioural or health-related variable, may have impacted on these results.

These results are limited by the small sample size. Confounding may have been an issue in this study, as controls were not reported to have been matched, aside from knowledge that the age distribution was similar between the two groups. It was not reported how or if cross-reactions were controlled for when testing human serum samples. Details on the nature of exposure of the workers, including important factors such as years in the swine industry were also not reported. It would be interesting to know if these types of variables impacted on the results. It is plausible that workers with several years of experience may be less likely to wear gloves or, more likely, that the number of years of cumulative exposure may have been the important indicator of likelihood of seropositivity as opposed to the act of wearing gloves. An analysis of the results for gloves by age may have assisted in this regard. The small sample size may have precluded such detailed analyses, though this is not stated.

4.6.5. Studies reporting results which were not statistically significant or where serological evidence of antibodies not found (n=5).

Chen[123] did not find any evidence of antibody prevalence to avian influenza viruses among those occupationally exposed to poultry, however they used hemagglutination inhibition to test single human serum samples, and this methodology has been deemed inappropriate for detecting human infections with avian influenza viruses since at least 1982.[65,69]

Vong[126] used appropriate laboratory methods, however found no positive results among those living among backyard flocks potentially infected with avian influenza A/H5N1.

Shu[131] and Zhou[132] studied persons with mixed exposures to ducks and pigs. Their findings were associated with avian viruses, however it is not discernable whether the evidence they present for human infection with avian viruses is associated with exposure to the pigs or the ducks, or both. Both studies used virus isolation and serological testing of humans and both found avian viruses in a small number of subjects. The serological methods (hemagglutination inhibition testing) were not recommended methods for detection of avian influenza virus antibodies in human serum. Ducks are not considered an animal of interest in this review, so these results are not meaningful in the context of this review.

Ayora-Talavera[133] studied 115 blood samples from a clinical laboratory bank in rural Mexico for the presence of antibodies to H1 and H3 influenza viruses. They assumed that all subjects had been exposed to pigs, because of the great proportion of households with backyard herds in that area of the country. They found that the relative risk (RR) of being seropositive for H1 or H3 viruses from exposure to pigs was 1.92 with human H1

(95%CI=1.2-3.0); 0.88 with human H3 (95% CI=0.55-1.4; not significant); 0.6 with swine H1 (95%CI=0.08-4.2; not significant), and 1.0 with swine H3 (95%CI= 0.62-1.6; not significant).

These results do not indicate an association between exposure and human infection with zoonotic influenza viruses, as the association stated for swine H1 indicates infection is less likely to occur in the exposed group and the association stated for swine H3 indicates no difference in risk among those exposed and those not exposed. The use of relative risk as a measure of association is inappropriate in this study, due to lack of sufficient information with which to calculate RR. Furthermore, the confidence intervals are unconvincing (and not significant), as they include 1. Unfortunately exposure of the subjects to pigs cannot be confirmed, as no data were collected on the subjects. The H3N2 swine virus used in the HI assays was a reassortant virus, so even if the results had been convincing, it may have been difficult to distinguish between swine and humans as the source of the virus.

4.7. Discussion:

To answer the research question, "Is there evidence of an association between exposure to domestic swine and / or poultry in an agricultural setting and human infection with an influenza virus of zoonotic origin? If so, what is the relationship, and given an exposure, what are the factors that modify (increase or decrease) the odds of becoming infected?" 16 reports of 15 studies were evaluated.

The methodological quality of the 15 included studies was weak on most quality indicators pertaining to study design and data collection. A total of five population-based studies reported a measured and/or statistically significant association between exposure to animals of interest and human infection. Of these five studies, one studied human infection in association with exposure to poultry and four pertained to swine exposure. Three studies evaluated seroprevalence without use of a measure of association or evaluation of statistical significance, and three studies provided evidence of factors which may modify such an association.

4.7.1. Is there an association between exposure and infection?

The study by Koopmans,[20] on the commercial poultry outbreak of influenza A/H7N7 in the Netherlands in 2003, included only symptomatic exposed individuals associated with the poultry outbreaks in the Netherlands in 2003. While this study provided the strongest direct evidence of human infection associated with exposure to poultry in an agricultural setting, it cannot tell us anything about the likelihood of becoming infected if exposed to infected poultry. In the context of the total number of exposed, estimated as 4500 people, it may provide insight into the proportion of people who develop symptoms of avian influenza virus disease if exposed, roughly 441 out of 4500 people (9.8%), however this was not the focus of this review.

Among the swine studies, there is sufficient evidence to accept increased odds of seropositivity among those exposed to swine vs. those not exposed to swine. Olsen[42] used laboratory methods sufficient to establish both indirect evidence of human infection with swine (H1N1) viruses (comparing pre-and post-season titres) and strong evidence of seroprevalence to same (pre-season titres) associated with exposure in a swine confinement setting.

The Olsen study[42] also presented evidence of animal infection which was rated as being of moderate quality. Three of 74 swine farm participants demonstrated seroconversion when pre and post season titres were compared. Comparing the pre-season swine farm participants to the urban control participants, a statistically significant association was found between exposure to swine in a confinement setting and having HI titres $\geq$40 ($p<0.001$). This study also identified specific factors related to the nature of exposure which were more strongly associated with seropositivity: being a farm owner or family member, living on a farm, entering a swine barn > 4 days a week, and being over age 50 were statistically significantly associated with HI titres $\geq$80. Having received the 1976-77 swine influenza virus vaccine or any other influenza vaccine was statistically significantly associated with HI titres $\geq$40 but not with HI titres $\geq$80. Key conclusions of this study were that exposure to swine was a more dominant factor than age, and that the overall frequency of contact with pigs was more important than the duration of contact at any one time. These results are limited by the small sample size of the exposed cohort, which precluded further analysis to segregate the effects of age and exposure to swine over time.

4.7.2. Is there an association between exposure and seropositivity?

Myers[33] studied specific occupational groups exposed to swine in comparison to unexposed controls. Using dichotomous comparisons, the results indicate extremely large odds ratios for seroprevalence to: both H1N1 and H1N2 swine influenza viruses associated with exposure to swine among both the farmers and the veterinarians, but not for meat processing workers. Using unconditional logistic regression, again large odds ratios for seroprevalence to both H1N1 and H1N2 swine viruses were found for both farmers and veterinarians and to swine H3N2 for meat processing workers. Finally, using proportional odds modeling and controlling for confounders, elevated odds for seroprevalence to both H1N1 and H1N2 swine viruses were found for all three occupational groups.

As the authors of the Myers[33] study acknowledge, it remains possible that the elevated titres identified in this study do not correlate with infection of these workers. However, in spite of the study's limitations, which include not collecting detailed exposure data on the farmer group and not identifying any illness associated with positive serological response, the elevated titres and high odds ratios for seropositivity among these three occupational groups provides convincing evidence that exposure to swine is associated with seropositivity. It is not clear how much exposure is required.

Wells[129] reported convincing evidence of seropositivity among junior swine exhibitors in association with an agricultural fair at which ill swine were exhibited. Even though only single serum samples were used, precluding any firm conclusions about the temporal relationship of the specific exposure and outcome, the fact that significantly more exposed than unexposed exhibitors experienced influenza-like-illness in the month of the fair, adds weight to a temporal association between the ill pigs and the seropositivity of the exhibitors. This is further strengthened by the data collected on the index case, a 32 year-old female who died shortly after visiting the fair and had swine H1N1 virus isolated from her respiratory specimen. The young age of the exhibitors and consequent limited antigenic experience with influenza relative to adult populations, may also lend support to the authors' claims that the swine at the fair were the source of the virus causing the serological response among exhibitors.

The Olson[130] study yielded evidence of seropositivity to swine H1N1 virus, at a very low titre cut point ($\geq$1:10) in association with exposure to swine on a farm setting belonging to the Taiwan Sugar Corporation. This study has several limitations, not the least of which is lack of data collection on exposures of the study population and the very small sample sizes within each age group studied. Statistically significant differences between the exposed and unexposed study groups were reported by study authors with respect to seropositivity with antibodies to swine influenza virus, however none of the results were significant at the p=0.05 level. It is possible that this may have been due to the small sample size, however, when the results for the exposed group as a whole (not separated by age group) were compared to each of the unexposed groups using chi square statistics, the distributions were found to be equal. This study does not present convincing evidence of an association between exposure and seropositivity.

The Bosman[124, 125] study on the commercial poultry outbreak of A/H7N7 influenza virus in the Netherlands in 2003 was poorly reported and rated as being of poor methodological quality. It was not possible to definitively identify from the two published reports, which subjects, of those tested for antibodies, were exposed to infected vs. uninfected poultry and which, if any, used antiviral drugs or personal protective equipment. It appears to be assumed that the workers tested were exposed to infected poultry. This study used a modified HI test, and cited a seroprevalence rate of approximately 50% among those exposed. However, published reports on recommended laboratory methods for detecting antibodies to avian influenza viruses in humans clearly state that HI testing is insufficient for this purpose, and recommend microneutralization testing instead.[46, 69] The aims of this study, general approach and substantial sample size (n=500) are highly relevant to all components of the research question for this review, however due to the poor reporting and possibly poor methodology, these results are less than convincing.

Three studies demonstrated evidence of seroprevalence without a measure of association or statistical significance, of persons exposed to poultry (n=2) and swine (n=1).

Buxton-Bridges[128] noted a 3% seroprevalence rate for avian influenza A/H5N1 among government workers culling chickens on farms during the 1997-1998 H5N1 poultry outbreaks in Hong Kong. They used strong laboratory methodology and evaluated paired

sera for 78% of the 293 government workers studied, therefore this is strong evidence of a 3% seroprevalence rate associated with the culling operation in question. However, statistics on the proportion of these workers who used personal protective equipment, they type of equipment used and the degree to which they used the equipment consistently, were not provided, only a statement that these workers tended to wear such protective equipment. This is a missed opportunity for evaluating the protective value of such equipment, a finding which would have been highly relevant for other countries preparing for a response to avian influenza outbreaks among domestic poultry in anticipation of continued spread of the highly pathogenic H5N1 avian influenza virus via migratory waterfowl.

The Buxton-Bridges[128] study fully explored exposures among the poultry workers in the market settings through a nested case-control study, but much less detail was provided for government workers in the farm settings. A key reason for this appears to be the relative lack of cases among the government workers compared to the poultry workers, precluding a similar nested case-control study, however further detail on exposures and use of personal protective equipment would have helped to put the seroprevalence rate of 3% into context.

Puzelli[127] noted a similar seroprevalence rate (3.78%) among poultry workers associated with 2 of 6 commercial poultry outbreaks in Italy during the time from 1999-2003. Seven out of 185 persons were found to have unequivocal serologic evidence of H7 avian influenza viruses. Due to the delay in collecting human serum samples from one of the six outbreaks and the stringent laboratory methodology employed, this result may be an underestimate of seroprevalence. Again, a missed opportunity in this study was collection and analysis of specific factors which may modify the likelihood of infection such as the use of personal protective equipment or antiviral drugs, and the ability to associate seroprevalence with the poultry outbreaks by testing paired serum samples as opposed to single samples.

Schurrenberger[96] found a fairly high prevalence of antibodies to swine H1N1 virus among various occupational groups exposed to swine (range 19.9%-46%). The study concluded that the prevalence of antibody to swine viruses increased in association with the degree of swine contact. Unfortunately, a high seroprevalence value was also found among the unexposed groups in the community after a specific date, leading the authors to conclude that a similar circulating human strain may have caused the results through cross-reaction. Among the exposed, a correlation between swine influenza virus and vaccination status was noted by the authors. Adjusting for age decreased this effect but did not eliminate it. Laboratory methods included treatment of serum samples to inactivate non-specific inhibition, however there were no mention of controls for cross-reactions. There is also a possibility that the 1918-1919 pandemic influenza virus (H1N1) may have impacted on the reported finding that infection with a swine influenza virus or a closely related agent occurred commonly in Illinois before 1920 but rarely after 1935. Therefore, this study does not present convincing evidence of seroprevalence among those who work with swine.

4.7.3. Are there factors which can modify the odds of becoming infected?

Smoking was identified by 2 studies as a factor which increases the likelihood of seropositivity to zoonotic influenza viruses. Buxton-Bridges[128] identified smoking as a statistically significant risk factor among government poultry workers (P=0.03) and based on a similar assessment of poultry workers with unprotected exposures in market settings, concluded that smoking only increased risk among those without pre-existing antibody titres. Ramirez[94] also identified smoking as a risk factor for seropositivity among swine confinement workers in the American Mid-West and found an extremely large odds ratio (18.7; CI, 2.5-141.3) for elevated antibody titres to swine H1N1 viruses among those workers who smoked ≥5 packs of cigarettes in the past year. However, the Ramirez[94] study had a small sample size and neither study controlled for age or exposure to swine over time, so the effect of these potentially confounding variables on the seemingly strong results cannot be determined.

Nonetheless, in spite of the small number of studies, subjects within them, and limitations described above, it seems reasonable that smoking may be a factor which is associated with antibody prevalence to zoonotic influenza viruses, of both avian and swine origin. This is because the behaviour of smoking could cause one to self-contaminate, thereby introducing virus to one's oral mucosa. Further study wherein confounding variables are controlled for and the practices of workers who smoke with respect to personal hygiene practices and practices in removal of any personal protective equipment worn, is necessary to identify if smoking is associated with seroprevalence. And, if so, to determine whether smoking is in and of itself a risk factor, or if the risk comes from self-contamination through smoking, after contact with animals and before performing hand hygiene.

Ramirez[94] found strong evidence that wearing gloves more frequently is associated with lower levels of antibody prevalence. However while they report using multivariate analysis, they did not appear to control for the effects of age and multiple exposures to swine over time in long-time industry workers, and their sample size was small. In light of the Olsen[42] study and our knowledge of the relationship between age and antigenic experience with influenza viruses among humans, it seems reasonable to expect exposures over time to impact on seroprevalence rates. Further study of this potentially protective factor would need to be done to determine how significant this result is for swine workers. Again, common sense suggests that barrier equipment such as gloves could decrease opportunity for direct exposure to virus. However, since influenza viruses are introduced into the body via mucous membranes, the important factor is preventing self-contamination, e.g., upon removal of personal protective equipment.

Bosman[124, 125] found a protective effect of using the antiviral drug oseltamivir, against conjunctivitis. The authors also noted that no protective effect was found for the use of goggles and masks. However, they acknowledge in their 2005 report that the overall compliance with protective equipment was low and antiviral therapy was taken by 48% of one group and 75.5% of another and interrupted by 71%.

4.7.4. Can the probability of human infection with zoonotic influenza viruses associated with exposure to domestic swine or poultry in agricultural settings be quantified?

Due to the wide variation in study objectives, methodology, settings, participants and measured outcomes among the included studies in this review, further analysis of the data using quantitative methods, e.g., a meta-analysis was not feasible.

4.8 Limitations and potential sources of bias in the review

4.8.1 Limitations and potential biases in the studies included in this review:

Many of the included studies had a small sample size, which limited researchers' ability to perform multivariate analysis in a number of cases. The design of included studies (cross-sectional) was not the optimal choice for establishing a temporal association between exposure and an outcome; this would have been more appropriately achieved using a case-control or cohort study design. The cross-sectional studies included in this review did, however, provide some insight into the potential magnitude of the problem, e.g., seroprevalence of antibodies to zoonotic influenza viruses among those who work with poultry and swine.

As noted in the description of the methodological quality of included studies, the studies included in this review were all cross-sectional studies, bringing with them a certain degree of bias. Most of the studies were opportunistic and retrospective, as they took place in association with an identified outbreak among the animal population. In terms of how representative the study population is of the target population, the selection of subjects would be considered to be biased and not representative of the target population of "all poultry workers" or "all swine confinement workers". However, depending on methodology, the subjects in individual studies may be representative of the workers in a specific industry setting in a particular country at the time the study was completed.

Allocation bias was identified as an issue for all included studies, due to their cross-sectional design. In a cross-sectional study retrospectively assessing human infection associated with an exposure, it is not possible to prevent this type of bias. This is because both the exposure and outcome are assessed at a single point in time, and as such they cannot be independently evaluated. This is not overly concerning with respect to this review; so long as one bears in mind the nature of the studies and the limitations on the interpretation of the findings as a result of study design.

The laboratory methodology used by many of the included studies was sufficient for establishing seroprevalence (single serum samples), but insufficient for establishing a temporal relationship between a specific exposure and presence of antibodies in human serum samples, i.e., previous vs. current infection. Among the studies using serological methods for identification of infection (previous or recent) among human subjects, there was inconsistency across avian studies with respect to the test methods used, and across swine studies with respect to HI titre cut-points and laboratory controls used. The studies

were inconsistent in their approach to establishing evidence of infection among the animals of interest in the various studies.
No single included study adequately addressed all potential confounders, though four studies scored moderate for their management of confounders. Overall, there was inconsistency and lack of detail gathered on exposure histories including "unintended" exposures, e.g., exposures outside of the context of the study, and exposures of workers over time. A small number of studies acknowledged mixed exposures, but did not control for this through analysis. Two of the avian studies and one of the swine studies had a large time gap between the known outbreak among animals and the serological study of humans exposed to the infected animals. Another study had an adequate sample size but did not clearly articulate their exposure status. This lack of information limits the interpretation of the seroprevalence findings, as well as the findings with respect to the protective nature of personal protective equipment and use of antiviral drugs.

Overall, detailed exposure data were lacking among included studies, and less data reported on comparison subjects than exposed subjects. Data on the specific nature of exposures and exposure history of subjects over the course of their career in the relevant industry was often lacking, limiting interpretation of seropositivity and the potential impact of preventive measures such as personal protective equipment. The poor data collection and/or analysis with respect to exposure histories in a number of the included studies likely led to a certain degree of misclassification of exposure status. This non-differential misclassification could introduce bias by causing positive results to be weaker than they ought to be. This is most concerning, as it could have been managed in all studies, through appropriately detailed exposure histories and subsequent data analysis.

Some studies commented on illness among the human subjects exposed to animals of interest, but the majority did not evaluate outcomes among humans. While exploring human illness as an outcome was identified as being beyond the scope of this review, it is acknowledged that identification of even a small number of symptomatic individuals among those exposed, associated with seropositivity to zoonotic influenza viruses lends credibility to claims of a temporal relationship between exposure and infection.

It is possible that positive data were more likely to be reported in the literature than negative findings, due to under-representation of negative result studies in the literature. This would introduce bias into the review. However, several sources of grey literature were also reviewed to identify any relevant studies.

4.8.2 Limitations and potential biases in the review itself:

This review was completed by a single reviewer, with input from committee members with expertise in relevant subject matter and research methodology, but not specifically in systematic review processes. The literature search and screening of resulting search records against eligibility criteria were completed by the single reviewer, introducing the possibility of reviewer bias in the final resulting set of included articles. The gold standard in systematic review methodology as developed and followed by the Cochrane Collaboration is to use a review group wherein each step of the review process is

completed by the entire group and decisions are made by consensus, and explicitly stated dispute resolution mechanisms are in place. This review was conducted as a Master's thesis, so multiple reviewers would not have been appropriate.

The committee was consulted on inclusion criteria, and helped to refine the study eligibility screening tool by using it to re-screen full text articles which the reviewer had already screened into the review. The committee also provided input on the quality assessment tool. While these processes aided in minimizing reviewer bias in the analysis of the final set of included studies, it is not nearly as robust as a process whereby a review group goes through all steps in the review process and all decisions about the review are made based on group consensus. The majority of the work in data collection and quality appraisal of included studies was not double checked by committee members, so it is possible that errors may have occurred and key pieces of data may have been missed or misinterpreted.

The search methodology included a thorough online literature search (performed between March and August 2006), a secondary literature search (performed between August and October 2006), identification of articles by colleagues, and notices from list serves of a small number of electronic journals. It is possible that some studies could have been missed between August and October because the search was not repeated. Studies published after October 2006 were not assessed or included due to the time limit set for the search.

Only those search engines and data sources which were publicly available and free of charge were used. A language restriction was not applied to the search, however due to lack of translation resources only articles published in English were accessed, assessed for relevance and if relevant, retrieved for further assessment. It is therefore possible that relevant studies may have been missed, particularly publications in countries currently affected by outbreaks of avian influenza among poultry. However, while forty-two records of studies were in languages other than English, it is likely that the true number of non-English records is lower due to overlap in search terms and results.

Another potential source of bias in the review itself is that due to time and resources, no attempts were made to contact authors of included studies for further information. Having done so may have yielded additional information and possibly provided clarification on some reports which at face value were not clear.

4.9 Agreements and disagreements with other studies or reviews

There seems to be general agreement in the literature, among included studies, excluded studies and background articles, that working with swine or infected poultry presents an occupational risk factor for infection with zoonotic influenza viruses. Several articles acknowledge the pandemic potential of such viruses; however this appears to be the first systematic review of the literature to evaluate the evidence of human infections with zoonotic influenza viruses in association with exposure to swine or poultry in agricultural settings.

4.10 Implications for practice

4.10.1 Poultry workers:

Evidence collected and evaluated in this review suggests that one might expect a seroprevalence rate of 3-4% among those working with infected poultry in commercial poultry outbreaks, however it is not clear if these rates represent seroprevalence among workers who consistently wore personal protective equipment, sometimes wore personal protective equipment, or never wore personal protective equipment. It is also possible that workers consistently wore personal protective equipment (PPE) but self-contaminated themselves through activities such as smoking, after removing some PPE but perhaps not washing their hands. This is purely speculative, however it seems prudent to reinforce adequate infection prevention and control measures among poultry workers, particularly when outbreaks of influenza are suspected or known to be occurring, regardless of which types of PPE are used. One study noted a much higher seroprevalence rate among workers, approximately 50%, so further exploration of this issue is urgently required, with particular attention to laboratory methodology and exposure histories including the use of any PPE, as well as infection prevention and control practices.

Proper training in the use of, including removal and disposal of PPE is also a common sense recommendation. The use of antiviral drugs, oseltamivir in particular, appears to have a protective effect against conjunctivitis associated with exposure to infected poultry. No protective effect was noted for the use of goggles and masks; however the same study acknowledged that a very small proportion of workers actually used the equipment consistently, so this finding should not impact on practice recommendations. More importantly, only approximately a quarter of the workers thought the preventive measures were feasible, with several workers citing problems such as misting up of goggles. This information should be considered by those making recommendations for personal protective equipment to be worn by workers involved in controlling commercial poultry outbreaks, so equipment can be identified which would both protect workers and be considered more feasible to wear by the workers. Workers should be involved in selecting any equipment to be used in an outbreak and have opportunities to test the equipment for fit and function.

The evidence documented by one study[124] regarding the protective effect of oseltamivir had several limitations which need to be addressed by further study. However, of immediate use to those preparing to respond to future avian influenza virus outbreaks among commercial poultry operations are the data on the consistency with which workers took the drug and their reasons for missing doses. This information could be used by avian influenza planners to increase compliance with antiviral prophylaxis should it become necessary.

4.10.2. Swine workers:

Evidence collected and evaluated in this review suggests that swine workers are at increased odds of becoming infected with zoonotic influenza viruses. It is not clear how frequently one needs to be exposed to swine to develop antibodies to swine influenza viruses over time, though one study based on a small number of swine confinement workers suggests exposure 4 days a week or more is associated with seropositivity to swine H1N1 virus. The use of gloves may have some protective value against seropositivity to swine influenza viruses, however further study with more subjects is required to draw firm conclusions about this potentially protective factor. Smoking was also implicated as a potential risk factor, associated with increased odds of seropositivity in one study of a small number of swine workers. However, this study had limitations including its small sample size and lack of controls for confounders such as age and exposure over time to swine, so further study is required to be certain of the association between smoking on seropositivity.

4.10.3. General issues and recommendations:

In spite of the limitations of the studies, which identified smoking as a potential risk factor for both poultry and swine workers, it seems prudent to recommend against smoking, eating or drinking while working with poultry or swine or immediately afterwards, until PPE has been appropriately removed and discarded and proper hand hygiene has taken place.

Above all, the take away practice message for poultry and swine workers, their employers, occupational health providers, and public health officials is that primary prevention is key to minimizing the likelihood of human infections with zoonotic influenza viruses during routine work but particularly when influenza is suspected or known to be circulating among the animals. While further study is required on the appropriate type of PPE to recommend and the critical times to use the various types of equipment, it is reasonable to recommend basic hygiene practices such as hand hygiene when working with animals, and especially before activities such as eating, drinking or smoking.

Lack of available official statistics on Manitoba's poultry and swine workers precluded a firm assessment of the number of people who may be exposed to and possibly infected with zoonotic influenza viruses, should there be an outbreak among domestic poultry or swine in Manitoba. While more statistics were available for animals than for humans involved in agriculture, data were lacking for small flocks and herds, e.g., backyard flocks and clarity was lacking re: the number of each type of animal on mixed farms.

Whereas the results of this review were inconclusive with respect to establishing and describing an association between exposure to swine or poultry and infection with influenza viruses of zoonotic origin, it remains problematic to not be able to identify those potentially at risk for communication, preparedness and response purposes, should such an association ever be established or should an outbreak occur. Future research may

refute the findings of this review, and so it is important to identify those employers and workers interested in the results, and potentially affected by the findings. It is also important to gain a clear understanding of the total animal population and the degree to which the animals co-mingle on mixed farms, given the propensity of influenza viruses to reassort.

4.11 Implications for research

Several research gaps have been identified through this review. There were no population based studies of Canadian poultry or swine workers, and no population based studies of poultry workers anywhere in North America. This may be due to the relatively small number of outbreaks of influenza among domestic poultry in North America, but such outbreaks have occurred sporadically, so this suggests missed opportunities for studies of the workers.

As an animal of interest, turkeys were underrepresented in the literature on poultry outbreaks. In fact, only the Puzelli[127] study included turkeys among the poultry infected. Turkeys are known to be susceptible to both avian and swine strains of influenza and are often let outdoors where they may have opportunity to co-mingle with wild waterfowl, so it would be prudent to consider research on humans exposed to domestic turkeys.

Specific studies on swine and poultry workers' routine use of personal protective equipment would be helpful not only to study the association between wearing the equipment and evidence of infection with zoonotic influenza viruses, but also to inform planning for outbreak control measures. Knowledge of what equipment, if any, is routinely used and accepted by workers in non-outbreak conditions would be useful for outbreak response planning, to guide recommendations of PPE that not only protects the workers, but that they are also likely to wear consistently.

The protective effect of oseltamivir noted in one study in this review is limited by the possibility of exposure misclassification of the workers in that study and the inconsistency with which the drug was taken. Therefore, further study of the effectiveness of antiviral drugs in preventing symptoms (e.g., conjunctivitis) among those exposed to infected animals such as poultry would inform future practice recommendations related to care of workers involved in animal outbreak control measures.

Further research on the association identified in two studies included in this review between smoking and seroprevalence with antibodies to zoonotic influenza viruses would help identify the true association, whether it is smoking itself, or a breach in personal hygiene practices. Commonsense recommendations for prevention have been suggested above; however evidence to support or refute such recommendations would be gained through further study.

Any future studies on this topic should pay particular attention to study design. While cross-sectional studies are known to be inadequate for establishing a temporal

relationship between exposure and outcome, they can be used to describe the odds of past or recent infection among those exposed, provided accurate exposure histories are obtained and appropriate laboratory methods used. Cross-sectional studies can also produce a prevalence odds-ratio,[108] if an unexposed group of subjects is also included. Case-control studies would allow calculation of an odds ratio, which would represent an approximation of the relative risk of becoming infected if exposed. Establishing convincing evidence of a temporal relationship between the exposure and outcome of interest would require a cohort study design. However, if gold standard laboratory techniques are used to establish recent or active infection among exposed subjects, and infection is probable or confirmed among the animals of interest at the time of exposure, this would be considered compelling evidence of human infection with zoonotic influenza virus associated with a specific exposure to animals of interest. Identifying and controlling for potential confounders through either design or data analysis is critical, so thorough data collection techniques and efforts to ensure an adequate sample size must be addressed.

Detailed exposure histories should be obtained from participants to allow for a more complete picture of factors associated with human infection, and to gather data on potential confounders. Questionnaire components from the Olsen[42] study, paired with details gathered on personal protective equipment in the Ramirez[94] study and perhaps comparing various occupational groups as per the Myers[33] study, would be a good starting point for any studies of human infection with zoonotic influenza viruses associated with exposure to swine, and these questionnaire items could be adapted to suit a poultry setting.

Researchers of this topic need to be clear about what it is they are trying to establish, whether it is recent infection associated with exposure at a particular site at a particular time, e.g., a poultry outbreak control operation, swine fair, etc., or if it is the presence of antibodies to influenza viruses associated with having ever been exposed to swine or poultry. Subsequent laboratory methodology and reporting of findings and conclusions should be consistent with the overall aim of the study. If wanting to establish a temporal relationship between exposure and infection, researchers should strive to evaluate evidence of recent infection associated with an exposure of interest. Viral isolation, nucleic acid testing or paired sera with appropriate laboratory methodology and controls should be used to determine if recent infection occurred. Inconsistencies or lack of clarity of these points were noted among the included studies in this review. Further work needs to be done to establish and collate laboratory standards for the identification of zoonotic influenza virus infections of people through retrospective research, and to communicate those standards which have been published. Researchers of this topic area should familiarize themselves with published laboratory recommendations and include a laboratory expert in virology on their research teams.

5. CONCLUSION:

The result of synthesizing the outcomes of 15 studies on zoonotic influenza infections of humans associated with exposure to poultry or swine in agricultural settings is inconclusive in determining an overall association between exposure and outcome, though some evidence exists of an association between exposure and seropositivity. There appears to be some degree of association between exposure to swine and presence of antibodies to zoonotic influenza viruses among human subjects in a number of the swine studies. Evidence regarding factors modifying the likelihood of becoming infected in association with exposure is also suggested in the literature. However, these results are clouded by several potential sources of bias and confounding. Quantitative analysis of the results was not considered feasible due to the wide variation among the included studies.

More rigorous research methodology and reporting is required to understand the true nature of the associations presented among the studies included in this review. There seems to be a desire for such knowledge, as evidenced by the large number of background and editorial articles on the topic declaring it an important public health issue and calling for such research, so this should be pursued. Perhaps too much emphasis has been placed in the literature on describing the pandemic potential of zoonotic influenza viruses and not enough effort to establish the likelihood of such an event occurring. After all, in order for a novel influenza strain to cause a human influenza pandemic, it must first demonstrate an ability to infect humans, cause illness and spread in a sustained manner from person to person.

Future research studies should pay particular attention to the nature of the exposure of interest, measurement of the outcome of interest, and control for confounding variables identified in the literature, either through study design or analysis of the results.

6. APPENDICES:

Appendix 1: Nomenclature of Influenza A Subtypes

Appendix 2: Forms and supporting material

2.1 Data collection form
2.2 Quality assessment tool
2.3 Potential confounders in studies of human infection with zoonotic influenza viruses

Appendix 3: Laboratory Diagnosis of Influenza

Appendix 4: Tables of Included Studies: Data Collection Forms

Appendix 5: Tables of Excluded Studies

Appendix 1: Nomenclature of Influenza A Subtypes, 1980 System

The following table by Kendal[25] appears in the text: Laboratory Diagnosis of Viral Infections, edited by Lennette (1985, p. 345):

Hemagglutinin		Neuraminidase	
Subtypes (1980 system)	Previous subtypes[1] (1971 system)	Subtypes (1980 system)	Previous subtypes[1] (1971 system)
H1	H0, H1, Hsw1	N1	N1
H2	H2	N2	N2
H3	H3, Heq2, Hav7	N3	Nav2, Nav3
H4	Hav4	N4	Nav4
H5	Hav5	N5	Nav5
H6	Hav6	N6	Nav1
H7	Heq1, Hav1	N7	Neq1
H8	Hav8	N8	Neq2
H9	Hav9	N9	Nav6
H10	Hav2		
H11	Hav3		
H12	Hav10		

[1] sw=swine; eq=equine; av=avian

Appendix 2: Forms

2.1. Data collection form
2.2. Quality assessment tool
2.3. Potential confounders in studies of human infection with zoonotic influenza viruses

2.1. Data Collection Form:[114, 118, 119]

Reference:	
Study design	•
Objectives of study	•
Setting:	•
Study population	•
Subject selection	•
Exposure of interest	•
Virus	•
Outcome of interest	•
Methodology	•
#People with exposure of interest	•
Laboratory methodology	•
#People tested	•
Results	•
Age & Gender with outcome of interest	•
Data analysis methods	•
Possible sources of bias; confounding	•
Provisions for minimizing influence of confounding	•
Limitations	•
Key findings	•
Conclusions	
Notes	

2.2. Quality Assessment Tool

Ref ID:_____
Author:_____
Year: _____
Reviewer:_____

QUALITY ASSESSMENT TOOL

A. SELECTION BIAS

Q1 Are the individuals selected to participate in the study likely to be representative of the target population?

The authors have done everything reasonably possible to ensure that the target population is represented.	Very Likely
Participants may not be representative if they are referred from a source within a target population even if it is in a systematic manner (e.g. patients from a teaching hospital for adults with asthma, only inner-city schools for adolescent risk.	Somewhat Likely
Participants are probably not representative if they are self-referred or are volunteers (e.g. volunteer patients from a teaching hospital for adults with asthma, inner-city school children with parental consent for adolescent risk) or if you can not tell.	Not Likely

Q2 What percentage of selected individuals agreed to participate in the study, e.g., to answer questions, have a lab specimen collected from them?

The % of subjects in the control and exposure groups that agreed to participate in the study before they were assigned to intervention or control groups.	80 - 100% Agreement
	60 – 79% Agreement
	Less than 60% Agreement
There is no mention of how many individuals were approached to participate.	Not Reported
The study was directed at a group of people in a specific geographical area, city, province, broadcast audience, where the denominator is not known, e.g. mass media intervention.	Not Applicable

Rate this section (see dictionary)	**Strong**	**Moderate**	**Weak**

B) ALLOCATION BIAS

"For observational studies, raters assess the extent that assessments of exposure and outcome are likely to be independent." (Cochrane QA Dictionary, 2003).

Q1 Indicate the study design.

Investigators randomly allocate eligible people to an exposure or control group.	RCT
Cohort (two group pre and post) Groups are assembled according to whether or not exposure has occurred. Exposure may or may not be under the control of the investigators. Study groups may not be equivalent or comparable on some feature that affects the outcome.	Two-group Quasi-Experimental
Before/After Study (one group pre + post) The same group is pre-tested, exposed, and tested immediately after the exposure. The exposed group, by means of the pretest, act as their own control group. *Case control study* A retrospective study design where the investigators gather 'cases' of people who already have the outcome of interest and 'controls' that do not. Both groups are then questioned or their records examined about whether they had the exposure of interest. *No Control Group* *Cross-sectional study* A defined population is examined for both the exposure and outcome of interest at one point in time.	Case-control, Before/After Study or No Control Group Cross-sectional

Rate this section (see dictionary)	**Strong**	**Moderate**	**Weak**

C) CONFOUNDERS

"A confounder is a characteristic of study subjects that: is a risk factor (determinant) for the outcome to the putative cause, or is associated (in a statistical sense) with exposure to the putative cause."
Note: Potential confounders have been discussed within the Review Committee and decided a priori.

Relevant Confounders reported in the study:

_____ _____
_____ _____
_____ _____

Q1. Prior to the exposure were there between-group differences for confounders reported in the paper?

Yes or No (see options below)

The authors reported that the groups were balanced at baseline with respect to confounders (either in the text or a table)	NO
The authors reported that the groups were not balanced at baseline with respect to confounders.	YES
Not reported or unclear	Can't Tell
No comparison group--go to Q3	N/A

Q2. If there were differences between groups for confounders, were they adequately managed in the analysis?

Yes or No (see options below) **Not Applicable (skip to Q-3)**

Differences between groups for important confounders were controlled in the design (by stratification or matching) or in the analysis.	YES
No attempt was made to control for confounders.	NO

Q3. Within the exposed group, did the analysis take confounders into consideration?

Important confounders were accounted for in the analysis.	YES
No attempt was made to account for confounders in the analysis or not reported.	NO

Q4. Were there important confounders not reported?

Describe:			YES
All confounders discussed within the Review Committee were reported.			NO
Rate this section (see dictionary)	**Strong**	**Moderate**	**Weak**

D) EVIDENCE OF INFECTION

i. Human[121, 122]

Outcome of interest: Evidence of: ___recent infection ___antibodies / past infection

Q1 Were the laboratory methods used appropriate for the stated outcome of interest?

• Virus isolation (gold standard) or • Suitable nucleic acid test such as RT PCR test or NASBA; o +/-sequencing *Note: genetic analysis (sequencing) required to definitively differentiate H3 and H1 influenza viruses of zoonotic origin from those of human origin.*	Direct evidence of infection (gold standard)
• Antigen detection by immunologic capture methods, e.g., ELISA, FA specific for H5, H7, H9 or other H-type not circulating in humans. (note: if H3 or H1 this would not be sufficient)	Direct evidence of infection—non-human H-types. (less strong than gold standard)
Paired sera required to document change in immune response: • 4-fold rise (or HI titre rise from <10 to 20) of antibody to specific reference strains in paired sera—HI, neutralization • For avian influenza, microneutralization is recommended[112]39 • For H5 avian influenza infections, may need western blot to confirm; for H5 in children 15 and under, ELISA + WB.[70]13	Indirect evidence of infection (antibody response).
• Single serum sample—any test method • Single serum HI test—1:20 or better is stronger; 1:10 could be false positive result. • Complement fixation test, unless confirmed through HI or MN. (if yes, see criteria for weak evidence of infection).	Not evidence of infection; indicates seroprevalence.
• HI test used for avian influenza.	Not appropriate test method for AI.

Q2 Were appropriate laboratory controls used, e.g., to control for cross-reactions and non-specific inhibition?[121, 122]

• The study mentions the use of techniques to control for possible cross-reactions and non-specific inhibition. Should control for cross-reactions with currently circulating human strains and vaccine strains.	YES
• The study did not use techniques to control for possible cross-reactions and non-specific inhibition	NO
• There is no mention of controlling for cross-reactions and non-specific inhibition.	Not Reported

Direct Evidence-- Strongest	Direct Evidence--Moderate	Indirect Evidence
Strong evidence of seroprevalence	**Weak evidence of seroprevalence**	**Insufficient evidence:**

ii. Animal

Q1 Were attempts made to detect influenza infection in the animals?[122]

• Attempts made and acceptable methods used o Genetic analysis, RT PCR, Virus isolation or paired sera for detection of antibodies to specific reference strains (antisera) acceptable	YES (go to Q2)
• No attempts to test were made or inappropriate methods used.	NO (go to Q3)
• There is no mention of whether the animals were tested for influenza infection.	Not Reported

Q2: Was there laboratory evidence of influenza infection in the animals?

• Laboratory evidence that the animals were infected with influenza	YES
• Clear laboratory evidence that the animals were not infected with influenza.	NO
• Attempts at viral isolation unsuccessful but other methods not used	Inconclusive
• Laboratory results were not reported.	Not Reported

Q3 Were the animals found to have illness compatible with influenza at the time of the study?

• The animals were reported to have influenza symptoms compatible with influenza: • Swine: Acute febrile, respiratory disease characterized by fever, apathy, anorexia and laboured breathing. Coughing may be present. Clinical signs seen less frequently include sneezing, nasal discharge and conjunctivitis.[143] • Poultry: lethargy, increased mortality, decreased egg production, diarrhea, respiratory illness.	YES
• The animals were reported to not have illness compatible with influenza	NO
• There is no mention of evaluation of evidence of the animals having influenza – like illness.	Not Reported

Rate this section (see dictionary)	Strong	Moderate	Weak

E) DATA COLLECTION METHODS

Reliability and validity can be reported in the study or in a separate study. For example, some standard assessment tools have known reliability and validity.

Q1. Was a questionnaire used? Yes No (skip to F) Not Reported

Q2. If yes, how was it administered? By study personnel Self-administered

Q3. Were data collection tools shown or known to be valid for the outcome of interest?

The tools are known or were shown to measure what they were intended to measure.	YES
There was no attempt to show that the tools measured what they were intended to measure.	NO
No data collected; no tool used; or not reported whether a tool was used	N/A

Q4 Were data collection tools shown or known to be reliable for the outcome of interest?

The tools are known or were shown to be consistent and accurate in measuring the outcome of interest (e.g., test-retest, Cronback's alpha, interrater reliability).	YES
There was no attempt to show that the tools were consistent and accurate in measuring the outcome of interest.	NO
No data collected; no tool used; or not reported whether a tool was used	N/A

Rate this section (see dictionary)	**Strong**	**Moderate**	**Weak**

F) WITHDRAWALS AND DROP-OUTS

Q1 Indicate the percentage of participants completing the study (The percentage of subjects that had both specimens collected and questionnaires collected, if applicable).

The percentage of participants that completed the study.	80 -100% 60 - 79% Less than 60%
The study was directed at a group of people in a specific geographical area, city, province, broadcast audience, the percentage of participants completing, withdrawing dropping-out of the study is not known, e.g. mass media intervention.	Not Applicable
The authors did not report on how many participants completed, withdrew or dropped-out of the study.	Not Reported

Rate this section (see dictionary)	**Strong**	**Moderate**	**Weak**

G) ANALYSIS

(Q1) Was there an association identified between exposure and infection or seroprevalence?

Yes No Can't Tell N/A N/R

(Q2) Were statistics reported?

Yes No N/A

(Q3) **Were the statistical methods appropriate, e.g., measures of association?**

Yes No Can't Tell N/A N/R

(Q4) **Are the measures of statistical stability appropriate?**

Yes No Can't Tell N/A N/R

(Q5) **Is there a statistically significant difference between groups?**

Yes No Can't Tell N/A N/R

H) INTERVENTION INTEGRITY (Exposure)

Q1 What percentage of participants received the exposure of interest?

The number of participants among the exposed group which actually were exposed is noted.	80 -100%
	60 - 79%
	Less than 60%
Describe	Not Reported
Describe	Not Applicable

Q2 Was the exposure appropriately measured?
The authors should describe how exposure was measured or assessed, e.g., a method of measuring if all participants were exposed in the same way, or, if they were grouped by exposure level / type, this should have been consistent.

Describe	Yes
describe	No
describe	Not Reported

Q3 Is it likely that subjects received an unintended exposure that may influence the results?
The authors should indicate if subjects received an unintended exposure that may have influenced the outcomes. In this case, it is possible that the effect of the exposure may be over-estimated. Contamination refers to situations where the control group accidentally receives the exposure of interest in the study. This could result in an under-estimation of the impact of the exposure.

a. Exposed (or Cases):

Describe	Yes
Describe	No
describe	Not Reported
Describe	N/A

b. Un-exposed (or Controls):

Describe	Yes
Describe	No
describe	Not Reported
Describe	N/A

I) INTERPRETATION OF DATA:

(Q1) What were the major results of the study?

(Q2) Is the interpretation of these results affected by information bias, selection bias, and confounding?

Yes **No** **Can't Tell**

If yes, describe. Discuss both the direction and magnitude of any bias, as appropriate.

(Q3). Is interpretation of these results affected by non-differential misclassification?

Yes **No** **Can't Tell**

If yes, describe. Discuss both the direction and magnitude of any misclassification, as appropriate.

(Q4). Did the discussion section adequately address the limitations of the study?

Yes **No**

(Q5) Are the conclusions appropriate?

Yes **No** **Not Reported**

If no, describe why not.

(Q6) Can the results be generalized to other populations? If yes, describe.

Yes **No** **Not sure**

GUIDE TO COMPONENT RATINGS

A. SELECTION BIAS

Strong	Moderate	Weak
Q1 = Very Likely AND Q2 = 80-100% Agreement ***OR*** Q1 = Very Likely AND Q2 = Not Applicable	Q1 = Very Likely AND Q2 = 60 - 79% Agreement ***OR*** Q1 = Very Likely AND Q2 = Not Reported ***OR*** Q1 = Somewhat Likely AND Q2 = 80-100% ***OR*** Q1 = Somewhat Likely AND Q2 = 60 - 79% Agreement **OR** Q1 = Somewhat Likely AND Q2 = Not Applicable	Q1 = Not Likely ***OR*** Q2 = Less than 60% agreement ***OR*** Q1 = Somewhat Likely AND Q2 = Not Reported ***OR***; Q1= Not Likely AND Q2= Not Reported.

B. STUDY DESIGN—ALLOCATION BIAS

Strong	Moderate	Weak
Study Design = RCT	Study Design = Two-Group Quasi-Experimental	Study Design = Case Control, Before/After Study, No Control Group Cross-Sectional

C) CONFOUNDERS

Strong	Moderate	Weak
Q1 = No AND Q2 = N/A AND Q3 = YES AND Q4= NO; ***OR*** Q1 = Yes AND Q2 = Yes AND Q3 = YES AND Q4 = No ***OR*** Q1= N/A AND Q2=N/A AND Q3 = YES AND Q4 =NO	Q1 = Yes AND Q2 = Yes AND Q3 = Yes; AND Q4 = YES; ***OR***, Q1= Yes AND Q2= Yes; AND Q3= No; AND Q4 = Yes; ***OR*** Q1= N/A; AND Q2 = N/A;AND Q3 = Yes; AND Q4= Yes ***OR*** Q1= N/A; AND Q2 = N/A;AND Q3 = No; AND Q4= No	Q1 = Can't Tell OR Yes AND Q2 = No AND Q3 = Yes OR No, AND Q4 YES; ***OR*** Q1 = No AND Q2 = N/A AND Q3= NO, AND Q4 = Yes ***OR*** Q1= N/A; AND Q2= N/A; AND Q3= No; AND Q4 = Yes. ***OR*** Q1= Can't Tell AND Q2= Yes; AND Q3 = No; AND Q4 = Yes.

D) EVIDENCE OF INFECTION

i. HUMANS[121]

Direct Evidence-- Strongest	Direct Evidence--Moderate	Indirect Evidence
Q1 = Strong AND Q2= Yes	Q1 =Moderate AND Q2 = Yes ; ***OR*** Q1 = Strong AND Q2 = No or NR	Q1 = Weak AND Q2 = Yes
Strong evidence of seroprevalence Q1 seroprevalence AND Q2 = Yes	**Weak evidence of seroprevalence** Q1 = seroprevalence (weak) AND Q2 = No or No Response	**Insufficient evidence:** Q1= Not appropriate method AND Q2= No or No Response or Yes.

ii. ANIMALS

Strong	Moderate	Weak
Q1 = Yes AND Q2 = Yes	Q1 = Yes AND Q2= inconclusive AND; Q3=Yes ***OR*** Q1=No AND Q3= Yes.	Q1 = No or Not Reported AND Q3 = No or Not Reported; ***OR*** Q1 = Yes AND Q2 = Not Reported AND Q3= Yes or NO or Not Reported

STRENGTH OF EVIDENCE OF INFECTION
Comments:

Human_____

_____

Animal_____

_____

E) DATA COLLECTION METHODS

Strong	Moderate	Weak	Insufficient
Q3 = Yes AND Q4 = Yes	Q3 = Yes AND Q4 = No	Q3 = No AND Q4 = Yes *OR* Q3 = No AND Q4 = No	Q1=No or NR, so Q3 & Q4 =N/A

Notes re: questionnaire use (Q1 & Q2):_____

F) WITHDRAWALS AND DROP-OUTS

Strong	Moderate	Weak	Not Applicable
Q1 = 80-100%	Q1 = 60-79%	Q1 = Less than 60% *OR* Q1 = Not Reported	

G ANALYSIS
Comments _____

_____

H INTERVENTION INTEGRITY (EXPOSURE)
Comments

_____
_____
_____
_____

I. INTERPRETATION OF DATA
Comments_____
_____
_____
_____
_____

2.3. Potential confounders in studies of human infection with zoonotic influenza viruses:

The potential confounders identified for this review are:

1. Antigenic experience (age, immunization history, knowledge of circulating strains in the community, history of previous exposures to animals of interest);
2. Smoking;
3. Use of antivirals as pre-exposure prophylaxis (relevant for commercial poultry farm outbreaks);
4. Nature of exposure:
    - Identify all exposures
    - Use of personal protective equipment and related infection prevention and control practices;
    - Duration
    - Intensity
    - Frequency
    - Direct / indirect
    - Setting, e.g., confined space or outdoor interaction with animals

These variables are described below, along with rationale for their role as potential confounders in this review.

A. Confounding variables related to the host:

1. Antigenic experience:

Antigenic experience of the subject will influence serological test results for the diagnosis of influenza infection. If the subject has previously been exposed to the specific virus of interest, or a related virus of the same subtype, then initial testing (acute phase) will demonstrate that. If a single serum sample is used to diagnose infection, it cannot be known whether a positive titre resulted from a previous, recent or current infection. Antigenic experience increases with age due to exposure to annually circulating strains of human influenza. Antigenic experience will also be determined by date; years of major influenza subtype prevalence will impact on those subtypes the studied population can be expected to have experience with, e.g., H2N2 for persons born after 1952; H3N2 after 1968.

Antigenic experience can be affected by either natural infection or by immunization and can be considered a function of age. "At present, when the population is subject to both natural infection and vaccination with H3N2 and Hsw1 strains of virus, even a low level response of heterologous HAI antibody may create difficulties in the serodiagnosis of influenza."[142]

1976 swine influenza virus vaccine and age-related implications:

For studies interested in human infection with swine influenza viruses, particular attention should be paid to immunization with the 1976 swine influenza virus (sw/H1N1) vaccine. In their 1979 study on "The impact of swine influenza vaccine on serum antibody", Egerer, Blichfeldt, Wentworth, and Wilcox[144] reported the following:

- Approximately 25% of all persons residing in the 44 counties studied in Wisconsin received the swine influenza virus vaccine:
    - 3.5% of individuals less than 18 years of age;
    - 27.6%.....18-24;
    - 36.2%.....25-34;
    - 36.0%.....35-44;
    - 37.4%.....45-64; and,
    - 39.8% of those individuals 65 years and older.

- Monovalent A/NewJersey vaccine was administered predominantly to ages 18-44 (87.3%), and to 58% of ages 0-17 and 45-64 years.
- Persons 65 years or older were immunized almost entirely (95%) with the bivalent A/NJ and A/Victoria/3/75 (A/Vic) vaccines.
- Most of the vaccine was administered during the months of October through December, 1976.
- Prevalence rates in the 1976 sample confirmed the hypothesis that persons less than 40 years of age in Michigan have had virtually no experience with swine influenza strains.
- Antibody was found in 20 percent of persons 40-49 years of age and prevalence peaked after age 50.
- Similar age-specific rates for A/NJ antibody were reported for 147 residents of Atlanta and for 828 pre-vaccine sera of a U.S. population sample.

Age Group:	**Antibody prevalence:**	
	Pre-vaccine—1976:	**Post-vaccine: 1977**
15-19 yrs	0% (0/49)	9.7% (10/103)
20-29	2% (1/51)	29.9% (29/97)
30-39	6.1% (3/49)	33.3% (34/102)
40-49	20% (7/35)	36.5% (35/96)
50 or older	83.7% (41/49)	92.9% (104/112)

- The geometric mean titre (GMT) for seropositives in the pre-vaccine survey was 49.5 (52/233) compared to a GMT of 67.8 (212/510) in the post-vaccine sample.
- Persons 50 years of age or older were expected to and did have the highest rate of antibody to A/NJ, apparently by virtue of exposure to virus during the 1918 pandemic or in the subsequent years when this strain was prevalent in the U.S. In either survey, prevalence rates were significantly higher for persons 50 years of age or older than for younger age groups.

- Antibody to A/Vic was greatest in the age group where the least antibody was found to A/NJ, i.e., ages 15-19. This contrast indicates the lack of antigenic cross-reactivity between these strains.[144, p. 86]

Implications for assessing post-1977 and current-day studies:

- It is possible that due to the fact that the 1976 incident at Fort Dix where a swine influenza virus was isolated from a military member and was suspected to have been spread in a limited fashion from person to person, prompting a national swine influenza H1N1 immunization campaign, that persons who have a history of serving in the military may be more likely to have received swine influenza virus vaccine. This is speculative reasoning, however Olsen et al used military service since 1975 as an item on their questionnaire to evaluate possible factors associated with seropositivity to swine influenza viruses.[42]
- The above data were collected in the U.S., and it is not known what the extent of use of the 1977 swine influenza vaccine was beyond the U.S.
- The following table illustrates the age of individuals who may have received the 1977 swine influenza virus vaccine in modern day studies. It is unlikely that the group with the highest antibody prevalence, those 50 or older in 1977, would still be actively working in the industry in present day studies, however younger age groups could reasonably be expected to remain be in the workforce.

Age in 1977	**1987**	**1997**	**2007**
15-19	25-29	35-39	45-49
20-29	30-39	40-49	50-59
30-39	40-49	50-59	60-69
40-49	50-59	60-69	70-79
50 or older	60 or older	70 or older	80 or older

In their 2002 study, Olsen et al. found they could not "fully separate the effects of age and exposure over time to swine."[42p. 817] However, they concluded that exposure to swine was a more dominant factor in seropositivity than was age.[42]

Vaccination history—the impact of human influenza virus vaccine on seropositivity to swine influenza seropositivity:

Elevated titres against swine influenza viruses, specifically H1N1 and H1N2, have been demonstrated in persons who had received human influenza vaccine. This was a key finding by Ramirez et al. upon multivariate analysis including the variable "having received the 2003-2004 influenza vaccine" (data not shown).[94] Olsen et al. found that having received either the human or swine influenza virus vaccine in 1976 or having ever received any human influenza vaccine were significantly associated with swine virus seropositivity.[42] However, they found it likely that vaccination status alone did not determine swine influenza virus seropositivity among the exposed swine farm subjects.[42]

Cross-reactions between influenza viruses in laboratory testing may lead to false positive results, which may contribute to over-estimating the prevalence of antibody to swine influenza viruses. Therefore, immunization history as well as appropriate laboratory testing and controls are important factors to note in published studies on zoonotic influenza infections of humans.

3. Use of chemoprophylaxis (antiviral medication)

In some studies, subjects may have been given antiviral medication prior to exposure or after exposure. Taken prophylactically, antivirals can decrease transmission of and prevent infection with influenza.[145, 146] Taken early after the onset of symptoms, antivirals can reduce flu symptoms, shorten the length of the illness and potentially reduce serious complications.[146, 147]

Studies where this factor is most likely to be encountered are recent studies focusing on persons responding to avian influenza outbreaks among domestic poultry in developed countries. However, generally speaking, farm workers who have identified the outbreak among affected poultry, have already been exposed by the time such measures are implemented. Those subjects most likely to receive antiviral prophylaxis prior to exposure are those temporary workers brought in to control the outbreak. A similar pattern can also be expected regarding personal protective equipment.

The impact of subjects having received prophylactic antiviral medication would be most significant in those studies focusing on illness as an outcome or as a selection factor for inclusion. However, antivirals work by decreasing the virus' ability to reproduce, so they can reduce recovery of virus from human clinical specimens.[147] The use of antivirals should be noted in studies of human infection with zoonotic strains of influenza, including the timing of administration with respect to exposure, because antiviral use might lead to under-estimation of the proportion of exposed individuals that become infected.

4. Smoking:

Cigarette smoking has been documented as a risk factor for influenza A infection and disease. Research has indicated higher attack rates of influenza A/H1N1 during an epidemic when compared against non-smokers.[148] Studies on the relationship between smoking and influenza have shown "that current smokers have higher rates of both asymptomatic and symptomatic influenza than non-smokers."[22, p. 233] The severity of illness and absenteeism due to illness with influenza were also found to be increased among smokers compared to non-smokers.[148] Recently, one study also identified smoking as a risk factor for human infection with swine influenza infection, stating that swine workers who smoked had high odds ratio for elevated titres (18.7).[94]

Thus, the potential impact of smoking as a confounding variable in studies included in this review may be three-fold. First, smoking may impact on the probability of becoming infected with influenza, and therefore may lead to an over-estimation of an association

between exposure to swine or poultry and infection with zoonotic influenza viruses. Secondly, smoking may increase the likelihood of influenza A disease, so smokers may be overrepresented in studies where subjects are selected on the basis of symptoms of influenza-like-illness. Thirdly, smoking may also be associated with a breach in personal protective equipment, if, for example, a worker does not remove their equipment appropriately and/or fails to wash their hands before smoking, as they could self-contaminate with influenza virus and become infected. Therefore, the smoking habits of subjects should be included in study questionnaires related to influenza infection.

5. Personal hygiene practices:

Based on accepted principles of infection prevention and control, personal practices such as routine hand washing after exposure to animals and their immediate environment and before eating, smoking or drinking, can be expected to decrease risk of infection. The degree to which this practice is followed by subjects studied is likely to vary and it is unlikely that this is reported in the literature.

B. Confounding variables related to the environment:

1. Nature of exposure to animals of interest:

The following factors are determinants of the "nature of exposure" and can be expected to impact on the likelihood of a subject having been exposed to a zoonotic strain of influenza: the type of exposure, the intensity of exposure, the temporal relationship of exposure with respect to infection, and the degree of evidence that the animals were indeed infected by influenza virus(es) at the time the subject was exposed to the animals.

a. The type of exposure:

Regarding the nature of exposure, consideration should be given to whether the exposure was direct or indirect. Direct exposure refers to whether or not the person actually handled the animal(s), whereas indirect exposure refers to touching surfaces in the animals' environment, e.g., barn, which may have been contaminated by virus, if present.

The exposure may have been protected or unprotected, e.g. whether or not the subjects used personal protective equipment such as gloves, masks, protective outerwear. For example, Ramirez et al. found that swine workers who seldom wore gloves were more likely to have higher antibody titres than those who wore gloves (OR, 30.3).[94] If personal protective equipment was used, the consistency and appropriateness of use and type(s) of equipment used should be noted, as this will impact on the likelihood that the subject(s) were potentially exposed to virus, if present. If the equipment was used, but was used inconsistently or inappropriately, contamination may have occurred, which could result in exposure to the virus, if present.

The setting in which the exposure took place is also important. It should be noted whether exposure took place in a confined setting, e.g., a closed barn, in a personal home or in an outdoor setting, as the case may be with persons housing backyard flocks of

poultry or a herd of pigs. The conditions in the setting are also relevant. For example, it has been reported that influenza virus can survive for a fortnight (14 days)[149] in dust[22] so a dusty poultry barn may pose a greater risk than one's own backyard, particularly if a dust mask is not worn.

Mixed exposures may introduce a source of contamination into a study. It is possible that some subjects could have had exposure to a mix of animals, e.g., swine, poultry, even wild birds, or exposure to other sources of the same animal of interest, e.g., a neighbor's farm, a poultry market and their own backyard flock. Therefore, all exposures should be accounted for in study questionnaires, so appropriate consideration can be given to other possible sources of infection.

b. Intensity:

The intensity of exposure could also be expected to have an impact on the likelihood of exposure to virus, if present. If known, the frequency and duration of exposure, in combination with the nature of exposure, would provide insight into which subjects had the greatest overall exposure, e.g., greater opportunity for exposure to virus, if present.

c. Temporal relationship of exposure to outcome (infection):

In situations where farm workers are studied, they will most likely have had previous exposure to the animals of interest and this can be expected to increase with age.[42] However, if illness consistent with influenza is present among the animals, this would strengthen the evidence of an association between human infection and the animals as the source of the virus. Pre-and-post exposure testing of an exposed human cohort may facilitate an assessment of the timing of infection in relation to infected and ill animals. Ill animals, like humans, are more likely to shed virus into their environment and transmit it to other animals and possibly to humans.

In situations where outbreaks of influenza among the animals of interest are noted and temporary farm workers are brought in to control the outbreak, it is possible that the workers have only been exposed during the study period, though they also may have had exposure elsewhere. This approach is typical of studies of outbreaks of avian influenza among domestic poultry in developed countries.[19, 20, 127]

2. Circulating strains in the community and prevalence of influenza at the time of study (epidemic vs. non-epidemic):

Identifying concurrently circulating human strains of influenza is important, so these strains can be included in serological testing and consideration be given to this as a possible cause of infection. If not accounted for in the study, this can introduce a significant source of bias. In the case of swine studies, it is possible that swine could be infected with circulating human strains introduced by farm workers. When a concurrent human influenza epidemic is occurring in the community in which the study took place, this could pose a challenge in determining the source of infection of the subjects, particularly if the virus of interest was primarily or even partially of human origin. Even when the strain of interest is of swine or avian origin, if viral isolation and identification

or comparable lab tests are not used, the possibility of cross-reacting antibodies is a potential source of confounding. This underscores the importance of appropriate laboratory diagnostic techniques, including use of laboratory controls.

C. Confounding variables related to the agent:

Documented presence of the virus at the time of exposure, e.g., evidence that the animals may have been infected, such as signs of clinical illness consistent with influenza or by isolation of virus from the animals, strengthens the evidence that the animals were the source of human infection.

Appendix 3: Laboratory Diagnosis of Influenza

The following is a summary of laboratory techniques used for the diagnosis of influenza infections in humans, to provide additional background information to aid in the understanding of the methods used in the studies included in this review, the basic principles behind them, and some of the challenges and limitations of laboratory diagnosis of human influenza infections. As the studies included in this review have been published over approximately the past 35 years, it is expected that laboratory science and resulting accepted techniques used to diagnose viral infections such as influenza, including human infections with influenza of animal origin, have evolved during this time. It is therefore reasonable to expect that the scientific thinking around defining sufficient evidence of prior infection with specific subtypes and strains of influenza has also evolved. No attempt is made to summarize all test methods, laboratory techniques, their popularity of use, rank their sensitivity and specificity or comment on their appropriateness relative to the included studies. The World Health Organization's (WHO) *Manual on Animal Influenza Diagnosis and Surveillance* provides specific advice regarding the detection of influenza virus infections in both animals and humans, and was a key resource used in the development of this summary.[65]

Viral Isolation and Identification:

Influenza virus is isolated by first culturing clinical samples containing virus in a susceptible host medium, such as embryonated eggs of approximately 10-12 days of age, or specific types of cell cultures. Various cell cultures are available commercially; however, commonly used tissues include primary monkey kidney cells (PMKC) and Madin-Darby canine kidney cells (MDCK). The latter method is considered more sensitive for the detection of human influenza viruses.[60] In general, avian influenza viruses grow best in eggs and mammalian influenza viruses (human, swine, equine) grow best in cell culture lines, with MDCK being preferred.[65] Cell culture lines may have adventitious hemagglutinating or hemadsorbing viruses present, e.g., viruses of simian origin in the case of PMKC, so controls using uninfected cells must be carried out to detect them.[60]

Following isolation, the virus (or viral antigen) must be identified. There are several accepted methods for doing this: immunologic methods such as hemagglutination, hemagglutination inhibition (HI), enzyme immunoassay (EIA or ELISA), immunofluorescence, molecular techniques such as polymerase chain reaction (PCR) to rapidly identify and genetically characterize the virus, or non-immunologic methods such as direct antigen detection or electron microscopy.[59, 65]

Serologic Diagnosis of Influenza:

Serodiagnosis of influenza involves demonstrating a significant rise (generally accepted as fourfold) in antibody titre to a given viral antigen over the course of an individual's illness.[60] Serologic diagnosis of influenza is more economical than virus isolation. Serologic diagnosis can also be used to diagnose influenza infection when virus cannot

be isolated due to the short period of virus excretion, or when "clinical specimens are unavailable or the laboratory does not have the resources for virus isolation.".[65, p. 37] However, due to the delay in obtaining a convalescent serum sample, serologic diagnosis is of limited clinical usefulness.[60] Procedures for serologic diagnosis of influenza include hemagglutination inhibition (HI), neuraminidase inhibition (NI), complement fixation (CF), neutralization (Nt), microneutralization (MN), single radial hemolysis (SRH), ELISA, and Western Blot testing (WB).[60, 63] It should be noted that different antibody methods for the same agent may measure a different antibody and so the results of tests using different methods may not always correlate with each other.[63]

Overview of laboratory methods used in included studies:

Hemagglutination:
The attachment of HA to cells is an essential function of the influenza virus and the first step in viral infection. Influenza virus is known to be a hemagglutinating virus, meaning it has the ability to agglutinate certain species of red blood cells (erythrocytes).[59] Influenza virus can hemagglutinate RBCs of some animal species under certain conditions. Hemagglutination is a reaction between hemagglutinins, viral antigens, and receptor sites on the surface of RBCs.[63] This results in visible clumping of the RBCs. Hemagglutination testing uses this principle. First, fluid obtained from virus-infected cell cultures is mixed with RBCs. This mixture is left to sit until the RBCs have settled. The tubes are then inspected for the presence of hemagglutination. If hemagglutination has occurred, the RBCs will form a layer or shield of small clumps covering the bottom of the container. This indicates presence of a virus. Conversely, unagglutinated RBCs settle forming a button in the bottom of the tube. If the tubes are tilted, unagglutinated RBCs will flow in a teardrop pattern. Further specific testing is required to identify any virus present.[59]

Hemagglutination Inhibition Test:
When antibodies to the virus are present, they attach to the antigenic sites on the HA molecule. This interferes with the viral HA's ability to bind to receptors on the RBC, thereby inhibiting agglutination of the RBCs.[63] The hemagglutination inhibition (HI) test is based on this principle, and tests for the presence of viral antibodies.[65] HI testing measures the antibody which prevents attachment of hemagglutinin (HA) of the influenza virus to cells. The serum concentration of HI antibody is therefore indicative of immunity to infection with influenza.[60] HI is considered to be a subtype specific test because it measures antibody to the HA.

Reference antisera required for the HI test exist for 15 HA types of influenza A. These reference sera distinguish between subtypes, and within each subtype are broadly cross-reactive to detect as many different variants as possible.[65] Multiple antisera are used in testing of subtypes containing human, swine, and equine viruses, so the antigenic diversity within a subtype is reflected.[65]

The HI titre is "the reciprocal of the highest dilution of serum that completely inhibits agglutination of the red blood cells by virus."[60,p.616] When correctly performed, each

virus isolate tested will be only be inhibited by serum to one influenza type or subtype, and each control antigen used will only be inhibited by its homologous antiserum.[60] There is no established titre cut point at which immunity is assured. As a general rule of thumb, however, higher titre levels are more likely to yield protection. Titres of 32 or 40 are generally accepted as the lowest titre levels associated with significant protection.[60] It has also been stated that "titres of 20 are at the lower limit of specificity and are of doubtful reliability."[132, p.653] HI test results often approximate those of the neutralization test.[60]

False positive results may occur with the HI test, e.g., the isolate is inhibited by more than one serum. This could be due to the presence of factors such as: residual non-antibody inhibitors of HA, mixed infections or laboratory contamination resulting in a mixture of hemagglutinating viruses, or bacterial contamination.[60] To avoid false positive results with the HI test, nonspecific viral inhibitors of the HA and any natural agglutinins of the RBCs present must first be removed or treated.[63, 65] This is one of the disadvantages of HI testing. Furthermore, antigen must be standardized each time the test is performed and specialized expertise is required for interpretation of the results. The sensitivity and specificity of HAI tests depend on many variables,[63] such as the avidity for antibody of the particular influenza strains present.[60] Methods to increase HI test sensitivity include treatment of the antigen and using antigen grown in tissue culture vs. egg culture when contemporary strains have low avidity for antibody in human sera.[60] False-negative results are also possible when using the HI test, e.g., the isolate fails to be inhibited by any antisera. This can occur when significant antigenic drift of the isolate has occurred, or in the presence of a virus with an extremely low avidity for antibody.[60]

Neuraminidase inhibition assay:

The neuraminidase inhibition assay or NI test operates on the same principles as does the HI test, only it measures inhibition of the neuraminidase of the influenza virus. Neuraminidase is second to the hemagglutinin glycoprotein in abundance on the surface of the influenza virus. "Immunity to NA plays a role in protection against influenza virus infection, and anti-NA antibodies prevent virus release from infected cells."[65, p. 40] The NI test uses reference antisera for the nine NA subtypes.[65] As with the HI test, cross reactions are possible in the NI test and must be controlled for. However, few sera contain nonspecific inhibitors to NA, a problem that is more common for the HA glycoprotein. Experts recommend using both the HI and NI tests.[65]

Complement Fixation Test:

The complement system is a component of the immune system and plays a key role in mediating and amplifying immune and inflammatory reactions. Complement is activated after combining with antigen-antibody complexes, and this forms the basis of the complement fixation (CF) test for antibody.[63] There are two stages to the CF test. First, serum is mixed with known antigen in the presence of a specific amount of complement. If the serum contains antibody to the antigen, the two will react to form antibody-antigen complexes. This process causes the complement to become fixed and depletes it from the mixture. If antibody is not present, complement remains free in the reaction mixture (not fixed). Then, sheep erythrocytes that have been coated (sensitized) with anti-sheep

erythrocyte antibody (hemolysin) are added to the mixture. Complement is a lytic agent, so any active complement remaining in the mixture will be lysed (hemolyzed). The absence of hemolysis indicates that the complement has been depleted or "fixed", confirming that an antigen-antibody reaction had occurred in the first step.[63]

A fourfold or greater increase in antibody titre measured in the CF test with nucleoprotein (NP) antigen is interpreted only to mean infection, or vaccination, with type A or type B influenza virus. CF testing uses the type-specific NP antigen of the influenza (A or B) virus. Because the NP antigen is identical for all viruses of a given type, this technique does not provide information identifying the HA subtype causing the disease, in contrast to HI testing. Antigenic variability of prevalent influenza virus strains and non-specific indicators do not influence CF test results. CF testing is advantageous when new virus subtypes appear that "may induce low (primary) antibody responses to HA, but larger secondary antibody responses to the NP antigen."[60, p.620]

CF and HI test results do not always correlate. For this reason, and because the effectiveness of each test depends on a number of variables, using both methods increases the sensitivity of diagnosis. Variables impacting on test effectiveness include "the previous antigenic experience of the individual, the appropriateness of the strain used in the HI test, and the interval between collection of acute and convalescent sera." [60, p. 620]

Hemolysis in Gel (or Single Radial Hemolysis):

The hemolysis in gel (HIG) or single radial hemolysis (SRH) is a serologic method based on the antigen-antibody reaction. Antigen-sensitized RBCs are suspended in an agarose gel containing guinea pig complement.[63] Then, a treated serum sample (to inactivate native complement) is allowed to diffuse into the gel from a well. If specific antibody is present in the serum sample, it will react with the antigen-sensitized RBCs, causing hemolysis. When this happens, a zone of hemolysis can be observed around the well.[63] "The diameter of the concentric zone of hemolysis is proportional to the concentration of specific antibody in the serum." [63, p. 89] One of the benefits of this technique is that it is more specific than corresponding HI tests because it is not affected by most nonspecific inhibitors which interfere with the virus hemagglutinins, and so does not require special serum treatment to remove the nonspecific HA inhibitors prior to testing.[63]

Neutralizing Antibody Assay:

In neutralization testing, incubation of the virus with its specific antibody prior to inoculation of the virus into susceptible cell cultures neutralizes the virus and renders it incapable of infecting susceptible cells.[59] "Loss viral activity through neutralization of viral infectivity or by HI provides confirmation of the identity of the virus."[59, p. 56]

The neutralization (Nt) test is used to measure antibody to a wide variety of viral agents, and for the influenza virus, primarily detects antibodies to the hemagglutinin. The Nt assay is both highly sensitive and specific.[65] Neutralizing antibodies persist well beyond the initial illness, and when detected in the absence of symptoms of recent infection with a specific virus, are widely accepted as evidence of immunity to the virus. As neutralizing antibody can persist for many years, Nt tests are useful in seroepidemiologic

studies to determine the virus infection history of given population.[63, p.79] There are two steps in the Nt assay: first, the virus is mixed and inoculated with appropriate antibody reagents and then the mixture is inoculated into the appropriate host system, e.g., cell cultures or embryonated eggs.[65]

There are several advantages of the neutralization assay. Similar to the HI test, the Nt test can identify strain-specific antibodies in animal and human serum. The Nt assay can be used to confirm HI test results, due to its sensitivity and specificity and the fact that neutralizing antibody is less cross-reactive between antigenically related viruses than HI antibody. Due to the fact that infectious virus is used, the Nt assay can also be developed rapidly when a novel virus is detected, usually prior to the availability of purified viral proteins for use in other assays. When used in combination with the HI test, Nt testing provides additional information on the identity of the infecting virus. Nt testing is considered to be the most accurate viral identification method and the most sensitive and specific serodiagnostic test for influenza. This is because by measuring neutralization of viral infectivity, it unequivocally correlates with protection.[60] As such, neutralization is the reference standard for viral identification procedures.

Microneutralization is considered an alternate neutralization procedure,[65] however is based on these same principles. For details on microneutralization laboratory procedures, please refer to the *WHO Manual on Animal Influenza Diagnosis and Surveillance*. Microneutralization is a recommended technique for the detection and diagnosis of human infections with avian A/H5N1 influenza virus.

Enzyme Immunoassay (EIA or ELISA):

Enzyme immunoassay (EIA) methods to detect antiviral antibodies are more sensitive than complement fixation and in many cases also more sensitive to hemagglutination inhibition testing. This method is detects antiviral antibodies and results can be available in as little as 2-3 hours. In EIA methods, lysates of virus-infected cells are used as antigens. The purified or semi-purified viral antigen is adsorbed to plastic beads, tubes or microtitration wells. This creates a solid surface capable of capturing and binding specific antibody in the test serum sample, known as the solid phase.[63] The serum sample is then incubated with the (purified) solid-phase antigen, which allows any specific antibody present in the sample to be captured and bound to the solid surface. Then, unreacted components are removed, the surfaces washed to separate bound and free antibodies. By coating the solid phase with purified strain specific HA protein, the HA-specific antibody response can be measured, which correlates with resistance to infection.[60] If the solid phase is coated with whole virus, antibody to both type and strain-specific antigens can be measured.[60]

The consistency with which the solid phase is coated with the antigen greatly affects the reproducibility of test results. "The sensitivity of any enzyme immunoassay is directly related to the properties of the enzyme label used."[63, p. 92] "Over the past several years, EIA has replaced other traditional test methods for the serodiagnosis of many common viral infections."[63, p. 93] When interpreting antibody data from immunoassays, the nature of the antigen including its purity, must be documented. Immunoassays are very

sensitive, so there is a possibility of measuring antibody to contaminating proteins, such as NA in a "pure" HA preparation instead of that of the antigen of interest.[60]

In the 1997 Hong Kong outbreak of avian A/H5N1 influenza in which 18 human cases were confirmed, a variety of lab methods were used. The performance of direct detection of virus with H5-specific immunofluorescent antibody (IFA) was found to depend highly on the quality of specimens used. In order for IFA results to be reliable, researchers found that at least 10 influenza virus-positive cells were required in each clinical sample, as determined by the presence of influenza A virus-specific monoclonal antibodies.[51]

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

While hemagglutination, hemagglutination-inhibition, and immunofluorescence are routinely used in the detection, typing and sub typing of influenza viruses, molecular techniques such as RT-PCR are used to rapidly identify and genetically characterize influenza A viruses. PCR is a powerful technique in that it can be used to identify influenza virus genomes, even if present at very low levels.[65] The sensitivity of PCR methods is comparable to virus isolation.[62] Results are generally available in 2-3 hours.[62] In the 1997 Hong Kong outbreak of A/H5N1 involving18 humans, RT-PCR and shell vial culture consistently demonstrated a high degree of sensitivity and specificity.[51] The rapid nature of this method was found to be of high clinical value in managing the outbreak due to the opportunity it provided for early antiviral administration, establishing appropriate infection control precautions and investigating contacts.[51]

Briefly put, the RT-PCR technique involves synthesis of a DNA copy (cDNA) of the influenza virus genome (a single-stranded RNA). Reverse transcriptase (RT) is a polymerase used to make the cDNA. Primer pairs designed and used in the PCR test are based on known sequences, for instance primer pairs specific for the hemagglutinin gene of currently circulating influenza viruses may be used. The choice of primer sequences in RT-PCR based methods is crucial to the resulting sensitivity and specificity. "The subtype of a virus can be determined by sequencing of the PCR products and comparison of the sequences with sequences deposited in databases."[65, p. 55]

Western Immunoblotting (Western Blot testing):

The Western Blot (WB) test is extremely sensitive and specific for characterizing antiviral antibodies. In this technique, antigens are separated and antibodies to specific viral proteins identified using gel electrophoresis. These proteins are then transferred from polyacramide gels to an immobilizing matrix, commonly nitrocellulose paper. This transfer process is known as "blotting". These blots then undergo immunologic and biochemical analysis.[63] Western Blot testing has been used as a confirmatory test in a small number of the included studies. The use of WB with purified H-type specific hemagglutinin excludes the possibility of non-specific cross-reactions with antibodies to human influenza viruses[64].

Limitations of antibody detection methods:

Problems and pitfalls of antibody detection, in general, include "insensitive and non-specific serodiagnostic tests resulting from heterologous cross reactions, immunologic interference, substandard reagents, or improper test performance."[63, p. 76] Nonspecific inhibition can occur and lead to false interpretation. Non-antibody molecules contain sialic acid residues which can mimic receptors on red blood cells and compete for the influenza hemagglutinin. Therefore, laboratory techniques to inactivate nonspecific inhibitors in sera of different species are recommended.[65]

Caution should be exercised in the interpretation of serologic results, particularly in the absence of virus isolation.[60] In diagnostic serological testing, the specific antigen used does not necessarily identify the infecting virus. Anamnestic responses an earlier antigen frequently occur, so the previous immunologic experience of the patient must be taken into consideration. It is possible for the antibody response to be greater to an earlier antigen than to the current infecting virus.[60]

It is accepted best practice in laboratory methodology to ensure serum being tested for hemagglutinating antibodies to specific antisera has been treated to inactivate nonspecific inhibitors. This must occur for an HI test to be considered valid.[65] Negative control sera need to be used to control for cross-reactions which can occur between some neuraminidase subtypes. Free sialic acid has the ability to mask antibody. This sialic acid may be present if low dilutions of antiserum are used, e.g., 1:10, 1:20. Using a more dilute antiserum or dialyzing the antiserum are techniques to control for nonspecific reactions. There are several methods for inactivating nonspecific inhibitors in sera, and it is beyond the scope of this project to outline them in detail here. Well documented studies on influenza infections should note that sera were treated to control for nonspecific inhibition and that controls for cross-reactions were used.[65]

Hemagglutinin present in the polyclonal antiserum may block neuraminidase activity, a non-specific reaction. A commonly used technique to control for this problem is to use antiserum against two different viruses. "For example, for N9 identification with polyclonal sera we could use an A/H1N9 and an A/H6N9; if only one serum inhibits then we would suspect that it was due to the antibodies to hemagglutinin; if both inhibit then it is likely to be antibodies to the neuraminidase, and we could conclude that this virus sample tested possessed a neuraminidase of the N9 subtype.[65, p.47]

Special considerations for avian influenza virus infections of humans:

Routine procedures for the detection of human influenza A viruses may be less effective in the detection of influenza of avian or swine origin. This could result in a false-negative diagnosis.[150] Research from the early 1980s suggests that differences exist between avian and mammalian influenza viruses in the hemagglutinin's accessibility to antibodies and / or in the biological outcome of antibody attachment. These differences result in the inability of the HI test to detect antibody to intact avian viruses, and a lack of

correlation with virus neutralization. Therefore, seroepidemiological studies using the HI test could yield misleading results.[69]

Avian influenza virus infections of humans typically result in poor antibody response. For instance, a lack of serological response has been documented for human cases of avian influenza A/H7N7 infection.[20] A combination of culture and antigen detection by ELISA are increasingly being recommended by experts due to their increased sensitivity.[65, 70]

Recently, a new set of PCR primers have been developed for the detection of influenza A viruses from multiple species. The resulting PCR was found to be fully reactive to a variety of isolates representing all known subtypes of influenza A viruses obtained from birds, humans, pigs, horses, and seals. This PCR is considered to be "up to 100-fold more sensitive than classical virus isolation procedures."[150, p. 4096.]

Laboratory tests recommended by the WHO to identify avian influenza A/H5N1 virus in specimens from humans include the following for identification of avian influenza A subtypes: immunofluorescence assay using clinical specimens or cell cultures with confirmatory testing with H5 monoclonal antibody (for detection of H5), virus culture and PCR. For serologic identification of influenza A/H5 infection, the microneutralization assay is recommended.

Research into serologic detection of antibodies to avian influenza A (H5N1) following the 1997-1998 outbreak of the same virus among a small number of humans in Hong Kong revealed that serologic detection of avian influenza viruses is best performed by microneutralization assay or H5-specific indirect ELISA testing, as these methods were found to be more sensitive than the HI assay. Furthermore, the sensitivity and specificity of the microneutralization test in the detection of anti-H5 antibody in those aged 18-59 were maximized, 80% and 96% respectively, by the addition of the Western Blot test. Detection of anti-H5 antibody in sera from children under 15 years of age using ELISA and Western Blot tests maximized both sensitivity and specificity at 100%.[70] Current advice published by the World Health Organization recommends that serological identification of influenza A/H5 infection be performed using the microneutralization test.[65, 65, 151]

Due to the fact that antibody to avian influenza subtypes is presumed to be low or absent among most human populations, it is considered acceptable practice to use single serum samples to screen for prevalence of antibody to avian influenza viruses. However, microneutralization testing using paired sera provides more definitive diagnosis of infection and should be used if infection of humans with avian influenza viruses is suspected.[65]

Lab diagnosis of influenza in humans: A historical overview of laboratory methods and their use in diagnosing human infection with human, avian, and swine influenza viruses.

1. Influenza virus infection of humans:

Pre-1940:

- Influenza virus first isolated from an avian source in 1902, however at the time of the 1918 human influenza pandemic, the causative agent was not known. The first isolation of influenza virus from humans occurred in 1933.[60]
- Classical neutralization tests using either cell monolayers and hemadsorption or plaque reduction were the earliest techniques used for assessing serological responses to influenza virus.[152,p. 303]

1940-1970

- Hemagglutination testing was first described by Hirst in 1941. Hirst found that hemagglutination inhibition testing paralleled the results of neutralization tests, but were easier and cheaper to perform[152].
- In 1956, Liu was the first to demonstrate fluorescent antibody staining of influenza infected nasal epithelial cells.[60]
- The neuraminidase component of influenza virus was discovered in 1957 by Gottschalk, however understanding of the genetic independence of the viral components responsible for hemagglutination and neuraminidase activities occurred later, as described by Laver and Kilbourne in 1966.[60]

1970-1990

- Single radial diffusion test described by Schild et al in 1972 and 1974.
- In 1975, Mostow, Schild, Dowdle and Wood indicated that HI, NI and complement fixation methods have historically been used to diagnose influenza infections.[153]
- In the late 1970s, a few attempts were made to detect the subtype of flu viruses by methods other than HAI: staining infected cells using polyclonal or monoclonal antibodies or by using viral nucleic acid detection techniques.[60]
- In the early 1980s, solid-phase enzyme immunoassays were described as recent addition to serological methods available to diagnose human influenza infections. They were found to be more sensitive than complement fixation testing; and some also reported that they were, in many cases, also more sensitive than HI. The greatest advantage to the EIA test methods, however, was that they could measure the HA-specific antibody response (correlating with resistance to infection) and could measure antibody to both type and strain specific antigens[60].
- Monoclonal antibodies were developed by the CDC and used in an indirect fluorescent antibody procedure and found to give type specific results.[60]
- Kendal described neutralization as having greater sensitivity than HI testing, and that with HI testing, "a rise in titre of fourfold or from <10 to 20 is evidence of infection." [25, p. 353]
- French and Leland described that a fourfold or greater rise in antibody titre is indicative of a very recent or current infection and if the first sample is positive for

antibody, then this is evidence that the person has had experience with the antigen in question.[25]

1990-2006

- Zambon concluded that "The techniques of hemagglutination and hemadsorption remain as crucial to routine influenza diagnostic laboratories today as they were 50 years ago."[152, p. 296]
- "The hemagglutination inhibition titre will continue to be a 'gold standard' for evaluation of susceptibility or protection from influenza for the foreseeable future, although a reliable test for use on a single serum sample to diagnose recent influenza infection is badly needed."[152, p. 307]
- deJong and Hien state that viral isolation is still the gold standard, and subtyping performed by subtype specific RT PCRs or HI and NI assays using a panel of reference antisera.[27]

2. Avian influenza virus infection of humans:

1980-1990

- Hemagglutination inhibition tests with intact avian influenza viruses are not successful at detecting antibody and do not correlate with neutralization of the virus and therefore seroepidemiologic studies of avian influenza infections of humans using conventional HI assays may yield misleading results.[69]

1990-2000

- In 1991, Beare and Webster concluded that "restricted replication of AI viruses can take place in humans without induction of detectable HI antibodies."[92]
- Also in 1999, Rowe[70] found that:
    - microneutralization and western blot testing found to be very sensitive and specific for anti-H5 antibody detection; more sensitive than HI testing.
    - In children ≤15 years of age, ELISA and western blot testing achieved maximal sensitivity and specificity.
    - Concluded that neutralization assays are the methods of choice for detection of antibodies against AI viruses in humans.
    - Single radial hemolysis may lack specificity for antibodies to hemagglutinin.

2000-2006

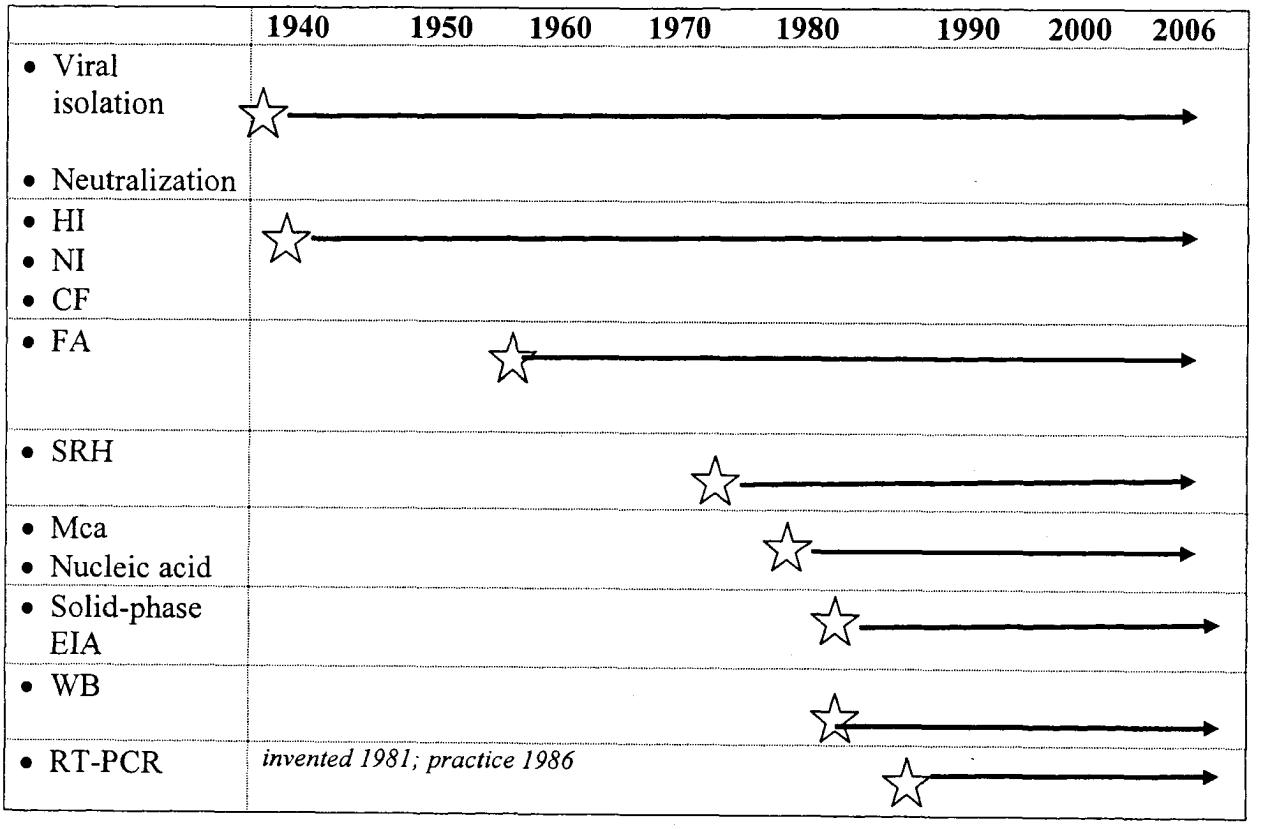
- Western blot testing used to test humans exposed in the 2003 H7 outbreaks among poultry in Italy. In their 2005 review article, Hayden and Croisier[64] state that the use of the WB test using purified H7 hemagglutinin "excludes the possibility of nonspecific cross-reactions with antibodies to human influenza viruses."[p.1311] They also point out that "Definitive evidence for active infection would include the detection of virus or viral RNA at the time of exposure or illness."[p. 1311]
- In 2006, deJong & Hien[27] indicated that:
    - viral isolation is still the gold standard;

- Antigen detection has limited usefulness in AI detection due to low sensitivity;
- RT-PCR is sensitive and specific for detecting viral nucleic acids and has been shown to have increased diagnostic sensitivity for many viral pathogens when compared to culture or antigen detection methods;
- HI assays are of limited usefulness for detection of antibodies against AI viruses;
- "Several studies have shown a failure to detect HI antibodies against AI viruses in mammals, even in cases where infection was confirmed by virus isolation."[27, p.8]

3. Swine influenza virus infection of humans:

- No specific description on the best practices related to diagnosis of swine influenza virus strains in humans was found in the literature. However, this is not surprising, as swine influenza viruses, like human influenza viruses, are mammalian viruses and types and subtypes of influenza A infecting both humans and mammals are similar to one another, e.g., H3N2, H1N1. It is likely reasonable to conclude that laboratory methods to detect human strains of influenza in human specimens could also be expected to detect swine influenza virus strains in human specimens.

Table: Historical overview of availability of laboratory diagnostic techniques for influenza[60, 71, 154]



Legend:

HI= hemagglutination inhibition
NI= neuraminidase inhibition
CF= complement fixation
FA= fluorescent antibody staining
SRH= single radial hemolysis
Mca=monoclonal antibody
EIA= enzyme immunoassay
WR= western blot
RT-PCR=real-time polymerase-chain reaction

Appendix 4: Tables of Included Studies: Data Collection Forms

1. Studies of influenza-infected humans associated with exposure to poultry.
2. Studies of influenza-infected humans associated with exposure to swine.

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Buxton-Bridges et al 2002 : Risk of influenza A (H5N1) infection among poultry workers, Hong Kong, 1997-1998**
Study design	• Cross-sectional with nested case-control study for poultry workers.
Objectives of study	• To assess H5N1 infection among government workers and among poultry workers involved in poultry slaughter required due to H5N1 outbreaks among poultry.
Setting	• Hong Kong 1997-1998; outbreak of H5N1 among poultry.
Study population	• 293 GWs and 1525 PWs • GWs often wore protective clothing such as gowns, masks and gloves when working directly with poultry during the culling operation and most were not involved in depopulating poultry in markets, most worked only on farms. (markets more likely than farms to have infected chickens). • Therefore, the GW cohort is the only cohort of interest in this review (market settings excluded)
Subject selection	• From December 29, 1997 to January 15, 1998, GWs and PWs involved in the slaughter of poultry were targeted and invited to visit any of 14 HK Government outpatient clinics and participate in a study of H5N1 infection among persons exposed to poultry.
Exposure of interest	• Infected poultry.
Virus	• A/H5N1
Outcome of interest	• Human infection with A/H5N1 avian influenza virus. Evaluated serologic evidence of antibodies to A/H5N1 avian influenza virus.
Methodology	• A retrospective cohort study conducted among government workers and poultry workers associated with the outbreak and control activities Paired serum samples: 1) 0-7 days post culling; 2) 2 weeks later. • Random sample tested by western blot due to resource constraints, from age groups 15-29; 30-44; 45-49. People >60 or <14 and those not stating age were excluded from analysis because western blot les specific in these age groups. • Data collected: Questions asked: age, sex, type of work with poultry, exposure to persons with H5N1 illness, respiratory illness since November 1, 1997 and whether they had observed >10% mortality among poultry with which they had worked since 1 Nov, 1997.
#People with exposure of interest	• 293 GWs and 1525 PWs.293 government workers involved in culling ops (paired sera) and 1525 poultry workers (single serum samples).
Laboratory methodology	• microneutralization followed by western blot testing on paired serum samples. Considered positive by MN if anti-H5 titres of ≥80 were obtained. If positive by MN, confirmed by WB. Positives by both MN and WB were considered positive.
#People tested	• 1818
Results	• Among GWs, 9 (3%) were both mn and wb positive on ≥1 sample. 78% of GWs (229/293) had paired samples. • Positives by age group: 0% (0 of 30) ages 22-29; 4% (6/166) among 30-44 yr olds and 3% (3/97) among 45-58 yr olds. • Of 229 GWs with paired serum samples, 1 seroconverted.
Age & Gender with outcome of interest	• GWs: all under 60 yrs; median age =41, range 22-58 • 85% of GWs were male

1. Studies of influenza-infected humans associated with exposure to poultry.

Data analysis methods	• To assess risk factors, a nested matched case control analysis weans done, where cases=positive result by MN and WB; controls--all PWs who tested negative to MN and those testing positive to MN but negative by WB. (not included were those testing positive by MN but not tested by WB). • Estimated seroprevalence of H5 among PWs based on percentage of PWs who tested positive by MN and the percent expected to test positive by WB, weighted by age group; • For GWs, all MN positive samples tested by WB and results analyzed on the basis of a cohort design
Possible sources of bias; confounding	• GWs wore protective clothing and most were not involved in depopulating in markets; however it is not stated how many GWs may have had a mixed exposure or a poultry market exposure vs. a farm exposure, either on the job or outside of the job. • 22.5% of GWs smoked. • no details provided on demographics or nature of exposure in occupational groups, or exposure outside of the "workplace". • PWs had more prolonged exposures than GWs; • Timing of PW exposure to potentially H5N1 infected poultry could not be accurately identified, so they were only asked to submit one serum sample.
Provisions for minimizing influence of confounding	• Stratified analysis; exposure history taken; sensitive lab methods used with confirmatory testing.
Limitations	• Single serum sample used in the PW study, thus the timing of infection with H5 virus cannot be known with certainty. • It is possible that the anti-H5 antibody detected in at least some of the PWs may have been a result of prior infection with a related H5 virus. • It is not known how many of the GWs had exposure in the markets, either as a result of their job or daily shopping activities, however it is stated that "most" worked on farms. • It is not known what proportion of GWs wore PPE consistently or if they took antiviral drugs.
Key findings (related to GWs).	• H5 seroprevalence rates of 3% (GWs) and 10% (PWs) suggest that a substantial number of mild or asymptomatic infections occurred in these occasionally exposed populations. • Smoking was found to be a risk factor for H5 antibody among GWs but not PWs, however smoking appeared to increase the risk only among those without preexisting antibody titres; thus this same risk factor may not have been seen among PWs because of preexisting antibody from prior exposures to H5 avian viruses.
Conclusions	• The serologic evidence for infections in PWs and GWs presented in this study further demonstrates the potential of avian influenza viruses to infect humans. • The findings of this study highlight the need to conduct additional seroprevalence studies in human populations in Asia and elsewhere that are exposed to domestic poultry in live bird markets.
Notes	

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Koopmans et al; 2004: Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands.**
Study design	• Cross-sectional
Objectives of study	• To assess the extent of transmission of influenza A/H7N7 virus from chickens to humans.
Setting	• The Netherlands Commercial poultry farms;
Study population	• Workers in poultry farms, poultry farmers and their families on farms affected by the avian influenza outbreaks among domestic poultry. Sampled those workers who became symptomatic.
Subject selection	• exposed people with health complaints were asked to report to officials and then were tested for AI infection. • Background: late seasonal increase in rate of human influenza viruses noted at time of outbreak; 2 veterinarians involved in outbreak control measures were simultaneously confirmed to have A/H3N2 human and A/.H7N7 avian influenza virus associated conjunctivitis, so active case finding ensued.
Exposure of interest	• Poultry infected with A/H7N7
Virus	• A/H7N7
Outcome of interest	• Infection with A/H7N7—identification of human cases of A/H7N7 avian influenza – case = symptoms following exposure to infected poultry and lab confirmation of infection.
Methodology	• Outbreak investigation: case finding study. • Symptomatic persons identified and subsequently tested for evidence of infection. • Population at risk defined as people living or working in the Netherlands after Feb 28, 2003 who had direct contact with poultry or poultry products that could have been infected with H7 or who had close contact with an H7 infected person; followed up on all such persons with health complaints; PHNs or doctors from the Municipal health service administered questionnaire and MHS workers took eye swabs and nose/throat swabs for testing. • Active case finding performed by visiting farms in the region. • Questionnaire included type of symptoms, duration of illness, possible exposures to infected poultry, background demographic data.
# People with exposure of interest	• In this study, 453 exposed symptomatic people; 450 of these were evaluated for infection. • ~1400 poultry farmers / farm family; ~1800 cullers; ~180 veterinarians; ***Total approx: 3380***. *Medical personnel and others not counted for this review, as these people would not likely have been exposed to poultry, but rather to human cases, and secondary cases were identified in this study).*
Laboratory methodology	• Virus isolation (cell culture) and RT PCR used (RT-PCR for influenza A virus followed by subtype H7 specific RT-PCR). • After first 25 cases confirmed by culture, RT PCR used as initial screening method. • Antigenic sub typing done by HI. • For RT-PCR, negative control included for every 4 samples tested.

1. Studies of influenza-infected humans associated with exposure to poultry.

	Positive control used for every PCR run. • Samples that could not be typed were assayed by cell culture and subtyped by HI test. • Most positive samples had been collected within 5 days of symptom onset.
# People tested	• 453 reported health complaints (testing data only shown for 450; data missing for 3) tested: 322 men and 128 women; 83 contacts of human cases also tested.
Results	• 82 primary cases confirmed by RT-PCR, virus isolation or both. • These were symptomatic exposed people, with the vast majority experiencing conjunctivitis (75) and conjunctivitis + ILI (5); only 2 had solely ILI. • The questionnaire was not filled out on 2 of these cases and 2 people with positive viral isolation did not meet the case definition. • A/H7 infection was confirmed in 3 contacts of human cases (not of interest in this review)
Age & Gender with outcome of interest	• Mean age of people included on case register = 32.8 y (SD 16.4; 0-103); Among confirmed cases, average age=30.4 (12.3; 13-59).
Data analysis methods	• Descriptive epidemiology using MS-Excel 97. • Chi square test with continuity correction to compare proportions of persons with symptoms of A/H7 positive and negative people.
Possible sources of bias; confounding	• Coincidental late seasonal increase in A/H3N2 at the time of the A/H7N7 outbreak may have influenced the number of those with symptoms coming forward; • After the first 2 cases of A/H7N7 associated conjunctivitis were detected, active case finding started as did the reinforcement of physical prevention measures and mandatory vaccination of workers (human flu vaccine). After 19 cases were confirmed, March 14, 2003, preventive measures were stepped up—personal protective equipment, hand hygiene, treatment with oseltamivir for people with conjunctivitis and prophylaxis with oseltamivir daily for people handling potentially infected poultry. • Municipal health service performed active case finding by visiting families and workers on all poultry farms in the region. This may have impacted on the high attack rate noted, as people with mild illness were found and assessed. • Confirmed cases were interviewed by telephone and contacts identified; written consent required for nose and throat swabs. • It has been postulated that the detection of influenza A virus in eye swabs by RT-PCR may be the result from mechanical contamination by virus-containing dust.
Provisions for minimizing influence of confounding	• A positive control sample was included in each PCR run (A/Parrot/NorthernIreland/VF-73-67/73). And for RT-PCR, a negative control (virus transport medium) used for every 4 clinical samples. • Virus isolation procedures done using standard protocols. • lab methods followed to minimize risk of cross-contamination, e.g., dedicated pipettes, thermo cycling and amplicon detection done on a separate floor.

1. Studies of influenza-infected humans associated with exposure to poultry.

Limitations	• only investigated symptomatic people; mild cases or asymptomatic infections would have been missed, impacting on the assessment of risk of infection with A/H7influenza. • Viral load data from eye swabs not stated.
Key findings	• Attack rates far exceeded those reported previously, but reasons for this are unclear—could be unique properties of the virus, type of poultry work during the outbreak, or active case finding. • Most cases were detected in workers who were culling chickens. • All viruses characterized were completely of avian origin.
Conclusions	• Concluded that veterinarians and people who cull infected poultry have the highest risk of A/H7 infection. • The association of positive virus tests with recent onset of illness, and the finding that contacts had ocular shedding, led the researchers to conclude that the conjunctivitis was caused by replicating avian influenza A viruses.
Notes	• A follow-up cohort study is underway to test anti-H7 antibodies and identify potential risk factors—see Bosman et al; RIVM study.

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Bosman, 2005: Final analysis of Netherlands avian influenza outbreaks reveals much higher levels of transmission to humans than previously thought (see RIVM report below for further detail).**
Study design	• Cross-sectional
Objectives of study	• Serosurvey to determine possible infection with the avian flu virus, in people associated with outbreak control measures.
Setting	• outbreak in the Netherlands in 2003—commercial domestic poultry farms.
Study population	• 1300 people: 400 poultry workers and their families and approximately 900 people were involved in the outbreak control efforts.
Subject selection	• The above population participated in a questionnaire survey and blood specimens were taken from 500 of the above population. • Additional studies were performed for 62 household contacts of 25 persons with avian flu virus infection. (not of interest in this review).
Exposure of interest	• Poultry; all exposed to poultry; some exposed to poultry infected with A/H7N7
Virus	• A/H7N7
Outcome of interest	• Human infection with A/H7N7
Methodology	• Cohort study: serosurvey • See further information in RIVM report below. • Data collection not described in detail; • Data collected on adherence to PPE use and on mental health issues.
#People with exposure of interest	• Study population above (n=1300 exposed); the 500 persons from whom blood samples were collected were exposed. • It is not clear to what extent the individuals in each of the 3 groups were exposed. • Total number exposed was estimated at approximately 4500.
Laboratory methodology	• A modification of the HI assay was developed, based on observations that AI viruses favour binding to RBCs from horses rather than turkeys. • "measurable antibodies" not defined.
#People tested	• 500 of 1300 in study population.
Results	• 50% of the people exposed to infected poultry had H7 antibodies detectable with the modified assay.—interpreted to mean 50% of 500, or 250 persons. Specific antibody titres not stated.
Age & Gender with outcome of interest	• Not stated
Data analysis methods	• RR calculated for having measurable antibodies associated with conjunctivitis and for antibody development associated with having taken antivirals. • Detailed data not shown.
Possible sources of bias; confounding	• Timing of data and sera collection in relation to the outbreak not specified.
Provisions for minimizing influence of confounding	• Specificity of the unconventional assay was confirmed by the absence of reactivity in sera from 100 controls recently vaccinated with influenza vaccine (2002/2003; specificity 100%). • Assay specificity further supported by the results of the cohort study.
Limitations	• It is not clear what proportion of the 500 persons tested came from the poultry farmer, family or other worker groups.

1. Studies of influenza-infected humans associated with exposure to poultry.

	• Laboratory cut point not stated; • Results data not shown • Details on contents of questionnaire not stated
Key findings	• Estimated that a/H7N7 infection occurred in at least 1000 people and perhaps up to 2000. • Seroprevalence of H7 antibodies in people without contact with infected poultry, but with household contact to an infected poultry worker, was 59%. • Having measurable antibodies was associated with having conjunctivitis (RR 1.72; 95%CI= 0.99-2.99); a lower proportion of those who took prophylactic antivirals developed antibodies (corrected OR, 0.48; 95% CI 0.25-0.89). • Oseltamivir protected against conjunctivitis (corrected OR=0.14; 95% CI=0.08-0.27) as well as against infection without specific symptoms. • No protective effect was demonstrable for safety goggles or mouth-nose masks. • Neither poultry farmers nor those engaged in controlling the epidemic complied satisfactorily with preventive measures.
Conclusions	• Seroprevalence of 59% in those household contacts of persons in contact with infected poultry suggest that the population at risk was not limited to those with direct contact with poultry and that person to person transmission may have occurred on a large scale. • Influenza viruses crossing the species barrier between poultry and humans is less rare than previously recognized and avian influenza virus adaptation occurs rapidly and if such jumps occur, human behaviour in the broad sense may accelerate dissemination.
Notes	• Overlaps with RIVM report—this is a summary version of the same study.

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Bosman et al--RIVM report—Executive summary: Avian Flu Epidemic 2003: Public Health Consequences; Bosman et al; further to summary report above.**
Study design	• Cross-sectional
Objectives of study	• 7 objectives, including to estimate the risk of infection with the A/H7N7 virus in humans after exposure to infected poultry.
Setting	• The Netherlands 2003—outbreak of H7N7 avian influenza in domestic poultry with associated human illness and infection.
Study population	• Study 1: persons in regions affected by avian flu with complaints or symptoms consistent with flu or conjunctivitis and who could have had contact with infected poultry. 453 persons with symptoms were investigated—this is duplicated by Koopmans study so will not be repeated here). 3 groups of people with health complaints were identified: poultry farmers and family, medical personnel and others; persons involved in flu control. Each group was divided into those with direct contact and those with no direct contact. • Studies 2 and 3: broader population: persons who had intensive contact with infected poultry, persons who were involved in or affected by the culling operations, and poultry farmers who were confronted with transport restrictions in the so-called 10 km zone. There were 2 affected areas; initially the study started in the first area affected, then expanded to include the second area
Subject selection	• Study 1== people coming forward with symptoms (covered by Koopmans et al, 2004). • Studies 2 and 3: A total of 1259 persons –owners of poultry farms, were invited to participate. These were all owners of poultry farms that were cleared because of AI and their partners. At least one person (owner and/or partner) of almost 33% of the farms agreed to participate in the study (total of ~400 persons). Persons keeping poultry as pets were excluded. • 50% of the 1749 invited persons who were involved in the control of the avian flu outbreak participated in this investigation (n=874). • In total (from the 1300 who agreed to participate), 500 persons donated blood and saliva samples to gain insight into the extent of transmission of AI to humans. 23 interviews were also carried out.
Exposure of interest	• Poultry. Some participants were exposed to infected poultry (A/H7N7) or manure; others were exposed to poultry which were not infected. (human contact investigations were done for lab-positive cases—not of interest in this review).
Virus	• A/H7N7
Outcome of interest	• Human infection with A/H7N7
Methodology	• 3 investigations were completed: o 1)surveillance of conjunctivitis and flu-like illness; o 2)Risk factors for transmission of AI virus and psychosocial health, well-being, and o 3)health care needs. • Investigations 2 and 3 were integrated as much as possible and as much use as possible was made of information from the Regional Crisis Center. • Data collected: Questionnaires, interviews, blood and saliva samples,

1. Studies of influenza-infected humans associated with exposure to poultry.

	information on the control of the outbreak and information from the crisis centre about affected poultry farms and indicators of exposure of other professional groups involved. • Questionnaire: demographic data, function and work performed during the epidemic; possible exposure to poultry or manure; informative arrangements and preparation for culling; experiences during culling; stress symptoms; fatigue and the quality of sleep; depressive feelings; use of care; social contacts and the support they provided; general health and the occurrence of symptoms; the degree of use of protective materials; and experience with agricultural assistance and advice. • Interviews to obtain qualitative information which is difficult to obtain via questionnaire. • Other sources of data were: the animal disease information system of the RVV; addresses of workers; and digital files such as the electronic archive system of the RVV.
#People with exposure of interest	• Estimated that 4500 persons had contact with poultry. 1300 of these participated in the study and 500 of these provided serum samples for testing, not counting the 453 exposed symptomatic individuals worked up in investigation #1.
Laboratory methodology	• Altered HI test
#People tested	• 453 symptomatic people tested; (investigation #1) • 500 people cullers and other responders were tested. (investigations 2 and 3).
Results	• Investigation #1: Of the 453 symptomatic people: 89 persons tested positive: A/H7N7 found in tear fluid of 78 (26.4%) persons with conjunctivitis only; 5 (9.4%) persons with both ILI and conjunctivitis, in 2 (5.4%) persons with ILI only and 4 (6%) persons with other symptoms. Investigation #2 and 3: Bosman (2005) reported that at least 50% of the people exposed to infected poultry had H7 antibodies.
Age & Gender with outcome of interest	• Not specified.
Data analysis methods	• RR; OR • Details not provided.
Possible sources of bias; confounding	• Due to active case finding and additional sampling of the eye, the likelihood to detect cases was greater than in routine flu surveillance, in which people are only examined when they themselves report with complaints and only a throat swab is obtained. • Not all groups were evenly represented in the different studies; that applies equally to the cullers and foreign hired personnel. These groups were underrepresented in the investigation. •
Provisions for minimizing influence of confounding	• Specificity of the unconventional assay was confirmed by the absence of reactivity in sera from 100 controls recently vaccinated with influenza vaccine (2002/2003; specificity 100%). • Assay specificity further supported by the results of the cohort study.
Limitations	• It is not clear what proportion of the 500 persons tested came from the poultry farmer, family or other worker groups. • Laboratory cut point not stated; • Detailed results by age group, vaccination history, antiviral use, PPE use not

1. Studies of influenza-infected humans associated with exposure to poultry.

	provided. • Antibody test results not provided, cut-point not defined. • It is not stated what the results of antibody testing was, e.g., values; cut-point not stated. • It is not specified which study groups investigated or the proportions of them, comprised this 50%. For example, some of the 400 poultry farmers and their family may have been from uninfected farms cleared as a preventive measure—this is not clearly stated.
Key findings	• Largest number of virologically confirmed AI infections in people ever described in the medical literature. • The percentage of poultry farmers who developed eye complaints was about 5 times higher on infected farms than on non-infected farms (14% vs. 2.4%). • In all analyses of the questionnaires, the extent of contact with manure from infected poultry emerged as the exceptional risk factor for conjunctivitis, based on the question of whether the work clothing became soiled. • Study 2: Conjuncitivits was more often observed in poultry farmers and others in the same household on infected farms (14%) than on non-infected farms (2.4%, RR=5.2; 95% CI 2.35-11.59). Antibodies against A/H7N7 were quite frequent in poultry farmers (63%) and in workers exposed to infected poultry (50.6%). • The results of the epi study suggest that oseltamivir protects against conjunctivitis (OR=0.14; 95%CI=0.08-0.27) as well as against infection without specific symptoms (OR=0.47; 95% CI=0.25-0.88). No protective effect was demonstrated for safety goggles or mouth-nose masks. • Contact with chicken manure was the only factor which, after correction for all other factors, was found to carry an elevated risk for conjunctivitis (OR=1.99; 95%CI=1.00-3.93). Persons who screened poultry on infected farms had an increased probability of H7 antibodies (OR=2.12; 95% CI=1.10-4.07) after correction for other risk factors. • Analysis of isolated virus from swabs of people with conjunctivitis revealed that there was scarcely an indication of alteration of the genetic material of virus (mutations), compared with viruses isolated from poultry. • Investigators deduced that at least 1000 persons who had contact with infected poultry developed an infection with the avian virus. • Persons with symptomatic AI infection shed virus for more than 3 days. • The majority of the AI infections in examined groups were asymptomatic.
Conclusions	• The methods routinely used for demonstration of human flu virus and antibodies are not suitable for demonstrating infection with AI. • Safety goggles and mouth-nose masks were found to have no protective effect, whereas prophylactic use of oseltamivir was noted (protects against infection) • Infection with AI is a professional risk for persons working in the poultry sector. • AI A/H7N7 appears to be transmissible from person to person in a household situation.
Notes	•

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Puzelli et al 2005 Serological analysis of serum samples from humans exposed to avian H7 influenza viruses in Italy between 1999 and 2003**
Study design	• Cross-sectional study.
Objectives of study	• Analysis of serum samples from humans exposed to AI H7 between 1999 and 2003 to evaluate the potential for avian-to-human transmission of low pathogenic AI and highly pathogenic AI viruses that were responsible for several outbreaks of influenza in poultry in Italy from 1999 to 2003.
Setting	• Italy; Veneto and Lombardy regions • poultry outbreaks between 1999-2003
Study population	• poultry workers in several categories of labor at different farms in the affected regions
Subject selection	• Recruitment methods not specified, however, informed consent process for participation
Exposure of interest	• Poultry infected with AI A/H7N3 (LPAI) and A/H7N1 (HPAI and LPAI)
Virus	• H7N3 (LPAI) and H7N1 (HPAI and LPAI)
Outcome of interest	• Evidence of anti-H7 antibodies in serum samples from subjects exposed to poultry.
Methodology	• Cohort study. • Between August 1999 and July 2003, 983 serum samples were collected from workers in several categories of labour at different farms located in the Veneto and Lombardy regions of Italy. • All subjects were asked to complete a questionnaire noting the type of work they did with poultry and any respiratory illnesses they had during the AI epizootics. for epizootic 4, serum samples were collected >1 year after the last outbreak of AI caused by an H7N1 LPAI virus in Veneto, due to delays in the ethical approval process. Informed consent obtained from participants. • serum samples were collected at least 15 days after the onset of each epizootic and stored until tested for antibodies to H7N1 or H7N3 virus. • Each serum sample was tested by HI, MN assays. • If a sample was considered to be positive for either test, a Western Blot analysis was performed. • each serum sample was tested at least twice in separate MN assays that were performed in duplicate. • serum samples that repeatedly had titres >20 were considered to be reactive in the MN assays. a titre of 10 was considered to be a positive result in the HI assays. • Questionnaires noted type of work with poultry, any respiratory tract illnesses they had during the AI epizootics.
#People with exposure of interest	• All 983 subjects were farm workers from the 2 regions affected; representing several categories of labour.
Laboratory methodology	• A variety of serological techniques were used in this study. • Each sample tested by HI and microneutralization (MN) for antibodies against H7N1 and H7N3 viruses; any positives for either test were then tested by western blot (WB). • Serum samples that repeatedly (tested twice) had titres >20 were considered to be reactive in the MN assays. • A titre of 1:10 was considered to be positive in the HI assays. • Single radial hemolysis testing also used and a reactive zone of >3.5mm

1. Studies of influenza-infected humans associated with exposure to poultry.

	considered positive. • Antiserum from immunized animals were used as positive controls in the HI, MN and SRH assays and monoclonal antibody specific for H7 HA was also used in the WB analysis.
#People tested	• 983 • epizootic 1: LP H7N1, Lombardy-- 85 samples tested. No positives. No 2: HP H7N1, Lombardy-- 513 samples; 0 positive. No 3, HP H7N1, Veneto, 159 samples--0 positive; No 4, LP H7N1 Veneto, none positive; No 5, LP H7N3, Lombardy, 43 samples, 1 positive by both HI and WB; 3 additional positive by MN and WB. ; No. 6, LP H7N3, 142 tested, 3 positive by HI and WB; same 3 also tested pos by MN and WB.
Results	• 7 positive in total; 7/185 (3.8%) reactive by MN to both viruses, with higher titres to H7N3, and 4 of 7 samples were reactive as assessed by HI assays, with higher titres to H7N3. • When these 7 samples were tested by WB against purified H7N1, H7N3 and baculovirus-expressed HA from H7N1, A/Ty/It/3889/99 or H7N3, A/Ty/It/214845/02, all showed clear reactivity to H7 HA, in contrast to serum samples that did not show any reactivity by HI and MN assays
Age & Gender with outcome of interest	• all were 35-62 yrs old; data missing for 1; • 3 male; 3 female; data missing for 1.
Data analysis methods	• descriptive analysis of laboratory test results
Possible sources of bias; confounding	• for epizootic 4, serum samples were collected >1 year after the last outbreak of AI caused by an H7N1 LPAI virus in Veneto. • No data were collected regarding sickness due to any other cause during the AI epizootics.
Provisions for minimizing influence of confounding	• Hayden and Crozier (2005) in their commentary on the transmission of AI viruses to and between humans state that the variety of test methodology used in this study and the confirmatory WB analysis using purified H7 HA excludes the possibility of nonspecific cross-reactions with antibodies to human influenza viruses is one of its strengths
Limitations	• samples collected from those workers involved in epizootic 4 were collected >1 year after the outbreak. • Only broad description of data collected on the questionnaire in association with lab results, e.g., exposure, environment in which exposure occurred; no specific analysis of risk factors. • Specific information on the nature of exposure within each occupational group not provided.
Key findings	• All seropositive subjects had close direct physical contact with either turkeys or chickens in poultry housing, which was described as being a dusty environment. only one of the seropositive subjects reported clinical symptoms at the time of the AI epizootics (conjunctivitis); 6 of seronegative subjects also had history of conjunctivitis but did not show any serological reactivity to H7 HA. • None of the 7 seropositive subjects reported ILI; 14 of seronegative subjects reported having ILI.
Conclusions	Conclusion: unequivocal serological evidence of exposure to or infection with H7 viruses.
Notes	

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Vong et al (2006): Low frequency of poultry-to-human H5N1 virus transmission, Southern Cambodia, 2005**
Study design	• Cross-sectional study
Objectives of study	• Retrospective survey of poultry deaths and a seroepidemiologic investigation to understand the transmission of avian influenza H5N1 virus to humans.
Setting	• Cambodian village in the Banteay Meas District, where a 28 year old man was infected with H5N1 virus in March 2005.
Study population	• 300 participants in the affected village • Among 93 households that were surveyed, Of 354 people, 351 participated and 3 refused; an average of 4 people resided in each household, median age=23 yrs (range 1 mo- 81 yrs), and 150 (42.7%) of the sample were male; 207 (59%) were farmers of both crops and livestock. The rest of the participants were students (29.3%), had no stated occupation (18.8%), or were construction or factory workers (0.9%).
Subject selection	• Four investigation teams of 3 members each visited all households in 4 different directions, starting from the household of the confirmed patient, until 300 participants were enrolled. Each household visited once and no further attempts made to interview absent adult household members.
Exposure of interest	• Poultry suspected of having H5N1 virus infection; all participants exposed to poultry; some exposed to suspected infected poultry.
Virus	• A/H5N1
Outcome of interest	• Presence of antibodies to A/H5N1 / transmission of H5N1 from poultry to humans.
Methodology	• Cross-sectional serosurvey. • Retrospective poultry death survey conduced in the district, March 25-57, 2005. All households within a 1km radius from the case-patient's household were mapped and positioned with a GPS. Info collected on illness suggestive of H5N1 among animals in each household by interviewing the head of the family with a standardized questionnaire. • Households where the head of the family was not at home or could not be found were omitted. • Chicken flocks were considered likely to have been infected by H5N1 during the past 6 months if all of the following were reported: flock death >60%; 100% case-fatality ratio, and sudden death of young and mature birds within 1 or 2 days of becoming sick. • Sick poultry and carcasses were collected for testing; cloacal swabs of 10 to 14 randomly selected live healthy birds were also collected from each household where birds remained. • Seroepidemiologic investigation; June 3-7, 2005 (~ 2 mos after poultry deaths). • Standardized 39 item questionnaire administered: demographic info, specific exposures to animals and the environment during the last 12 months; blood specimens collected. • See subject selection for recruitment methodology. • written informed consent was obtained from adults or from a parent or guardian for children <18 years. • Data collected included: exposure data; demographics; specific poultry handling behaviours, poultry purchasing behaviours,

1. Studies of influenza-infected humans associated with exposure to poultry.

#People with exposure of interest	• A substantial proportion of the surveyed population had regular, high-intensity contact with these animals in the 12 months before the survey; this included collecting, processing, and eating sick birds or birds that had recently died when H5N1 viruses were thought to be circulating among flocks in the village. • In the cohort study, the exposures of 96 residents from households who had a high probability of an outbreak among their flock were compared to 166 occupants from households where no chickens died.
Laboratory methodology	• Human blood specimens were tested for H5N1 neutralizing antibodies by MN assay and confirmatory WB assay. Serologic evidence of H5N1 virus infection was defined as H5N1 neutralizing antibody titre $\geq$80 with a confirmatory western blot assay.
#People tested	• 351.
Results	• No positives found.
Age & Gender with outcome of interest	• n/a
Data analysis methods	• Space-time statistic to determine cluster of households most likely to have been affected by H5N1 virus in previous 6mo, using SaTScan. Cases assumed to follow a Poisson distribution. • Individual and household data were entered into EpiData and validated with a duplicate data file. STATA used for all statistical analyses. • Independent associations between demographic and behavioral data and households that were likely to be affected by H5N1 in poultry were also analyzed by logistic regression models. • The cluster effect of households was accounted for with STATA's "cluster" option for logistic regression, which specifies that observations are independent across households but not within households. • For multivariate analysis, variables with a p value $\leq$0.1 were retained in the models. • Selected variables whose correlation coefficient was >0.4, which indicates colinearity between these variables, were not included in the logistic regression model.
Possible sources of bias; confounding	• Recall bias • Temporality of specific exposures and poultry deaths. • Only 3 refusals. • A small chance exists that previous H5N1 virus infection might have been missed if levels of H5N1 neutralizing antibodies had declined; for example, some human influenza virus infections do not invariably result in a detectable serum antibody response.
Provisions for minimizing influence of confounding	• Used standardized questionnaire. • Investigated infection among poultry as well as humans • Used accepted testing methods • Surveyed regarding exposures and animal handling practices. • Interviews were conducted soon after poultry outbreaks occurred (~2months) Limitations were documented
Limitations	• Recall period of 12 months • Did not document more temporally relevant exposures immediately before or

1. Studies of influenza-infected humans associated with exposure to poultry.

	during the outbreak. • Long recall period may increase the probability of exposure to potential risk factors, making the households with and without suspected H5N1 virus infection in flocks more similar. • The temporal association between behavioral risk factors and H5N1 virus infection in poultry was difficult to establish. • Without confirmation of H5N1 virus infection, the sensitivity and specificity of the case definition (infected flocks) could not be known and the degree of misclassification, if present, could not be quantified. • Households not visited were not mapped, but this bias is likely to have been non-differential because the proportions of non-visited households were similar for all 4 investigation teams that surveyed in 4 directions. • Poultry specimen collection had limitations—tracheal swabbing yields higher concentrations of virus vs. the cloacae but the former was not performed due to objection of farmers. • Findings are limited to the period of 2005.
Key findings	• Primary finding: transmission of H5N1 viruses from infected poultry to humans appears to have been low in a rural Cambodian population with confirmed and suspected H5N1 poultry outbreaks and where a human case occurred in 2005. • According to the definition applied, 42 households were likely to have had an outbreak of H5N1, for an overall attack rate of 27% among households with chickens. 11 households with a high likelihood of H5N1 (35%) in chickens also owned ducks, although only 2 of these described simultaneous deaths of ducks, however overall, raising ducks with chickens was not associated with deaths in chickens (p=0.57). • None of the villagers interviewed reported having a febrile or respiratory illness during the same period, and none of the 351 participants had neutralizing antibodies suggestive of H5N1 virus infection on microneutralization assay. • Bivariate analysis showed that households that purchased live poultry in the preceding year were almost 4 times more likely to have had H5N1 in their flock than households that did not buy live chickens. • After controlling for poultry purchasing the following behaviors appeared to reduce the risk for H5N1 virus infection in their flock by half: cleaning cages or stalls, cleaning up poultry feathers, and handling live poultry. • Slaughtering chickens was not a significant risk factor after controlling for exposures that were significant on multivariate analysis.
Conclusions	• Asymptomatic and mild H5N1 virus infections had not occurred in the population investigated. • The seroprevalence of H5N1 antibody in the Cambodian population surveyed was substantially lower than was found in poultry workers in Hong Kong in 1997 with the same microneutralization assay.
Notes	

1. Studies of influenza-infected humans associated with exposure to poultry.

Reference:	**Chen et al, 2001; Ref# Title: Surveillance of influenza viruses in Guangdong Province, China in 1998: a preliminary report**
Study design	• Cross-sectional study
Objectives of study	• Surveillance of influenza viruses in Guangdong Province, China in 1998.
Setting	• Guangdong Province, China in 1998: • 8 cities during March-October 1998. • Occupational group raised and slaughtered chickens; however the exact setting is not clearly described.
Study population	• One of the groups studied was the occupational group of raising, selling and slaughtering chickens; of interest in this review; and also a "general" group (exclusive from the outpatients with ILI group, inpatients with respiratory infections, occupational group, all other groups tested). Chickens in farming markets or chicken farms were also tested
Subject selection	• Not described
Exposure of interest	• chickens-- occupational group; no exposure in general group.
Virus	• H9N2 found; H5N1 not found
Outcome of interest	• Serologic evidence of avian influenza infection (antibodies) among persons working with poultry; • Isolation of avian influenza viruses from outpatients with influenza-like-illness and inpatients with bronchitis and pneumonia or other lung infections—*this outcome not of interest in this review.* •
Methodology	• Pertaining to occupational group and general group: sera collected and tested for antibody to influenza viruses by HAI. • Data collected-- Lab test results described
#People with exposure of interest	• Occupational group (n=1512) • Comparison-- General group (n=885); exclusive of occupational group and the 2 groups of patients with respiratory illness.
Laboratory methodology	• HI titres and viral isolation. • H5N1 tested by HI; titre of 1:20 or more was diagnosed to be positive; According to the serological test, HI antibody of A/H9N2 was found to be positive only in one case, while the others were not detectable. The blood collection time was in intervals of about 2-3 months after their onset of diseases and the ages of the cases were distributed widely.
#People tested	• 1512—occupational group • 885—general group • outpatients—8563 samples taken from patients (inpatients and outpatients).
Results	• H9 was isolated from 9 patients and HI antibody to H9N2 tested in 8 of the 9 and only 1 of the 9 had 1:120 to 1:160 and others had 1:20. **No exposure data is cited for positive individuals, so they should be excluded from any analyses in this review.** • Occupational group and general group: sera tested by HAI; no positives.
Age & Gender with outcome of interest	• Not stated
Data analysis methods	• Description and data lacking.

1. Studies of influenza-infected humans associated with exposure to poultry.

Possible sources of bias; confounding	• HI method not recommended for avian A/H5N1 testing, which may have impacted on the lack of positives found in the 1512 samples from the occupational group and 885 from the group of general individuals tested. • Only single serum samples tested. • Measures to control for cross reactions not described.
Provisions for minimizing influence of confounding	• All sera treated before testing to remove nonspecific inhibitors.
Limitations	• It is not clear if the occupational group was tested for human strains of influenza; lab methods selected and cut point for positivity not in keeping with current WHO recommendations. • no details provided on demographics or nature of exposure in occupational groups; occupational groups not tested for other strains (aside from H5N1); HI not considered adequate by other researchers for detection of H5 antibodies. • Author acknowledges that viral neutralization testing should be done for further confirmation of the result; however HI titre of 1:40 corresponds generally with detectable virus infectivity neutralizing activity. The impact of cross-reacting H9 with H3, H2, and H4 must be fully considered when detecting HI antibodies—this is stated as important, however it is not clear if this was done.
Key findings	• No H5N1 antibodies detected in sera of occupational group. • After they were infected by A/H9N2, the HI antibody of A/H9N2 should have been produced and could not have disappeared completely in the serum.
Conclusions	• Conclusion of authors-- needs further study.
Notes:	• a preliminary report. • Other groups not described here: outpatients with ILI; inpatients with bronchitis, pneumonia or other infections in lungs (no exposure history noted).

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	Zhou et al 1996 ; Ref# ; Influenza infection in humans and pigs in southeastern China
Study design	• Cross-sectional
Objectives of study	• 1) to establish the frequency of interspecies transmission and reassortment of flu A viruses among pigs and humans living in or near Nanchang City; • 2) to establish seroprevalence of flu in the Nanchang region as compared to other cities.
Setting	• China • Nanchang region of central China
Study population	• 4 groups were studied; 3 human; 1 swine • people who lived or worked in close contact with pigs (268 slaughterhouse workers and 200 women who raised pigs--also raised ducks and worked in rice fields), ages ranged 18-50, mean=31.3 yrs. • people who had little or no contact with pigs (200 university students at local medical school; 19-22 yrs; mean= 20.7 yrs; • people living and working in the US (32 employees at St. Jude Children's research Hospital); • 4) slaughtered pigs from suburban areas or neighboring counties of Nanchang City. • Groups 1 and 2 had never been vaccinated against influenza; group 3 had not been vaccinated in the current year
Subject selection	• Recruitment methods not outlined.
Exposure of interest	• Pigs, ducks and chickens; Swine husbandry in Nanchang is a family business in which pigs as well as poultry are raised in the yards and houses of farmers. Women who raised ducks and pigs also spent considerable time in rice fields.
Virus	• Nanchang/3332/93 (H3N2); Texas/36/91 • H1N1); Swine/Beijing/47/91 (H1N1)--classical sw virus; Swine/Italy/786/88 (H1N1) (recent avian origin);Swine/Italy/809/89 (H3N2)-- a Port Chalmers/73 (H3N2-like strain; Duck/Nanchang/1681/93 (H3N8); Duck/Nanchang/1904/93 (h7N4); Duck/Nanchang/1941/93 (H4N4); Duck/Nanchang/1749/93 (H11N2); Japan/305/57-A/Bel/42 (H2N1), A reassortant strain; Duck/Nanchang/1904/93-A/Bel.42 (H7N1) a reassortant strain
Outcome of interest	• Presence of antibodies to swine influenza viruses and duck viruses; transmission of influenza viruses from animals (ducks, pigs) to humans.
Methodology	• Serological studies done December 1993 to June 1994. Blood samples collected twice, at 6 month intervals, from slaughterhouse workers and once from other subjects. • Strains used: Nanchang/3332/93/(H3N2); Texas/36/91 (H1N1); Swine/Beiging/47/91/(H1N1)--classical swine virus; Swine/Italy/786/88 (H1N1) recent avian origin; Swine/Italy/809/89/(H3N2)-- a PortChalmers/73/H3N2-like strain; Duck/Nanchang/1941/93(H4N4); Duck/Nanchang/;1749/93(H11N2); Japan/305/57--A/Bel/42 (H2N1), R a reassortant strain; Duck/Nanchang/1904/93--A /Bel/42(H7N1), R, a reassortant strain-- this was used to assay antibodies to H2 and avoid any cross reactions with current N2 strains. • The A/Bel/42(N1) neuraminidase was used, because it is sufficiently

2. Studies of influenza-infected humans associated with exposure to swine.

	different antigenically from current N1 strains. Nanchang viruses were isolated from humans and ducks in the region in which this study was done. • Data collected: General exposure profiles, lab data.
#People with exposure of interest	• 468 • 268 slaughterhouse workers; 200 women raising pigs; 205 student controls and 32 Memphis controls.
Laboratory Methodology	• Virus isolation and serologic testing (twice at 6 month intervals) for slaughterhouse workers and once for other subjects. • Isolated viruses were grown in embryonated chicken eggs and allantoic fluids were measured for hemagglutination titres and used as antigens in HI (using purified HA protein) and NI tests. • Serology used HI and NI assays to test for antibodies against human, swine and avian influenza viruses, according to WHO standards (treated serum samples). In the swine virus testing, NI testing was not performed on the 205 student controls, but was done on the exposed populations and the 32 Memphis controls. • A modified ELISA method was employed for detection of influenza virus antibodies of low titre, especially avian virus antibodies in humans and pigs. • Serum from Nanchang and Memphis controls also tested by ELISA. • Duck/Nanchang/1904/93-A/Bel.42 (H7N1) reassortant strain was used to assay antibodies to H2 and avoid any cross reactions with current N2 strains. • The A/Bel/42 (n1) neuraminidase was used for it is sufficiently different antigenically from current N1 strains; Serologic assays also done, using HI and NI assays;
#People tested	• 186 slaughterhouse workers and 191 women who raised pigs were tested for serum antibody to swine viruses (377) exposed; 232 unexposed.
Results	• Highest reactivity to H7 duck virus was found in women raising pigs (25% by ELISA; with a maximum titre of 800); the remaining groups had low or negligible rates. • A 26% positivity rate was demonstrated with an H11N2 virus and the NI assay in samples collected from Nanchang slaughterhouse workers and the Memphis controls but this may reflect cross-reactivity with human N2 strains. • Reactivity with the N8 antigen of Nanchang/1681/93 was detected in two slaughterhouse workers; the assay was repeated with a reassortant virus (equine H7N8) to confirm the reactivity of human sera to the N8 antibodies.
Age & Gender with outcome of interest	• Age and Gender not stated for positives.
Data analysis methods	• Descriptive
Possible sources of bias; confounding	• Mixed exposures likely for Nanchang groups, given the described setting and lifestyle. Specific exposure histories for each positive individual not stated. • Vaccination history== Nanchang subjects had never been vaccinated, whereas controls in Memphis had just not been vaccinated in the current season. • Serologic studies were performed from December 1993 to June 1994, with samples collected twice, at 6 month intervals from slaughterhouse workers and once from other subjects.

2. Studies of influenza-infected humans associated with exposure to swine.

	• Control groups used which did not have similar exposures and vaccination history for the current year.
Provisions for minimizing influence of confounding	• Control strains were used to avoid cross-reaction with current N2 and N1 human strains and animal strains. • Standard WHO lab procedures were used for HI and NI testing, with controls. • ELISA method used to avoid false-positive results which may occur with HI tests.
Limitations	• Methodology not thoroughly outlined; • Mixed exposures possible; not detailed, • Duck feces sampled may have represented visiting wild birds and/or resident domestic ducks
Key findings	• Low incidence of antibody to swine influenza among Nanchang residents having contact with pigs may be explained by the small size and isolation of individual pig farms. • Since results for the human-like swine virus may reflect the triggering of antibody memory generated in response to exposure to recent H3N2 strains, the serologic data do not indicate transmission of swine viruses to humans in Nanchang. • H7 duck virus-- highest reactivity rates found in women raising pigs and ducks in their homes and who spent considerable time in rice fields (25% with maximum titre of 800); remaining groups had low or negligible rates. H11N2 virus: Nanchang slaughterhouse workers and Memphis controls: 26% positivity rate with H11N2 virus and the NI assay in Nanchang slaughterhouse workers; may reflect cross reactivity with human N2 strains. N8 antigen of Nanchang/1681/93 detected in 2 slaughterhouse workers (H3N8). Since virus used was H3N8, assay was repeated with HA of H7N8 (A/equine/Prague/1/56and N8 NA (H7N8) as the antigen, to confirm reactivity in human sera to N8 antibodies. Rates of reactivity and antibody titres with characteristic swine viruses were essentially the same in slaughterhouse workers and pig farmers as in students who were not exposed to pigs. • Slaughterhouse workers who lived in suburban areas of Nanchang, where numerous duck farms are located, had the second highest rates, followed by university students and Memphis controls, neither of whom had contact with living ducks. • The NI assay yielded evidence of antibodies to N4 and N8 avian subtypes, supporting the theory of direct avian-to-human transmission. • specific results in tables in the study-- Table 2:
Conclusions	• Because swine husbandry in Nanchang is usually a family business, the size of each pig farm is relatively small and farms are usually isolated from each other; perhaps explaining the apparent low incidence of antibodies to swine influenza viruses in pigs in Nanchang residents having contact with pigs. • This study yielded evidence to support the theory of direct avian-to-human transmission of avian influenza viruses.
Notes:	•

2. Studies of influenza-infected humans associated with exposure to swine.

Study group	Sw/BJ/47/91 (H1N1)		Sw/Ita/786/88 (H1N1)		Sw/Ita/809/89 (H3N2)	
	HI	NI	HI	NI	HI	NI
Slaugherhouse workers (186)	2/186 (20)	0	3/186 (20)	11/186 80 (1) 40 (4) 20 (6)	151/186 >160 (3) 160 (2) 80 (26) 40 (51) 20 (69)	108186 80(19) 40 (35) 20 (54)
Women raising pigs (191)	6/191 (20)	2/191 (20)	0	6/191 40 (3) 20 (3)	149/191 >160 (4) 160 (3) 80 (22) 40 (43) 20 (77)	138/191 80 (46) 40 (30) 20 (62)
Student controls (205)	3 (20)	ND	10 40 (1) 20 (9)	ND	175/205 >160 (39) 80 (60) 40 (42) 20 (34)	ND
Memphis Controls (32)	5 80 (1) 40 (2) 20 (2)	2 80 (1) 40 (2)	3 80 (1) 40 (1) 20 (1)	2 80 (1) 40 (1)	27 >160 (4) 80 (6) 40 (10) 20 (7)	27 80 (7) 40 (10) 20 (10)

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	• **Wells et al 1991: Swine influenza virus infections. Transmission from ill pigs to humans at a Wisconsin Agricultural fair and subsequent probable person-to-person transmission.**
Study design	• Cross-sectional
Objectives of study	• To detect other persons who were possibly infected by contact with the ill swine at the exhibit, prompted by the single case of the 32 year old pregnant woman who died as a result of SIV infection.
Setting	• U.S. Wisconsin Agricultural fair (Rota et al 1989)
Study population	• 50 junior swine exhibitors who were 9-19 years old • Comparison group: 50/136 junior swine exhibitors in a neighboring county • The patient's family and close contacts including health care providers were studied but details not summarized here, as secondary transmission is not the focus of this review.
Subject selection	• All 156 junior swine exhibitors were considered exposed, and 50/156 were randomly selected for the survey. • A comparison group of 50/136 junior swine exhibitors in a neighboring county were selected and matched by age +/- 1 year to exposed exhibitors.
Exposure of interest	• Ill pigs at a Wisconsin county fair
Virus	• Swine H1N1
Outcome of interest	• Infection or illness following exposure to swine at the fair.
Methodology	• Standardized telephone questionnaire was administered to the parents of the exhibitors from November 2-18th; information collected noted above. • Questions were asked regarding: receipt of 1976/77 swine influenza virus vaccine, history of ILI in exhibitors, their family or pigs, history of living on a farm where pigs were raised; age
#People with exposure of interest	• All 156 junior swine exhibitors were considered to be exposed because the swine were all kept in the same barn and 50/156 were randomly selected for survey.
Laboratory methodology	• HI titres $\geq$20 considered positive. • Lab testing done on 25 of the exposed and 25 unexposed junior swine exhibitors.
#People tested	• 25 exposed; 25 unexposed (control group from neighboring county).
Results	• among the 25 exposed who provided serum samples, 19 were positive for swine influenza virus (76%), including 5 of 6 first time exhibitors; none of the controls had HI levels detectable at a dilution of 10 and were reported as less than 10.
Age & Gender with outcome of interest	• mean age all jr. exhibitors =14; range 9-19 • Gender not stated
Data analysis methods	• statistical methods – reported P values were derived using Fisher's exact test, two-tailed. Relative risk was calculated for all comparisons and 95% confidence intervals were obtained using Taylor series.
Possible sources of bias; confounding	• low titre cut point, however authors note that younger persons can be presumed to have a decreased likelihood of having detectable antibody to SIV; seroprevalence increases with age, history of swine influenza vaccination and previous swine and avian (esp. turkey) exposure.

2. Studies of influenza-infected humans associated with exposure to swine.

Provisions for minimizing influence of confounding	• controls were matched by age +/- 1 year to exposed exhibitors. • Community influenza surveillance data, including school absenteeism data, public health virological reports were reviewed for the time surrounding the initial case was detected (reported in Rota et al, 1989). No evidence of a community influenza outbreak was detected (no virus isolated aside from the index case; no increase in absenteeism from the preceding year and no unexpected number of outpatient visits for ILI. • Data collected on immunization history with the 1976-77 swine influenza virus vaccine—none of the junior exhibitors reported having received it. • More
Limitations	• all 156 exhibitors were considered exposed due to the swine being kept in the same barn, however, information on illness among the specific swine of those exhibitors tested, and the movement of exhibitors and swine within the barn is not provided, not is information about the swine on the exhibitors' home farms. Single serum sample testing prevents linking the fair with the antibody response among the exhibitors.
Key findings	• More unexposed exhibitors had lived on a farm where pigs were raised than had exposed exhibitors (P=0.05) and the mean number of years of exhibiting pigs was greater for those who were unexposed (P>0.05). • Significantly more (31 of 50) exposed exhibitors than unexposed exhibitors (3 of 50) reported having exhibited a pig with ILI either at the time of their county fair or afterward (P<0.0001). • Among the 25 exposed exhibitors providing a serum specimen, 19 (76%) including 5 of 6 who were first-time exhibitors, had an SIV Hi titre of 20 or more, while none of the unexposed exhibitors had levels detectable at a dilution of 10 and therefore were reported as less than 10 (P<0.0001, chi square, Yates corrected) • Significantly more exposed (7/50) than unexposed (1/50) exhibitors had ILI in September RR, 7.0; 95%CI, 1.3-3.5; P=.03. Five of these 7 exposed who were ill reported onsets within 5 days of exposure to the pigs who were ill at the fair and also had an SIV HI titre of 20 or greater. • One exposed exhibitor with ILI and SIV HI titre of 80 also had 2 siblings (4 and 7 years of age) who visited the fair who also had ILI within 5 days of the fair and subsequent SIV HI titres of 80.
Conclusions	• The findings strongly implicate the swine exhibited at the county fair as the source of SIV in the patient, either directly from the pigs that were ill or by transmission to her husband and from him to her (ref to the 32 year old also reported in Rota et al). • The finding of a strikingly higher proportion of SIV HI titres of 20 or greater among exposed exhibitors including those who were first time exhibitors, strongly supports the conclusion that exposure to the ill swine at this particular fair led to a high rate of SIV infection in these children.
Notes	

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	Olsen et al 2002: Serologic evidence of H1 swine influenza virus infection in swine farm residents and employees
Study design	• Cross-sectional
Objectives of study	• To serologically assess the relative level of exposure to classical H1 swine influenza viruses among people involved in swine farming.
Setting	• U.S. Rural south-central Wisconsin—swine farm; Urban controls—Milwaukee, WI. •
Study population	• 74 swine farm owners, employees, their family members and veterinarians. 114 urban controls were also evaluated.
Subject selection	• Farm participants—names and contact info received by area veterinarians; farmers approached by researchers for interest in participating. Participation extended to other employees on the farm, spouses and children >7 yrs of age, and farm veterinarians. $100 honorarium provided. Control sera—people not specifically enrolled in the study; so additional info could not be collected.
Exposure of interest	• Exposure to swine
Virus	• swine H1N1 and human H1N1 and H3N2 influenza viruses: A/Johannesburg/82/96 (H1N1); A/Nanchang/933/95 (H3N2); A/Nebraska/01/92 (human isolate of swine H1N1 influenza virus); A/Swine/Indiana/1726/88 (H1N1).
Outcome of interest	• Detection of antibodies against swine influenza viruses in human serum samples.
Methodology	• The GMTs of the samples from preseason farm participants were compared to the GMTs of the urban control sera by using Wilcoxon rank sum analysis with normal approximation. The numbers of sera with an HI titre >40 to either swine virus were compared among the preseason farm participant samples vs. the urban control samples by chi-square analysis. Associations were examined for statistical significance by chi-square or two-sided Fisher's exact analyses. P values of <0.05 were considered significant. Multivariate analysis not done due to small numbers with elevated preseason titres to swine influenza viruses. • Data collected: Questionnaire recorded age, sex, overall health, nature of contact with swine, influenza virus vaccination history including receipt of 1976 swine influenza virus vaccine.
#People with exposure of interest	• 74
Laboratory methodology	• HI titres ≥40 considered positive. The farm cohort had pre and post season titres evaluated (4-fold rise). Human serum samples were treated to eliminate non-specific inhibitors of hemagglutination. Control sera also used in the HI panels.
#People tested	• 74 exp; 114 unexp.
Results	• 17/74 farm participants had HI s ≥40 (range 40-160) in preseason samples against either A/NEB or sw/IND; • 15/17 were seropositive to both swine viruses. • These included 7 farm owners (41-55 yrs), 7 family members of farm owners (7-54 yrs), a 33-year old farm employee, a 38 year old family member of a farm employee and a 43 year old vet.

2. Studies of influenza-infected humans associated with exposure to swine.

	• Only 1/114 controls (41 year old) had a positive HI titre against a swine virus (HI=40 against only A/NEB). • Difference in number of seropositive samples between the farm and control cohorts was statistically significant, ($p<0.001$).
Age & Gender with outcome of interest	• See above
Data analysis methods	• measures of association: factors related to a person's degree of contact with pigs to seropositivity were associated. Associations between preseason seropositivity to swine influenza viruses at HI titres >40 or >80 among farm participants and specific aspects of swine exposure or other variables were evaluated.
Possible sources of bias; confounding	• selection bias is possible; controls were not specifically enrolled in the study; so additional info could not be collected. Cross reactivity in HI assays between human and swine reference strains is a possible source of confounding. Previous immunization with the 1976-77 swine flu vaccine or other flu vaccine.
Provisions for minimizing influence of confounding	• Lab procedures demonstrated that no serologic cross-reactivity in HI assays between the human H1N1, H3N2 and swine H1N1 influenza viruses existed. HI titres compared using virus-specific sheep and ferret reference sera to show no serologic cross-reactivity between human H1N1, human H3N2 and swine H1N1 viruses. • Farm cohort asked re: previous flu imm history and specifically about 1976-77.
Limitations	• multivariate analysis was not done because of the small number of participants with elevated preseason titres to swine influenza viruses
Key findings	• Swine virus seropositivity was significantly ($p<0.05$) associated with being a farm owner or a farm family member, living on a farm, or entering the swine barn >4 days a week, being >50 and having received swine flu vaccine in 1976-77 (n=4) or other influenza virus vaccine was also associated with swine virus seropositivity. • 17/74 swine farm participants had significantly higher ($p<0.001$) positive HI antibody titres >40 to swine influenza virus than the 1/114 urban controls.
Conclusions	• Overall frequency of contact with pigs more important consideration than the length of contact at any one time. • These results support for the hypothesis that people associated with swine production are infected with swine influenza more regularly than the small number of zoonotic infections in the literature would suggest. • The number of hours per day was not a significant factor, and frequency was more important, which is consistent with the fact that influenza virus infections in pigs occur sporadically and pigs only shed virus for approximately 7 days after infection.
Notes	

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	Ramirez, 2006: Preventing Zoonotic Influenza Virus Infection
Study design	• Cross-sectional
Objectives of study	• Objective: To learn if persons who work in enclosed livestock buildings have among the highest risk of becoming infected with swine influenza virus and if so, to determine the factors that cause them to be at increased risk.
Setting	• Iowa swine industry. •
Study population	• 49 swine industry workers and 79 non-exposed controls. Similar age distribution for the 2 groups. Confinement workers more likely to be Hispanic and less likely to have received influenza vaccine.
Subject selection	• Eligibility for the study—if they had worked in a swine confinement facility in the past 12 months. Controls were enrolled during a concurrent study of U of Iowa faculty, staff and students.
Exposure of interest	• Swine and their immediate environment.
Virus	• Tested: 2 recently circulating swine strains, A/Swine/WI/238/97 (H1N1) and A/Swine/WI/R33F/01 (H1N2) and • 1 human influenza strain A/NewCaledonia/20/99 (H1N1).
Outcome of interest	• Antibodies to swine influenza virus.
Methodology	• Participants completed a questionnaire and blood specimens were collected on enrollment. • Data collected: Questionnaire: demographic, medical and occupational data including influenza immunization history, swine occupational exposures and use of protective equipment (gloves, masks).
#People with exposure of interest	• 49 confinement workers.
Laboratory methodology	• HI assay against strains listed above. • H1N1 titres were grouped as <10; 10, >10.
#People tested	• 128 (49 exposed; 79 controls--unexposed).
Results	• Multivariate analysis: • Persons who received the 2003-04 flu vaccine were significantly more likely to have elevated titres (≥10) against swine H1N1 virus as well as swine H1N2. • A cross-reaction with 1 of the viruses in the vaccine or a circulating flu virus may explain this; higher titres would have been expected for all vaccinated persons (including controls), but this was not observed. • Suggest this represents other behavioural or health-related confounders not included in the questionnaire for this study. • Workers who sometimes or never used gloves were significantly more likely (OR 30.3, 95%CI 3.8-243.5) to have elevated titres to H1N1 than the nonexposed controls. These workers were also significantly more likely to have elevated titres to H1N1 than the other confinement workers who used gloves most of the time or always. (OR 12.7, CI 1.1-151.1) • Workers who reported smoking also had high OR (data not shown) for elevated titres. Those who smoked (OR 18.7) most frequently had evidence of previous H1N1 swine virus.
Age & Gender with outcome of interest	• Distribution of ages was similar for the 2 groups but the confinement workers were more likely to be male.

2. Studies of influenza-infected humans associated with exposure to swine.

Data analysis methods	• Wilcoxon rank sum and chi-square statistic or 2-sided Fisher exact test were used to assess bivariate risk factor associations. • Depending on the nature of the data and modeling assumptions, proportional odds modeling or logistic regression was used to adjust for multiple risk factors. • Final multivariate models were designed by using a saturated model and manual backwards elimination.
Possible sources of bias; confounding	• If any cross reactions occurred between the 3 strains studied and those in vaccines received by the 2 groups, this could introduce confounding in the results reported, and there would have been a bias with the nonexposed controls having a greater HI response falsely attributed to the study strains, since this group was more likely to have been vaccinated than the exposed group. This would decrease the differences between the two groups.
Provisions for minimizing influence of confounding	• Multivariate analysis was done and participants in both groups answered a questionnaire so data on vaccination history, gender, etc. could be collected.
Limitations	• Not stated if controls were matched, so assumed not; however, age distribution was similar for the two groups. • Small sample size • Lab data on how/ if cross reactions were controlled for was not outlined. • Lab results were grouped but data (titre levels) not shown. • Language barriers were cited as an issue in communicating with swine confinement workers.
Key findings	• Use of gloves during swine confinement work noticeably decreases the risk for swine influenza virus infection. • While smoking has been documented as a risk factor for human influenza virus infection, however this is the first study identifying smoking as a risk factor for swine influenza virus infection.
Conclusions	• Conclusion—swine confinement workers are at increased risk for zoonotic influenza infection.
Notes	

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	**Olson et al 1977: Epizootic swine influenza with evidence of a low rate of human infection associated with occupational exposure to swine.**
Study design	• Cross-sectional
Objectives of study	• To determine whether transmission of the virus had occurred from infected swine to humans and whether secondary spread was evident.
Setting	• Taiwan Chu-nan ;Taiwan Sugar Corporation; April, 1976
Study population	• Exposed (61) and un-exposed (56); • farm employees at the Taiwan Sugar Corporation (both those with exposure to swine and those without swine exposure) and their family members. • blood specimens also obtained from residents of Taipei City who had no swine contact—outpatients in urban Taipei with no history of any contact with swine; presenting for reasons other tan upper respiratory illnesses.
Subject selection	• employees at the farm during the epizootic in swine were sampled a year later; these were people with exposure to swine. • Specific recruitment procedures not specified.
Exposure of interest	• farm workers had daily occupational exposure to infected swine; control groups also used.--TSC employees with no swine contact and urban outpatients in Taipei reporting for reasons other than upper respiratory illnesses.
Virus	• assessed for exposure to: A/MayoClinic/103/74 (HSw1N1); A/NewJersey/8/76 (Hsw1N1); A/Victoria/3/75 (H3N2); and B/HongKong/5/72.
Outcome of interest	• Prevalence of antibody titres in the study population; transmission from pigs to humans.
Methodology	• Prevalence and geometric mean titres of antibody to swine-like influenza viruses A/May Clinic/74 and A/New Jersey/76 were compared and contrasted among subjects. • Data collected: laboratory data; basic exposure status.
#People with exposure of interest	• 61
Laboratory methodology	• HI tests performed at the CDC Atlanta. Cut point was ≥1:10. Sera were collected nearly a year after the epizootic among swine
#People tested	• 61 TSC employees with daily close contact to pigs; 56 TSC employees without exposure; 167 outpatients.
Results	• titre ≥1:10 against to either swine virus: TSC exposed: 2/7 (20-29 y-o); 6/32 (30-39); 1/13 (40-49; 6/9 (50 and over); TSC controls: 1/15 (20-29); 1/17 (30-39); 1/10 (40-49); 8/14 (50 and over); Taipei controls 1/46 (20-29); 2/34(30-39); 8/26 (40-49); 49/61 (50 and over).
Age & Gender with outcome of interest	• age range of employees and outpatients: 20-over 50. Results reported by age group. Gender not stated
Data analysis methods	• Geometric mean titres calculated
Possible sources of bias; confounding	• gender not stated; • information not available on influenza activity in surrounding community • nature of exposure not described, e.g., activities and personal hygiene practices. • Controls not matched, but compared by age groups each spanning 10 years. • Influenza vaccination history not mentioned.

2. Studies of influenza-infected humans associated with exposure to swine.

	• Persons 50 and older would have been born on or before 1925; other studies have indicated that prevalence of antibody to swine influenza virus increases with age. • ?only 2 virus strains were used in serological testing. • Low titre cut point and single serum samples taken a year after exposure to known infected swine—results may reflect cross-reaction to other strains, especially at low titre levels and in the absence of acute and convalescent sampling. No mention of illness among the workers at the time of the outbreak among swine is described.
Provisions for minimizing influence of confounding	• unexposed control subjects used both from the farm site and from the community; • community group were ill but not with respiratory illnesses, decreasing likelihood but not eliminating possibility of infection with other respiratory viruses.
Limitations	• Definition of positive titre $\geq$1:10; substantially lower than current day studies • No virus isolation among humans • Single specimens were collected a year after the epizootic in swine.
Key findings	• Geometric mean titres also assessed and noted by age group in table 1. 20-29 year old exposed had higher prevalence (29%) than the TSC (7%) and Taipei (2%) control groups; significant at the $p<0.12$ and $p<0.07$ levels, respectively. In this age group, the GMT of that age group was higher than in either control group $p<0.04$.; • The 30-39 age group with exposure had higher prevalence of antibody (19%) than the TSC (6%) or Taipei (6%) controls; significant at the ($p<0.08$) and ($p<0.06$) levels, respectively. In this age group, the GMT of those with exposure to swine influenza was higher than in either control group ($p<0.08$, $p<0.008$). • Antibody prevalence among 40-49 year olds was not greater in the exp (8%) vs. unexp, TSC (10%); Taipei (31%). The GMT was slightly greater in the exposed workers (5.62) vs. unexposed TSC workers (5.37) but $p<.39$ indicates could have happened by chance. • Prevalence of antibodies in exposed 50 yrs and older was not statistically significantly different than unexposed, $p>0.3$. The GMT was lower in the exposed than either control group.
Conclusions	• Author concluded that this is evidence of transmission of a swine-like virus from swine to humans; the prevalences suggest this occurred at low levels despite daily occupational exposure to swine.
Notes	

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	Schnurrenberger 1970 : Serologic Evidence of Human Infection with Swine Influenza Virus
Study design	• Cross-sectional
Objectives of study	• To describe results of serologic studies on human populations in Illinois classified on the basis of presumed degree of exposure to swine.
Setting	• U.S. Illinois
Study population	• occupational groups
Subject selection	• none were sampled randomly.
Exposure of interest	• pigs
Virus	• A/Swine/Illinois/1/63. The serums obtained in 1966 from the vets and swine producers were also tested against A/Swine/1976/31. .
Outcome of interest	• Serologic evidence of infection—detection of antibodies to swine influenza viruses in serum samples of study population.
Methodology	• blood samples collected during annual meetings of Illinois Pork Producers Association and the Illinois State Veterinary Medical Association in January and February 1966 and from swine exhibitors at the Illinois State Fair in August 1966, and also at the February 1968 meeting of the Illinois State Veterinary Medical Association, but only age and type of practice were recorded. • General population (298 residents) were sampled in community clinics in Sept 1964 in conjunction with an arbor virus encephalitis survey; PH department participants were volunteers; • 518 persons whose serum samples were submitted for routine premarital testing were also included, but some were from clinically ill persons whose serum was submitted for agglutinin testing. • Samples collected in July 1966 from employees of a Peoria, IL packing plant in which both swine and cattle were slaughtered. Information concerning age, sex, work duties, length of employment, and absenteeism was obtained from the plant personnel records. • Data collected: age, type of veterinary practice, influenza vaccination history, service in the armed forces
#People with exposure of interest	• veterinarians (maximal exposure); regulatory veterinarians and GPs (moderate exp); all others- (minimal exp); also employees of hog packing plant who worked with live hogs, warm carcasses or viscera (maximal exp); those who contacted refrigerated pork (moderate exp) and all others (minimal exp). • Also studied: 298 residents of Hamilton county, Illinois, 24 employees of Dept of Public Health and 518 persons whose serum was submitted to the health dept lab (typically routine premarital or prenatal samples, though some were clinically ill).
Laboratory methodology	• HI testing used to test treated serum samples. 307 of 332 samples were tested against 2 swine viruses agreed within a twofold dilution (92.5%); and 328/332 (98.8%) within a fourfold dilution. A titre of $\geq$1:20 was considered reactive.
#People tested	• samples collected from 168 pork producers, 248 veterinarians or veterinary students, 551 packing plant employees, 24 hog buyers or vocational agriculture teachers, and 13 pork producers' wives or daughters. • A total of 307 of 332 samples tested against 2 strains of flu virus samples tested

2. Studies of influenza-infected humans associated with exposure to swine.

Results	• The reactor rate for veterinarians was 34.4% in 1966 and 35.3% in 1968. • 46 of the vets tested in 1966 were restudied in 1968; the serum of 4 had changed from <1:20 in 1966 to reactive at 1:20 in 1968. • The only change greater than fourfold was in a regulatory vet born in 1914 whose titre increased from 1:40 to 1:640. • HI antibody to A/Sw/Ill/1/63 reaction rates: 15% Hog producers, 17% hog buyers, 19% general public, 34% veterinarians, to 45% abattoir workers. 0% in producers' families; 28% overall. Age adjusted occupational group results decreased the results slightly (table 2).
Age & Gender with outcome of interest	• antibody detected in fewer than 3% of those born after 1935 in contrast to 73 percent of those born before 1920
Data analysis methods	• Descriptive antibody prevalence (percent positive).
Possible sources of bias; confounding	• none of the population groups was sampled randomly • possibility of cross reaction not incorporated into testing. • prevalence of titres against swine influenza increased directly with age; • correlation with immunization history likely due to a cross reaction, since at time of publication (1970) swine antigen had never been included in vaccine for civilians: • Nature of exposure: swine buyers were noted to see more swine than producers but they have less intimate contact; veterinarians have close contact with numerous ill swine. Abattoir workers have direct contact with more swine than does any of the other occupational groups. • No information is available on possible exposures among community participants (some may live or work on farms or visit Ag fairs); • Pork producers wives and daughters tested, presumably as controls, however only 13 were tested and no male controls were used; ?gender bias, ? age bias—daughters presumably younger than average age of participants. Only abattoir workers and the general population had adequate representation of both sexes. • The health service of the abattoir annually offered free influenza vaccine to workers, but no records were kept of those receiving vaccine.
Provisions for minimizing influence of confounding	• Assessing potential confounders: age, recent vaccination history, previous military work history. • Pork producers wives and daughters were tested (n=13) and none were reactive. • Results were age-adjusted.
Limitations	• Results cannot be generalized to the occupations represented in this study due to lack of random selection. • Controlled for non-specific inhibition but did not mention controlling for cross-reactions. • Less detailed personal information collected from Veterinarians when serum collected at the second meeting (1968), as compared to the first meeting studied in 1966. • Assessed record of community influenza outbreak at the time surrounding the second sample collection from the general population group; no outbreak of respiratory disease reported, but acknowledged that it could have been sub clinical or have gone unreported.

2. Studies of influenza-infected humans associated with exposure to swine.

Key findings	• Reaction rates varied by occupational group; 15% among producers to 45% among abattoir workers; age adjustment decreased the rates slightly. • Prevalence of titres against swine influenza virus increased directly with age; antibody detected in fewer than 3% of persons born after 1935 in contrast to 73 % of those born before 1920. • No difference was noted in the reactor rates for persons who were veterans of the armed forces and those who were not. No serum from the producers' wives and daughters was reactive. • Among persons who had been vaccinated in the year before sampling, the crude reactor rate was 41%, in contrast to 20% among unvaccinated persons. Age adjusting reduced but did not eliminate the difference by vaccination history. • Reactor rate for veterinarians was 34.4% in 1966 and 35.3% in 1968. • A small difference in reactor rates of vets with moderate or greater exposure and those with minimal exposure. • Marked difference in reactor rates within group 3 of the general population when examined by collection date: 1.2% for those collected before July 1965 and 41.8% after Oct 1966; age adjusted rates= 4.9% and 45.2% respectively—no reason could be identified for this finding. • No significant difference found between the abattoir workers in departments having close swine contact and those having no direct contact with animals or product. These rates were unchanged by age adjustment; and could have been due to vaccination or spread within the abattoir. • Abattoir workers and general population were the only groups in which both sexes were adequately represented to permit calculation of valid sex-specific rates: 196/451 males (43.5%) and 50/100 female abattoir workers; 21.1% (79/375) for men and 14.7% (61/416) for women in the general population. Age adjusting the data reduced the sex difference to 4% in both populations.
Conclusions	• The increase in antibody prevalence among but not within the various occupations coincides with the degree of swine contact except for the general population. • The correlation with vaccination status can only be explained as a cross-reaction, as the swine antigen has never been included in a vaccine for civilians in this country (this study done in 1970).
Notes	• "vet"=veterinarian • Dowdle & Hattwick (1977) cite this study as an example of minimal and circumstantial evidence of transmission to humans prior to 1974; and that the significance of the age difference in antibody prevalence is not clear. The possibility of cross-reacting antibody could result in the high positive rate; no significant difference in the frequency rate of antibody between the abattoir workers in departments having close contact with swine and those having no contact with swine, so this could indicate either human to human transmission or lack of an occupational hazard.

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	**1996 Shu et al; ref # : An epidemiological study of influenza viruses among Chinese farm families with household ducks and pigs.**
Study design	• Serial cross-sectional:
Objectives of study	• Epi study of flu viruses among Chinese farm families with household ducks and pigs; to examine the possibility of interspecies transmission and genetic reassortment of influenza viruses on farms in Southern China, and overall, to identify seasonal pattern of respiratory illness and the prevalence and types of influenza viruses among humans.
Setting	• China (1992-1993) outside Nanchang City.
Study population	• Farm families who raised pigs and ducks in their homes
Subject selection	• Recruitment procedures not described.
Exposure of interest	• pigs and ducks (note: ducks are not an animal of interest in this study)
Virus	• 6 influenza viruses isolated in humans and 5 in ducks.
Outcome of interest	• transmission of influenza viruses of zoonotic origin to humans; antibody prevalence.
Methodology	• From the weekly visits, one family member each week was selected who had respiratory disease symptoms, e.g., sore throat; and recorded their recent exposures to animals. A throat swab was collected from the person with the most severe symptoms. When no one was sick, the person with the most exposure to pigs was tested. • Serum samples collected from humans periodically from the person at each of the 20 residences who were identified as being the person responsible for care of pigs and ducks; 20 in Sept 1992, 18 in November 1992, and 18 in June 1993 (n=56). • In November 1993, blood specimens from 5 people at each residence were collected (n=100). • Additional samples were obtained from the local hospital whenever influenza virus was isolated from one of these families (not included in this review). • Obtaining swab samples from pigs at each residence was not feasible because of their large size and reluctance of farmers to allow weekly swabbing of pigs. Serological sampling of randomly selected pigs was done shortly before slaughter. 5 pigs from each residence (total of 100) were tested and also 55 pigs at a local slaughter house in July 1993. • Duck feces samples were collected from each of the 10 sites (total of 540 over the course of the study). It is not stated how frequently it is expected that avian influenza virus be yielded from fecal samples. • Data collected: Weekly interviews of family members and virus isolation studies of throat swabs and fecal samples; from September 1992 to September 1993. Genotype analysis of duck and human isolates provided no evidence for reassortment.
#People with exposure of interest	• 20 farm families;
Laboratory methodology	• virus isolation studies of human throat swabs, duck fecal samples and nasal swabs from pigs. Inoculated into embryonated chicken eggs then isolates tested by HI and NI with a panel of monospecific antisera. • Human serum samples tested-- persons responsible for the care of pigs and ducks. Serum samples also collected from pigs and tested. • Isolates were identified by HI testing with a panel of monospecific antisera.

2. Studies of influenza-infected humans associated with exposure to swine.

	Sera tested with HI and NI assays for antibodies against human, pig and duck influenza viruses. • An HI or NI titre of ≥1:20 considered positive. • Genetic analysis done on viruses isolated and no genetic reassortment noted.
#People tested	• (not always the same person in each family). 156 serum samples from humans were tested.
Results	• 8/156 serum samples inhibited the neuraminidase activity of two of the duck isolates, A/Duck/Nanchang/1904/92 (H7N4) with two of these samples also inhibiting the activity of A/Duck/Nanchang/1941/93 (H4N4) influenza viruses. • A 10-year-old boy had antibody titre of 40 to N4 duck virus. • Failure to isolated H1N1 flu viruses from farm families suggests that the positive assay results represent previous exposure to the virus. Antibodies specific for sw HI were not detected in humans. The antibodies to sw NI probably reflect cross-reaction with human H1N1 viruses.
Age & Gender with outcome of interest	• 1 stated: 10 year old boy
Data analysis methods	• Descriptive data; seroprevalence data. RR with 95% CI provided for seropositivity to H4N4 or H7N4 viruses.
Possible sources of bias; confounding	• Serum samples and throat samples from humans not always collected from the same person each time. • Pig sampling was random; human sampling was not. Duck swabs were not taken from the animals. • Individualized exposure and demographic data not outlined for each individual who tested positive. • Circulating human influenza strains—elevated antibody titres detected to H1N1 (64% of 100 persons sampled in November 1993); and H3N2 (32% of same sample Nov '93); but neither were isolated from families sampled, suggesting secondary immune response. • Antibodies specific to swine H1 viruses not detected in humans, so the antibodies to swine N1 probably reflect cross reactivity with human H1N1 viruses.
Provisions for minimizing influence of confounding	• Cross reactions between human and swine strains may have been detected (controlled for?) by lab testing with specific antibodies, although this is not clearly articulated.
Limitations	• The same person in each household was not tested at each visit. Therefore, each individual may have been tested more than once and some may not have been tested. • Detailed exposure histories not collected. • Sampling focused on symptomatic exposed individuals.
Key findings	• RR of one or more family members being seropositive for H4N4 or H7N4 viruses for exposure to ducks testing positive for one of these viruses was 1.1 (95% CI; 0.3-3.9). • While no evidence was found for genetic reassortment of viruses, findings do support the concept that intermingling of humans, pigs and ducks on Chinese farms is favorable to the generation of new, potentially hazardous strains of influenza virus.

2. Studies of influenza-infected humans associated with exposure to swine.

	• Patterns of influenza virus circulation among humans and among ducks were identified and described as being different times in humans and ducks living in close proximity on the farms. • 8/156 human serum samples inhibited the neuraminidase activity of two of the duck isolates, raising the possibility of interspecies transmission of these avian viruses.
Conclusions	• Reassortment of AI viruses with strains from other hosts in nature are rare events and not likely to be detected without extended surveillance studies in favourable settings.
Notes:	• Re: the 10 year old boy-- the serum sample was taken 10 months after the isolation of the H4N4 duck virus, which may explain why the titre was low.

More results:	
• 12/100 samples reacted to the HI of H1N1 human virus (max titre=80); same for human H3N2; none to H1N1 swine or duck viruses.	
• Throat swabs from farm families and from hospitalized subjects yielded flu/B and H3N2 isolates.	
Nov 93:	6/100 inhibited NI of H1N1 sw; max titre =40 (suspected cross-reaction).
	23/100—NI to H11N2 duck virus (max titre =40)
	2/100—NI of H4N4 duck (20) 6/100—NI of H7N4 duck (40)
June 93:	5/18 (40) NI of H11N2
	1/18 (40) NI of H7N4
Nov 92:	9/18 (80) NI of H11N2
	1/18 (20) NI of H7N4
Sept 92:	1/20 (40) NI to H11N2

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	Ayora-Talavera 2005 : Serologic Evidence of Human and Swine Influenza in Mayan Persons
Study design	• Cross-sectional
Objectives of study	• To describe the serologic evidence of antibodies against influenza strains from humans and pigs in indigenous Mayan persons from Yucatan.
Setting	• Mexico Yucatan-- Kochol, ~20 km from muni of Maxcanu.
Study population	• 1,207 residents; mostly dedicated to agricultural activities; living in crowded conditions
Subject selection	• the serum samples from 115 persons were made available by the health official of Kochol in 2000. these were samples from residents who came to the health service for any medical condition and required laboratory tests.
Exposure of interest	• animals eat, live and share space, water sources and even food with humans and may be found inside houses. Families have 1-18 pigs
Virus	• A/Bayern/7./95, A/Sydney/5/97; A/Swine/Wisconsin/238/97; and A/Swine/Minnesota/593/99
Outcome of interest	• Antibodies to swine influenza viruses in human serum samples.
Methodology	• serum samples used from health service and tested for presence of antibodies to the strains specified above. • Data collected: lab results and general area of residence (implied by catchment area); age
#People with exposure of interest	• 115 indigenous Mayan persons ; however exposure not individually assessed
Laboratory methodology	• HI titres $\geq$1: 40 considered positive. Serum treated to inactivate nonspecific inhibitors. Controls used to rule-out induction of non-specific hemagglutination.
#People tested	• 115 serum samples were made available to researchers by Kochol health officials
Results	• 31/115 were positive to H1 and 93/115 to H3.highest seropositivity rates across all age groups were detected with the A/Sw/Minnesota virus as antigen (an H3N2 reassortant). (HA, NA and PB1 genes are of human origin even though this strain was isolated from American pigs). seropositivity to swine H1 virus at the cutoff value in this study was noted in only 2 persons 43 and 59 years of age; lower titres were detected in 4 more persons 33-55 years of age.
Age & Gender with outcome of interest	• age range of those tested= 8-53
Data analysis methods	• seroprevalence among age groups
Possible sources of bias; confounding	• In this study, serum samples were not tested against avian viruses; • Single serum samples used • Vaccination history not known, but stated that generally Mexicans are not immunized. • Specific exposure and general health status data not stated and not individually assessed; • Community prevalence of influenza not specified, but believed to be low as evidenced by no virus detection of H1 viruses in ~1500 throat swabs taken in 5 years tested by immunofluorescence assay and only 5 viruses have been detected with RT-PCR testing (virus not specified).

2. Studies of influenza-infected humans associated with exposure to swine.

	• H3 virus used in the study (A/Minnesota) contained human HA, NA and PB1 genes. (?is it possible that this is circulating among the human population?).
Provisions for minimizing influence of confounding	• Laboratory controls used and appropriate cut point; • The results in this study agree with previous unpublished data on serologic tests of human serum samples from the Yucatan indicating that H1 viruses likely circulate at lower rates than H3.
Limitations	• It cannot be concluded that the seropositivity noted in this study is a result of exposure to swine; due to lack of detailed exposure histories.
Key findings	• The 15-24 year old age group were most commonly seropositive (H1 or H3 not specified). • Overall, 31 (26.9%) of 115 samples were positive to H1 and 93 (80.8%) were seropositive to H3; however applying the cutoff values in this study, seropositivity to swine H1 virus was only detected in 2 samples from persons 43 and 59 years of age. Weaker reactions were noted in 4 other persons 33-55 years of age, which could indicate previous exposure to viruses of swine origin, a situation that has not occurred in persons >30. *(I think they mean <30?)* • The weak reactivity to H1 virus could suggest a past exposure of adult persons to viruses of swine origin. • The RR of being seropositive for H1 or H3 viruses from exposure to pigs was 1.93 with human H1 (95%CI, 1.2-3.0); 0.88 with human H3 (0.55-1.4); 0.6 with swine H1 (0.08-4.2) and 1.0 with swine H3 (0.62-1.6). • A different seroprevalence study of pigs in central Mexico, H1 subtype is prevalent in 20% of pigs and in a previous study from Yucatan, the most prevalent subtype in pig farms is H3 (65%) and H1 (20%). • The highest seropositivity rates across all age groups were detected with the A/Sw/Minnesota virus as antigen, taken from American pigs. However, the NA, HA and PB1 genes are of human origin.
Conclusions	• Serologic evidence exists that influenza H3 is highly prevalent in the Yucatan. • Antibodies to swine influenza viruses are prevalent in humans in the Yucatan.
Notes	• did not test for antibody to avian influenza viruses due to lack of antigen availability; however the Yucatan is considered a free state for avian influenza virus, according to the Mexican Ministry of Agriculture (chicken farms sampled 3 times a year for serologic surveillance and 10% of the backyard flocks are sampled annually. No surveillance program exists for swine flu viruses. • The animal population in this study owned by persons in this study consisted of pigs (68.7%), chickens (73%) and ducks (17.3%). Any combination of 2 or 3 species was kept by 54.7%. the range of number of animals owned was 0-12 (mean 2.9) pigs; 0-60 (mean 7) chickens, and 0-23 (mean0.93) ducks. • Previous studies on pig farms in Central Mexico (not Yucatan), found that H1 is prevalent in 20% of pigs) and a previous Yucatan study found the most prevalent subtype of influenza among pigs to be H3 (65%) and H1 (20%).

2. Studies of influenza-infected humans associated with exposure to swine.

Reference:	**Myers et al 2006: Are swine workers in the United States at increased risk of infection with zoonotic influenza virus?**
Study design	• Controlled, cross-sectional seroprevalence study among swine industry workers
Objectives of study	• To serologically examine workers with occupational swine exposure, with a goal of identifying those at highest risk of a zoonotic influenza infection
Setting	• U.S. Iowa
Study population	• farmers, meat processing workers (pork producing facility), veterinarians; and control subjects; • 111 farmers; 97 meat processors; 65 vets; 79 control subjects
Subject selection	• Control subjects were volunteer participants from the University of Iowa and had no exposure to swine; vets were recruited at the 2004 Iowa Vet Medical Association spring conference; farmers were part of a large rural cohort, but specifics are not provided regarding recruitment. • The meat processing workers, veterinarians and control subjects were only allowed to participate if they were >18 years of age, had no immunocompromising conditions and were not pregnant. The same is not specified regarding the farmer cohort.
Exposure of interest	• exposure to swine
Virus	• Swine influenza viruses: A/Swine/WI/238/97(H1N1).;A/Swine/WI/R33F/01 (H1N2); A/Swine/Minnesota/593/99 (H3N2) (and 3 human isolates also tested)
Outcome of interest	• Seropositivity to swine influenza viruses (presence of antibodies).
Methodology	• Compared serologic evidence of infection among those exposed and those not exposed to swine. • Study participants completed occupational risk factor questionnaires. • HI test on serum samples—serum samples were tested against 6 isolates of recently circulating swine and human influenza A viruses, consisting of 3 each of the H1 and H3 subtypes • Data collected: demographic data, occupational risk factors, immunization history
#People with exposure of interest	• 111 farmers; 97meat processing workers; 65 veterinarians; 79 control subjects (not exposed).
Laboratory methodology	• HI titres of ≥1:40 considered evidence of previous infection. Single sample to determine seroprevalence. • Serologic HI testing was done according to CDC HI protocol. • HI titres for control antisera detected against reference virus strains.
#People tested	• 273 exposed and 79 unexposed.
Results	• In dichotomous comparisons, the OR for farmers vs. controls for H1N1 was 22.9 (95%CI, 3.9-∞); and, for H1N2 was 20.7 (95%CI, 2.5-172.1); for veterinarians vs. controls for H1N1 was 12.8 (95%CI, 1.9-∞); and, for H1N2 was 18.1 (95%CI, 2.3-138.8). • Age, sex and homologous human influenza strains were adjusted through unconditional logistic regression and the following ORs were found: for elevated H1N1 and H1N2 titres: for farmers, 30.6 (95%CI, 4.3-∞), and 16.0 (95%CI, 1.9-776.4), respectively. For veterinarians, elevated H1N2 titres, OR= 13.4 (95% CI, 1.5-670.5). Meat processing workers were found to have had elevated titres against the swine H3N2 virus, OR=5.8 (95% CI, 1.7-23.0).

2. Studies of influenza-infected humans associated with exposure to swine.

	• In the unadjusted proportional odds model, the OR for antibodies against both swine H1N1 and H1N2 viruses were elevated for all 3 occupational groups when compared against controls. • Age and sex were used in the model as confounders, and when confounders were controlled, the OR for each group to H1N1 and H1N2 swine viruses were as follows: Farmers: 35.3 (CI, 7.7-161.8) and 13.8 (5.4-35.4), respectively; veterinarians, 17.8 (CI, 3.8-82.7)and 9.5 (CI, 3.6-24.6), respectively; meat processing workers, 6.5 (CI, 1.4-29.5) and 2.7 (1.1-6.7), respectively.
Age & Gender with outcome of interest	• mean age: farmers: 47; mpws, 39.5, vets 48.4, controls, 35.3 yrs old. • farmers: 61male, 50 female; mpw's: 52 male; 45 female; vets: 50 m, 15 f; controls: 26 m; 53 f.
Data analysis methods	• measures of association: OR. • GMTs evaluated for each cohort • Bivariate analysis of possible risk factors for each cohort (data not shown); 38 risk factors for meat processing workers; 28 for veterinarians; • Logistic regression performed for swine H3N2 serology • HI test results first evaluated as dichotomous outcomes (HI titres of >1:40 were considered to be evidence of previous infection); later as ordinal outcomes, with the goal of examining the entire distribution of antibody titre level • Dichotomous outcomes were examined using chi-square statistic or 2-sided Fisher's exact test, with 95%CIs calculated for Ors. • Geometric mean HI titres (GMTs) were also calculated for each virus strain and compared by risk factor using the Wilcoxon rank sum test, with normal approximation. • Unconditional logistic regression used to examine multiple independent variables for their association with the outcomes. Covariates with bivariate P values <0.1 were considered for inclusion in all logistic regression models.
Possible sources of bias; confounding	• Selection bias; Lack of detailed exposure information for farmer group; It is possible that the elevated titres compared by proportional odds modeling do not correlate with infection. • elevated titres against swine H3N2 were associated with having elevated titres against human H3N2 strains, suggesting cross reactivity. Elevated HI titres against sw H3N2 isolate were associated with having received a 2003-2004 influenza vaccination as well as with presence of others in the household. • It is unclear if farmers with immunocompromising conditions and pregnancy were excluded.
Provisions for minimizing influence of confounding and bias	• Internal reliability testing of HI assays performed • Researchers noted above factors regarding cross reactivity considered in key findings; • Serologic risk factor data controlled for potential confounders, such as serologic response to human influenza virus and vaccine. • CDCP Atlanta HI serologic protocol was followed and internal reliability testing of HI assays. • Study laboratory findings were validated by a blinded external laboratory, and serologic assay results were corroborated by studies of virus-specific antisera. • Cross-reactivity was assessed through cross-testing of reference antisera (swine H3 antisera tested by HI against human H3 viruses).

2. Studies of influenza-infected humans associated with exposure to swine.

	• proportional odds modeling was used to better discriminate the effect of potential risk factors to the swine virus serologic outcomes. • People with immunocompromising conditions and pregnancy were excluded from participating in the meat processing, veterinarian and control cohorts. Unclear if the same applies to farmers.
Limitations	• lack of detailed exposure information for the farmer group. Study design did not allow researchers to determine whether individuals developed clinical symptoms with seroconversion; it is possible that the elevated titres compared by proportional odds modeling do not correlate with infection.
Key findings	• Increased odds of being seropositive with swine influenza viruses among those exposed than those unexposed; farmers then vets. Meat processing workers only had increased odds of being seropositive with H3N2. • All 3 exposure groups had a high prevalence of antibodies against the swine H3N2 isolate, but none of these prevalence values were significantly different than the controls' (data not shown). • elevated HI titres against sw H3N2 isolate were associated with having received a 2003-2004 influenza vaccination as well as with presence of others in the household. • among all groups, elevated titres against swine H3N2 were associated with having elevated titres against human H3N2 strains, suggesting cross reactivity.
Conclusions	• This study found seropositvity to H1N2 among farm workers that had not been previously detected. • Occupational exposure to pigs greatly increases workers' risk of swine influenza virus infection. Swine influenza virus infections frequently occur among swine workers.
Notes	

Appendix 5: Tables of Excluded Studies:

1. Excluded studies which failed to meet the study design criterion (n=11).

The following studies include information about human infections with zoonotic origin associated with exposure to poultry or swine in an agricultural setting. However, these single case studies, by design, cannot answer the research question regarding risk of infection.

Study:`	Reason for exclusion:	Information of interest:
Dacso (1984)	Single case study	Describes evidence of human infection associated with exposure to swine.
deJong et al (1988)	Single case study	Describes evidence of human infection associated with exposure to swine.
Gregory et al (2003)	Single case study	Describes evidence of human infection associated with exposure to swine.
Kimura et al (1998)	Single case study	Describes evidence of human infection associated with exposure to swine.
Nguyen Van-Tam (2006)	Single case study	Describes evidence of human infection associated with exposure in a commercial poultry farm setting.
O'Brian et al (1977)	Single case study	Describes evidence of human infection associated with exposure to swine.
Olsen et al (2006)	Single case study	Describes evidence of human infection with a triple-reassortant swine flu virus associated with exposure to swine—first report.
Rota et al (1989)	Single case study	Describes evidence of human infection associated with exposure to swine.
Smith et al (1976)	Single case study	Describes evidence of human infection associated with exposure to swine.
Thompson et al (1976)	Single case study	Describes evidence of human infection associated with exposure to swine.
Tweed et al (2004)	Did not state number of exposed—case finding based on investigation of exposed persons with ILI	Describes evidence of human infection associated with exposure in a commercial poultry farm setting.

2. **Excluded studies which failed to meet criteria related to exposure, setting, participants, +/- design (n=65: primary search, n=47; secondary, n=18).**

Study:	Reason for exclusion:
Banks et al (1998)	Characterization of avian influenza virus isolated from a human—case noted elsewhere in this review.
Cameron et al (2000)	Analysis of the H9N2 viruses responsible for human infection in Hong Kong in comparison to those in poultry in Pakistan. No human cases with appropriate exposures identified.
Capua et al (2002)	Review article on avian influenza and human health; summarized cases identified to date; captured elsewhere in this review.
Capua et al (2004)	No exposure data; a total of 7 cases of H9N2 are described as having occurred on 2 separate occasions; no reference for 5 of the 7 is provided and no exposure data described for the other 2.
Chan (1997)	Discusses human cases of A/H5N1; no exposure data.
Chan (2002)	Excluded by committee due to setting—market setting not agricultural; subjects were not agricultural workers
Chatterjee et al (1995)	No exposure data
Choi et al, (2004)	No exposure data – H9N2 virus evolution described.
Claas et al (1994)	No clear exposure data
Claas et al (1998)	1st case in the 1997/98 HK outbreak; no exposure to poultry.
Claas et al (2000)	Summary of cases in which influenza viruses containing avian-like gene segments were introduced into the human population is presented in this study. Background information presented; no new case reports.
deJong et al (2005)	Outlines atypical clinical presentations of A/H5N1; exposure data unclear.
Du Ry van Beest Holle et al (2005)	Explored human to human transmission among household members of infected poultry workers
Eason et al (1980)	Describes human H1N1 influenza epidemic; not zoonotic.
Gilsdorf et al (2006)	Outbreak report of two clusters of human infection of H5N1 in Azerbaijan in Feb-Mar 2006. Duplicates information in WHO reports considered separately in this review.
Gaydos et al (1997)	No exposure data
Gregory et al (2001)	No exposure data
Ha et al,(2002)	Background information and description of H5 avian and H9 swine influenza virus hemagglutinin structures—discusses possible origin of influenza subtypes.
Hien, et al (2004)	Describes clinical and epidemiological details on 10 patients with H5N1 in Vietnam. These have been captured by WHO reports.
Hirst et al (2004)	Study on the genome sequence of the H7N3 viruses isolated in the B.C outbreak in 2004.
Hodder et al (1977)	Lacking exposure data; investigated human to human transmission
Katz et al (1999)	Human to human transmission studied among household and social contacts (A/H5N1).

Kaye et al (2005)	Provides background information on the implication of avian influenza viruses for human health.
Kelkar et al (1981)	No exposure data
Koopmans et al (2004)	Duplicates study of Koopmans; provides background info on lab methodology.
Kurtz et al (1996)	Excluded by committee; ducks excluded as an animal of interest.
Liao et al (2004)	Insufficient data and lacks clarity re: exposure in agricultural settings.
Mase et al (2001)	Phylogenetic analysis of influenza A/H9N2 viruses that are genetically closely related to those transmitted to humans in Hong Kong.
Mounts et al (1999)	Setting was poultry market setting not agricultural and subjects were not farm workers. (excluded by committee)
Nicholson et al (2003)	Background article on Influenza.
Ouchi et al (1996)	Study of swine only; no human infection explored
Patriarca et al (1984)	Explores human to human transmission; swine virus isolated from human but no exposure data.
Peiris et al (2004)	Laboratory and clinical data only
Rimmelzwaan et al (2001)	Information on a 5 year old case in the Netherlands, but no details; provides duplicate information on 2 other cases reported and captured elsewhere in the review.
Saito et al (2001)	Provides information on antigenic analysis of a human H9N2 virus isolated in Hong Kong, but no exposure data
Saw et al (1997)	Excluded by committee due to setting—market setting not agricultural; workers were market workers not agricultural workers
Saw et al (1998)	Excluded by committee due to setting—market setting not agricultural; workers were market workers not agricultural workers
Suarez (1998)	Compares H5N1 viruses isolated from humans and chickens from Hong Kong.
Top et al (1977)	Descriptive speculation regarding source of human infection with swine flu virus, however mostly refers to human to human transmission and not zoonotic transmission.
Ungchusak et al (2005)	Index patient would be captured in WHO single case reports; the rest of the study explores probable human to human transmission
Uyeki et al (2002)	Assesses human to human transmission with H9N2; not primary infection.
Webster (2004)	Background information on wet markets in Asia as a source of severe acute respiratory syndrome and influenza.
Webster et al (2006)	Background information on the H5N1 outbreaks.
Wentworth et al (1994)	Setting was animal laboratory; participants were lab workers not farmers; excluded by committee.
Wentworth et al (1997)	Setting was animal laboratory; participants were lab workers not farmers; excluded by committee.
World Health Organization (2003-2006)	Several single case reports of avian influenza A/H5N1; not counted in excluded studies.
Yuen et al (2005)	Review article on human infection by avian influenza A/H5N1 in Southeast Asia.

Secondary search (n=18):	
Campbell et al (1970)	No exposure data
Centers for Disease Control (1976)	MMWR volume 25—Swine influenza in Missouri man—no exposure to swine.
deJong et al (1986)	Duplicate report of 2 cases from Switzerland and 1 from the Netherlands; captured elsewhere.
DeLay et al (1967)	Exposure data lacking
Department of Health and Human Services, Influenza Surveillance Reports No. 92 and 93	No relevant information
Goldfield et al (1977)	No exposure data
Guo et al (2000)	Article in Chinese; inadvertently requested full text.
HPA press statement, April 28, 2006	No additional information beyond what was already published by VanTam.
Kendal et al (1977)	Antigenic properties of two subpopulations in A/New Jersey/76 (HswlN1) isolates and also in other HswlN1 viruses isolated in 1976 from pigs and man; no exposure data.
Lin et al (2000)	No exposure data.
Nakamura et al (1972)	Refers to swine only; not humans
Peiris et al (1999)	No exposure data; refers to humans, poultry and pigs infected with H9N2.
Peiris et al (2001)	No exposure data
Stuart-Harris, (1976)	Background information—editorial on the Fort Dix event.
Subbarao et al (1998)	No close contact of the case with chickens, however there were chickens at the case's daycare. Duplicates WHO reports, however provides viral sequencing data.
Tam et al (2002)	Excluded by committee due to setting—market setting not agricultural; subjects were not agricultural workers
Taylor et al (1977)	Avian influenza Hav 1; Neq 1 confirmed by viral culture in 24 year old female laboratory worker in Australia. Exposure was to a laboratory specimen, not an animal source of the virus and the setting was not agricultural, and so this study was excluded.
Van Kolfschooten (2003)	Duplicate information on the Netherlands outbreak and associated fatal case.

3. Further detail on single case studies excluded from this review:

The following section provides additional detail on single case studies excluded from this review due to a failure to meet the study design criterion. These studies are prominent in the literature, so bear mentioning here. Table 3a outlines single human cases of influenza associated with exposure to swine; Tables 3b and 3c outline single human cases of influenza associated with exposure to poultry.

Table 3a: Single cases of influenza associated with exposure to swine, 1976-2006

Author / Year	Location	# Cases	Age and Gender	Virus	Lab testing	Outcome	Notes
1976 Smith	U.S. Minnesota	1	M, 16	H1N1	Viral isolation	Died	Hodgkin's disease and on chemo-therapy.
1976 Thompson	Virginia, U.S.	1	F, 40	H1N1	Paired sera; 4-fold rise HI titres. Viral isolation attempted; not successful	Recovered	
1977 O'Brian	Wisconsin, U.S.	1	M, 8	H1N1	HAI antibody and CF antibody	Recovered	Previously healthy
1984 Dacso	Texas, U.S.	2	M, 20 M, 6	H1N1	Viral isolation	Recovered	Separate locations, exposures
1988 deJong	Switzerland Netherlands	1 1	M, 50 M, 29	H1N1	Viral isolation and 4-fold rise HI titres	Both recovered	1 other case in Switzerland ; no exposure.
1989 Rota	Wisconsin, U.S.	1	F, 32	H1N1	Viral isolation and 4-fold rise HI titres.	Died	Pregnant
1998 Kimura	Minnesota	1	F, 37	H1N1	Viral isolation post-mortem	Died	Previously healthy
2003 Gregory	Switzerland	1	M, 50	H1N1	Viral isolation; HI titre of 40 (70 d post infection)	Recovered	
2006 Olsen	Ontario, Canada	1	Not stated (both)	H3N2 triple reassor tant	Viral isolation; did not seroconvert	Recovered	

Table 3b: Summary of confirmed human cases of influenza (A/H7N3) associated with exposure to poultry, U.K., 2006.

Date	Gender	Age	Exposure	Lab testing:	Outcome
2006	M	Not stated	Poultry—commercial farm	A/H7N3	Conjunctivitis; recovered

Table 3c: Summary of confirmed cases of A/H7N3 influenza virus associated with the 2004 outbreak among commercial poultry in the Fraser Valley region of British Columbia, Canada.[19]

Case	Gender and Age	Exposure	Symptoms	Tamiflu and Vaccine	PPE	Viral isolation	Antibody response: MN[2] testing
1	M--40	Direct conjunctival contact	Conjunctivitis	Tamiflu taken as treatment; No vaccine	Eye protection not used	A/Canada/ 444/04 Low Pathogenic	No
2	M—45	Direct conjunctival contact	Conjunctivitis	Tamiflu taken as treatment; Vaccine received.	Eye protection used	A/Canada / 504/04 High Pathogenic	No

[2] MN= microneutralization assay.

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