STUDY OF INFLUENZA A VIRUS IN WILD BIRDS

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INTRODUCTION

Influenza virus is a RNA virus that exists as different types and subtypes. Influenza A virus strains are known to cause disease in several bird and mammalian species. Wild birds are believed to constitute the natural reservoir for influenza A virus. In humans, influenza A virus causes yearly seasonal influenza epidemics of respiratory disease resulting in high morbidity and severe economic consequences. Due to the virus' ability to change its antigenic properties by mutation, yearly vaccination is required for protection from the disease.

There are many different subtypes of influenza virus which are characterized according to two surface structures - the hemagglutinin and neuraminidase proteins - , for example; H5N1. These subtypes have the ability to recombine, and thereby creating new variant combinations. If a subtype that the living population of humans has not encountered before starts to spread among humans, it can result in a pandemic. Pandemic outbreaks have occurred at irregular intervals throughout history and have had a devastating impact on mankind. For example, the Spanish influenza pandemic of 1918 is thought to have killed more than 50 million people.

Influenza A virus is also an important cause of disease in poultry where virus strains of some subtypes may change into forms that are highly pathogenic. These virus strains may transmit directly to man and multiple other species. This has been the case in the ongoing outbreak that started in Southeast Asia in 2003. All known subtypes of influenza A virus have been isolated from wild birds living in aquatic environments, mainly dabbling ducks. These species are considered to be the reservoir for influenza A virus. The virus causes sub clinical gastrointestinal infection in ducks. High amounts of virus are excreted in the feces and spread via the fecal-oral route through water where it can persist for a prolonged time. There are still many unknowns about the ecology of influenza virus in the wild bird reservoir. This paper includes five articles where data are presented that add new knowledge on this subject. We add proof that wild ducks are indeed the host for most influenza A virus subtypes by presenting data from a meta-analysis on all published screening data from wild birds and by presenting data from a four-year screening of migratory ducks that were caught and sampled at Ottenby Bird Observatory. Our investigations have shown that the prevalence of influenza virus in the wild duck population of western Eurasia shows temporal differences in comparison to the results found in studies in North America. The prevalence in western Eurasian ducks is high during the period August to December and also rises in the spring. These findings are of importance for the understanding of how influenza virus is perpetuated in nature. During the course of the study only low pathogenic subtypes were isolated. Of concern is the high frequency of isolation of virus strains of the H5 and H7 subtypes that are prone to change into highly pathogenic variants in poultry. Many of the strains isolated in our Study are similar to the ones that have caused influenza outbreaks in poultry in Europe during the

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last seven years. This indicates that wild bird surveillance for influenza A virus can be of major value as a sentinel system to prevent outbreaks in domestic poultry.

Studies on Black-headed Gulls (Larus ridibundus) revealed a previously unknown subtype, H16. This finding widened the spectra of known influenza A virus subtypes in nature. Influenza A virus was also isolated in samples from Guillemots (Uria aalge) in the Baltic Sea. This was the first-time influenza A virus was isolated from this species in Europe.

REVIEW OF LITERATURE

During the years 1918-1920 one of the most devastating disease outbreaks in world history took place. It would become known as the Spanish flu pandemic and left the world in horror as it caused the death of maybe as many as 50-100 million people (Johnson and Mueller, 2002). It beats even the Great Plague in the number of people killed. It is not known where this pandemic started although British army camps in northern France during the first world war have been suggested (Oxford et al., 2005). The first documented clinical cases were found in the United States and an alternative theory state that recruits traveling to the war brought the disease over to Europe. The Spanish flu spread from continent to continent and returned in three major waves during next years with increasing virulence. This horrific event sparked research into the field that eventually led to the discovery of the viral culprit. In 1933 Smith and co-workers discovered a filterable substance that caused influenza-like respiratory disease in humans which could be transmitted between ferrets and rendered them immune to reinfection (Smith, Andrewes, and Laidlaw, 1933). Smith and Stuart-Harris were later able to fulfill Koch's postulate by isolating the influenza virus from one of the researchers' throats when he developed influenza-like illness after he had accidentally been sneezed upon by one of the infected ferrets (Nicholson, Webster, and Hay, 1998). The results were published in Lancet in1936 (Smith and Stuart-Harris, 1936). By analyzing exhumed remains from of an individual buried in the arctic, where there had been permafrost since the outbreak, and by sampling tissues from victims stored in formalin, Taubenberger and Reid et al (Basler et al., 2001; Reid et al., 1999; Reid et al., 2004; Reid et al., 2002; Reid et al., 2000; Reid et al., 2003b; Reid, Taubenberger, and Fanning, 2004; Taubenberger, Reid, and Fanning, 2000; Taubenberger et al., 2001) were able to recover enough RNA to determine the subtype of the Spanish Influenza pandemic virus as H1N1.

The world experienced two more severe pandemics during the 20th century. Although less devastating than the Spanish flu, they still caused high morbidity and mortality with death tolls reaching 6 million worldwide (Oxford, 2000). During the years 1957-1958 there was a pandemic named the Asian flu with the H2N2 subtype, and between 1968 and 1970 the H3N2 subtype caused a pandemic known as the Hong Kong flu. After each pandemic the previously circulating strain disappeared for unknown reasons. Between 1977 and 1978 a very mild pandemic mainly affecting young people swept the world as the H1N1 subtype returned; possibly released by mistake during live vaccine trials in the Far East (Palese, 2004). This strain currently co-circulates in humans with the H3N2 subtype from the Hong Kong pandemic of 1968. Based on historical patterns, pandemics can be expected to occur on average three to four times each century, but still there is no way to predict when the next pandemic will hit the world. Considering the high

population density and modes of travel in today's world, a new pandemic could have devastating consequences. Influenza A virus does not only cause disease in man but also in animals. In 1878, the disease was first identified in animals in Italy by Eduardo Perroncito. He described an initially mild disease in domestic birds that after a while turned highly pathogenic, killing virtually all the birds in the area. In 1901 two other Italian scientists, Centanni and Savonuzzi, identified "Fowl Plague", as it was then called, to be a viral disease, but it was not until 1955 that influenza virus was identified as the causative agent. Between the years 1959 and 1999, 18 outbreaks of avian influenza with high mortality (HPAI) were reported in domestic poultry around the world. These outbreaks had devastating economic consequences for the affected countries. Millions of raised birds died from the disease or were culled in order to stop the outbreaks (Capua I, 2001). In recent years the frequency of outbreaks in domestic birds has increased (Munster et al., 2005).

The first documented outbreak of HPAI in the wild bird population was in 1961, when an outbreak in Common Terns (*Sterna hirundo*) killed about 1600 birds in South Africa (Becker, 1966). This outbreak put focus on wild birds as a possible reservoir for influenza A virus. When screening wild birds in search of Newcastle disease virus (known to be spread by wild birds), during an outbreak of Newcastle disease in poultry in California in 1974, Slemons et al (Slemons et al., 1974) revealed that low pathogenic influenza A virus (LPAI) could be isolated from wild birds. Further screening soon revealed that species living in aquatic environments such as ducks, gulls, geese and shorebirds harbored low pathogenic influenza A virus strains of many different subtypes and probably acted as a reservoir for these strains (Webster et al., 1992). It has since been shown that all known influenza strains infecting humans and other mammals originally circulated in the wild bird population (Ito and Kawaoka, 2000; Reid et al., 1999).

Research has shown that low pathogenic influenza A virus strains may, after circulation in poultry populations, sometimes mutate into highly pathogenic influenza virus strains (Alexander et al 2000). During an epizootic in Italy between 1999 and 2001, the H7N1 virus, initially of low pathogenicity, mutated within nine months to a highly pathogenic form (Zanella et al, 2001). More than 13 million birds died or were destroyed.

Influenza A virus also infects and causes epizootics in mammalian species such as horses, pigs, seals, whales, ferrets and mink (Webster et al., 1992) which will be discussed in more detail further on in the text. It was long thought that the disease outbreaks of influenza A virus in poultry were of no concern to humans even though there had been reports of people suffering from conjunctivitis after being in contact with animals sick with influenza A virus, or when working with highly pathogenic influenza A isolates in the laboratory (Capua and Alexander, 2004). Influenza A virus was not considered to be a zoonotic disease agent of any importance until transmission from birds to humans occurred in Hong Kong in 1997. The outbreak in Hong Kong was caused by a highly pathogenic H5N1 strain that caused severe respiratory disease in 18 humans. Six of the infected people died of the disease (Chan, 2002). The cases of human infection coincided with an epizootic of HPAI in Hong Kong's poultry population, caused by the same strain of influenza A virus. Investigation of the outbreak determined that close contact with live infected poultry was the source of human infection and that the virus had been acquired directly by humans from birds. Rapid destruction of Hong Kong's entire poultry population of 1.5 million

birds reduced opportunities for further direct transmission to humans. However, the world would soon again experience outbreaks of HPAI in poultry transmitting virus to and causing severe disease in humans. In 2003 an outbreak of highly pathogenic IAV of the H7N7 subtype, caused the death of one veterinarian and mild illness in 83 other humans in the Netherlands. More than 30 million birds were killed at the cost of several million euros.

The worst outbreak of HPAI in modern times is currently plaguing the world. Starting in February 2003, the outbreak has as of the 20th July 2006 led to the death or destruction of more than 100 million birds and has caused verified disease in 231 humans of which 133 have died (WHO) in ten countries. The outbreak of the same H5N1 subtype that caused disease in\ Hong Kong in 1997 started in Southeast Asia and subsequently spread to most parts of Eurasia and several countries in Africa. The virus has also transmitted to species that were previously not known to be susceptible to infection such as domestic cats, leopards and tigers (Keawcharoen et al., 2004; Kuiken et al., 2004; Thanawongnuwech et al., 2005).

METHODOLOGICAL CONSIDERATIONS

The screening of patient samples for the presence of influenza A virus using reverse transcription-polymerase chain reaction (RT-PCR) has been evaluated in many studies and been found to have a high specificity and sensitivity (Hindiyeh et al., 2005; Smith et al., 2003; Stone et al., 2004). Some studies have also evaluated methods more adapted to suit the detection of avian influenza virus strains (Cattoli et al., 2004; Fouchier et al., 2000; Lee and Suarez, 2004; Spackman et al., 2003a; Spackman et al., 2003b). The fecal samples collected in studies I-IV were screened using RT-PCR. Different RTPCR- methods were used at the different laboratories that cooperated in paper IV. In brief, RNA was isolated from the samples using commercially available RNA-isolation kits according to the manufacturers' instructions and from 2003 and onwards by the use of automated procedures. A reverse transcriptase (RT)-step creating a cDNA amplicon was carried out. Influenza A virus RNA was detected using primers directed at conserved regions of the M-gene of the influenza virus and amplified using PCR. From 2003 onwards all samples were screened with real time PCR technology allowing for quantification analyses and minimizing the risks of cross-contamination of samples caused by post amplification sample handling (Spackman et al., 2003a). At the Erasmus Medical Center in Rotterdam, the Netherlands, a real time PCR with Taqman™ probes was used, developed by Fouchier et al (Fouchier et al., 2000) (papers I, II, III and IV) while at the Swedish Institute for Infectious Disease Control (SMI) and at Kalmar University a real time PCR-method, developed and described by Karlsson and co-workers (Karlsson et al., submitted), using SYBR® Green was used. The Taq-man method uses a probe that is designed to bind in between the nucleotide sequence determined by two primers. When the polymerase replication takes place, the probe is cleaved, and fluorescent light emits. SYBR-green is a dye that only binds to double stranded DNA. When the PCR product determined by the primers is amplified and hybridized the dye binds and emits light. Both these methods can be used to measure the amount of the desired PCR products as the amount of light emitted is proportional to the PCR product. The amount of nucleotide template in the original sample can also be determined, since the more template molecules present at the beginning of the reaction, the fewer cycles it takes to reach the

point at which the fluorescent signal is first recorded. The specificity of the reactions was also controlled by the evaluation of melting curves and when using the SYBR-green technology, samples were analyzed in duplicates or triplicates. The different methods were evaluated in in-house tests and the two different SYBR-green methods were shown to have similar sensitivity. Using the Taq-man approach with the combination of specific primers and a specific probe should theoretically be more specific than using SYBR-green technology. It is, however, more expensive than the SYBR-green method, since more primers and probes are needed.

The SYBR-green method might have an advantage when it comes to detecting different variants of influenza A virus as it may allow for the amplification of strains with more variations in the nucleotide sequence. It might, however, be less specific due to the fact that unspecific binding may occur to non-specific reaction products including primer-dimers.

CONCLUSION

Since the first findings of low pathogenic influenza A virus among wild birds in 1974 (Slemons et al., 1974), investigations have pointed to wild birds as being a reservoir for many different influenza A virus strains. Early sampling showed that the largest amounts of influenza A virus isolations could be made from samples taken from birds living in aquatic environments such as ducks, geese, waders and gulls (Webster et al., 1992). We performed a meta-analysis on data from all published surveys (known to us) of isolations of influenza A virus from wild bird species. From this analysis we conclude that isolations are by far most frequent from dabbling ducks (9.5 %) and to a lesser extent from other species living in aquatic environments (>1.7 %). Isolations from non-aquatic species, represented here by the passerine species, exist (0.7 %), but are not common. It should be noted, however, that most existing data have been gathered from aquatic species and from species that are easy to catch. Much of today's knowledge of the ecology of influenza A virus in wild birds is derived from large studies on the prevalence in wild birds carried out in North America (Krauss et al., 2004). In order to assess the prevalence and distribution of influenza A virus in wild birds in Sweden, and thus a western Palaearctic bird population, we performed a large study at Ottenby Bird Observatory. There we caught and sampled Mallards, and to a lesser extent other dabbling ducks such as Eurasian Teals (Anas crecca), Northern Pintails (Anas acuta) and Shelducks (Tadorna tadorna). When analyzing the material from four years of sampling (described in paper IV), we found that the dabbling duck population had a similarly high prevalence of influenza A virus infection as had previously been found in other large surveys. Out of all Mallard samples collected at Ottenby Bird Observatory 14.5 percent were positive for influenza A virus as compared to 22.2 percent in wild ducks in the North American study (Krauss et al., 2004) and 8.7 percent in a German study (Suss et al., 1994). Our data thus strongly support the idea that dabbling ducks are the main reservoir for influenza A virus in nature. However, there may be reservoir species that are not dabbling ducks and dabbling ducks may not be a reservoir for all subtypes. Therefore, we sampled other species that we thought were potential carriers.

REFERENCES

1. *Austin, F. J., and Webster, R. G. (1993). Evidence of ortho- and paramyxoviruses in fauna from Antarctica. Journal of Wildlife Diseases **29**(4), 568-71Baigent, S. J., and McCauley, J. W. (2003). Influenza type A in humans, mammals and birds: determinants of virus virulence, host-range and interspecies transmission. Bioessays **25**(7), 657-71.*

2. *Banks, J., Speidel, E. S., Moore, E., Plowright, L., Piccirillo, A., Capua, I., Cordioli, P., Fioretti, A., and Alexander, D. J. (2001). Changes in the haemagglutinin and the neuraminidase genes prior to the emergence of highly pathogenic H7N1 avian influenza viruses in Italy. Archives of Virology **146**(5), 963-73.*

3. *Capua, I., and Alexander, D. J. (2004). Human health implications of avian influenza viruses and paramyxoviruses. European Journal of Clinical Microbiology & Infectious Diseases **23**(1), 1-6.*

4. *Englund, L., and Hard af Segerstad, C. (1998). Two avian H10 influenza A virus strains with different pathogenicity for mink (Mustela vison). Archives of Virology **143**(4), 653- 66.*

5. *Gambaryan, A., Yamnikova, S., Lvov, D., Tuzikov, A., Chinarev, A., Pazynina, G., Webster, R., Matrosovich, M., and Bovin, N. (2005). Receptor specificity influenza viruses from birds and mammals: new data on involvement of the inner fragments of the carbohydrate chain. Virology **334**(2), 276-83.*

6. *Hilleman, M. R. (2002). Realities and enigmas of human viral influenza: pathogenesis, epidemiology and control. Vaccine **20**(25-26), 3068-87.*

7. *Jefferson, T., Demicheli, V., Rivetti, D., Jones, M., Di Pietrantonj, C., and Rivetti, A. (2006). Antivirals for influenza in healthy adults: systematic review. Lancet **367**(9507), 303- 13.*

8. *Johnson, N. P., and Mueller, J. (2002). Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. Bulletin of the History of Medicine **76**(1), 105- 15.*

9. *Kaleta, E. F., Hergarten, G., and Yilmaz, A. (2005). Avian influenza A viruses in birds*

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