2. LITERATURE REVIEW

2.1 Avian influenza

2.1.1 Aetiology

Avian flu is caused by an influenza virus from the family *Orthomyxoviridae*. It is an RNA virus and is thought to have 8 single-stranded negative RNA segments of 890 to 2,341 nucleotides (Gürtler, 2006). It is spherical in shape with a diameter of 80-120nm (Agarwal *et al*., 2004). When sliced transversely, influenza virions resemble a symmetrical pepperoni pizza, with a circular slice of pepperoni in the middle and seven other slices evenly distributed around it (Noda, 2006).

It belongs to the Influenza A type of influenza viruses out of A, B and C which means that it has the capacity for antigenic drift or antigenic shift. On the surface are the three types of spike proteins: haemagglutinin (HA), neuraminidase (NA) and matrix (M2) protein. The HA and NA are the two most important surface antigens for the host's immune response (Ardans, 1999). There are 16 different HA (H1 to H16) and 9 different NA (N1 to N9) in influenza-A viruses and can be present in any combination. All these combinations of antigenically distinct subtypes of the virus have been found in birds and fewer combinations have been found in mammals (Alexander *et al*., 2004) Only three HA have been found in humans (H1, H2 and H3) and H1 and H3 are circulating in humans at the present.

The conventional nomenclature for influenza virus isolates requires connotation of the influenza virus type, the host species (omitted in the case of human origin), the geographical site, serial number, and year of isolation. For influenza virus type A, the

haemagglutinin and neuraminidase subtypes are added in brackets. One of the parental avian strains of the current outbreaks of H5N1 of Asian lineage was isolated from a goose in the Chinese province, Guangdong: accordingly, it is designated A/goose/Guangdong/1/96 (H5N1) (Xu, 1999).

2.1.2 Epidemiology

All bird species are thought to be susceptible, some domestic poultry species- chickens, turkey, guinea fowl, quail and pheasants- are known to be especially vulnerable to the sequelae of infection. Wild aquatic birds, notably members of the orders *Anseriformes* (ducks and geese) and *Charadriiformes* (gulls and shorebirds), are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses (Webster, 1992; Fouchier, 2003; Krauss, 2004; Widjaja, 2004).

Until the emergence of the Asian lineage H5N1 HPAI viruses, spill-overs of HPAIV into the wild bird population occurred sporadically and were locally restricted (with the single exception of a die-off among terns in South Africa in 1961 [Becker, 1966]), so that wild birds had not been assigned an epidemiologically important function in the spread of HPAIV (Swayne and Suarez, 2000). This might have changed fundamentally since early 2005, when a large outbreak of the Asian lineage H5N1-related HPAI was observed among thousands of wild aquatic birds in a nature reservation at Lake Qinghai in the North West of China (Chen, 2005; Liu, 2005). As a result of this, further spread of this virus towards Europe during 2005 may have been founded (OIE, 2005). These outbreaks have been linked to migratory wild birds spreading the disease as have outbreaks in India, Nigeria and Niger that occurred in 2006. Many other countries have reported H5N1 in either wild birds, domestic poultry or humans and a comprehensive timeline at the WHO website.
(http://www.who.int/csr/disease/avian_influenza/timeline.pdf)

Highly pathogenic avian viruses can survive in the environment for long periods, especially in low temperatures (i.e., in manure-contaminated water). In water, the virus can survive for up to four days at 22°C, and more than 30 days at 0°C. In frozen material, the virus probably survives indefinitely. H5N1 viruses isolated in 2004 have become more stable, surviving at 37°C for 6 days while isolates from the 1997 outbreak survived just 2 days (WHO, 20041029). The virus is killed by heat (56°C for 3 hours or 60°C for 30 minutes) and common disinfectants, such as formalin and iodine compounds.

Avian influenza viruses generally replicate in the gastrointestinal tract of wild ducks, are shed at high levels through the faeces and are transmitted horizontally through the faecal-oral route. Large quantities of virus of up to $10^{8.7}$ x 50% egg-infective dose (EID_{50}) per gram faeces can be excreted (Webster, 1978).

2.1.3 Clinical signs of poultry

Influenza-A viruses infecting poultry can be divided into two distinct groups on the basis of their ability to cause disease. The very virulent viruses cause a disease formerly known as fowl plague and now termed as highly pathogenic avian influenza [HPAI] in which mortality may be as high as 100%. It was believed that the infection generally runs an entirely asymptomatic course as influenza A virus biotypes of low pathogenicity co-exist in almost perfect balance with the natural hosts (Webster, 1992; Alexander, 2000).

Highly pathogenic avian influenza, or, as it was termed originally, fowl plague, was initially recognized as an infectious disease of birds in chickens in Italy, 1878 (Perroncito, 1878). Due to a former hot spot in the Italian upper Po valley it was also referred to as Lombardian disease. Although Centanni and Savonuzzi, in 1901, identified a filterable agent responsible for causing the disease, it was not before 1955 that Schäfer characterised these agents as influenza A viruses (Schäfer, 1955).

When low pathogenic avian influenza virus (LPAIV) strains are transmitted from avian reservoir hosts to highly susceptible poultry species such as chickens and turkeys, only mild symptoms are induced in general. However, in cases where the poultry species supports several cycles of infection, these strains may undergo a series of mutation events resulting in adaptation to their new hosts.

Due to excessive economical losses to the poultry industry, HPAI receives immense attention in the veterinary world and is globally treated as a disease immediately notifiable on suspicion to the authorities. Because of their potential to give rise to HPAIV, LPAI caused by subtypes H5 and H7 is also considered notifiable (OIE, 2005).

The OIE adopted the following criteria for the classification of an avian influenza virus as highly pathogenic: any influenza virus that is lethal for six, seven or eight of eight 4– to 8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid; any virus that has an intravenous pathogenicity index (IVPI) greater than 1.2; the amino acid sequence of the connecting peptide of the haemagglutinin must be determined. If the sequence is similar to that observed for other highly pathogenic AI isolates, the isolate being tested will be considered to be highly pathogenic.

Following an incubation period of usually a few days (but rarely up to 21 days), depending upon the characteristics of the isolate, the dose of inoculum, the species, and age of the bird, the clinical presentation of avian influenza in birds is variable and symptoms are fairly unspecific (Elbers, 2005).

In its highly pathogenic form, the illness in chickens and turkeys is characterized by a sudden onset of severe symptoms and a mortality that can approach 100 % within 48 hours (Swayne and Suarez, 2000). Spread within an affected flock depends on the form of rearing: in herds which are litter-reared and where direct contact and mixing of animals is possible, spread of the infection is faster than in caged holdings

but would still require several days for complete contagion (Capua, 2000). Often, only a section of a stable is affected. Many birds die without premonitory signs so that sometimes poisoning is suspected in the beginning (Nakatami, 2005). It is worth noting, that a particular HPAI virus isolate may provoke severe disease in one avian species but not in another: in live poultry markets in Hong Kong prior to a complete depopulation in 1997, 20% of the chickens but only 2.5% of ducks and geese harboured H5N1 HPAIV while all other galliforme, passerine and psittacine species tested virus-negative and only the chickens actually showed clinical disease (Shortridge, 1998).

In industrialized poultry holdings, a sharp rise followed by a progressive decline in water and food consumption can signal the presence of a systemic disease in a flock. In laying flocks, a cessation of egg production is apparent. Individual birds affected by HPAI often reveal little more than severe apathy and immobility (Kwon, 2005). Oedema, visible at feather-free parts of the head, cyanosis of comb, wattles and legs, greenish diarrhoea and laboured breathing may be inconsistently present. In layers, soft-shelled eggs are seen initially, but any laying activities cease rapidly with progression of the disease (Elbers, 2005). Nervous symptoms including tremor, unusual postures (torticollis), and problems with co-ordination (ataxia) dominate the picture in less vulnerable species such as ducks, geese, and ratites (Kwon, 2005). During an outbreak of HPAI in Saxonia, Germany, in 1979, geese compulsively swimming in narrow circles on a pond were among the first conspicuous signs leading to a preliminary suspicion of HPAI.

The symptoms following infection with low pathogenic AIV may be as discrete as ruffled feathers, transient reductions in egg production or weight loss combined with a slight respiratory disease (Capua and Mutinelli, 2001). Some LP strains such as certain Asian H9N2 lineages, adapted to efficient replication in poultry, may cause more prominent signs and also significant mortality (Bano, 2003; Li, 2005).

2.1.4 Pathology

Gross pathological and histopathological alterations of HPAI reveal similar dependencies to those listed for the clinical presentation. Different classes of pathological alterations have been tentatively postulated as following: (Perkins and Swayne, 2003).

Peracute (death within 24.36 hours post infection, mainly seen in some galliforme species) and acute forms of disease reveal no characteristic gross pathological alterations: a discrete hydropericardium, mild intestinal congestion and occasionally petechial bleedings of the mesenterical and pericardial serosa have been inconsistently described (Mutinelli, 2003a; Jones and Swayne, 2004). Chickens infected with the Asian lineage H5N1 sometimes reveal haemorrhagic patches and significant amounts of mucus in the trachea (Elbers, 2004). Serous exudates in body cavities and pulmonary oedema may be seen as well. Pinpoint bleedings in the mucosa of the proventriculus, which were often described in text books in the past, have only exceptionally been encountered in poultry infected with the Asian lineage H5N1 (Elbers 2004). Various histological lesions together with the viral antigen can be detected throughout different organs (Mo, 1997). The virus is first seen in endothelial cells. Later on virus-infected cells are detected in the myocardium, adrenal glands and pancreas. Neurons as well as the glial cells of the brain also become infected. Pathogenetically, a course similar to other endotheliotropic viruses may be assumed, where endothelial and leukocyte activation leads to a systemic and uncoordinated cytokine release predisposing to cardiopulmonary or multi-organ failure (Feldmann, 2000; Klenk, 2005).

In animals which show a protracted onset of symptoms and a prolonged course of disease, neurological symptoms and, histologically, non-suppurative brain lesions predominate the picture (Perkins and Swayne, 2002a; Kwon, 2005). However, virus can also be isolated from other organs. This course has been described in geese, ducks, emus and other species experimentally infected with an Asian lineage HPAI H5N1 strain. In laying birds, inflammation of the ovaries and oviducts, and, after follicle rupture, so-called yolk peritonitis, can be seen.

In ducks, gulls and house sparrows, only restricted viral replication was found. These birds showed mild interstitial pneumonia, airsacculitis and occasionally lymphocytic and histiocytic myocarditis (Perkins and Swayne 2002a, 2003). In the experiments described by Perkins and Swayne (2003), pigeons and starlings proved to be resistant against H5N1 infection. However, Werner were able to induce protracted neurological disease, due to nonsuppurative encephalitis (Klopfleisch, 2006), in 5/16 pigeons using a recent Indonesian HPAI H5N1 isolate.

Lesions vary with the viral strain and the species and age of the host. In general, only turkeys and chickens reveal any gross and microscopic alterations especially with strains adapted to these hosts (Capua and Mutinelli, 2001). In turkeys, sinusitis, tracheitis and airsacculitis have been detected, although secondary bacterial infections may have contributed as well. Pancreatitis in turkeys has been described. In chickens, mild involvement of the respiratory tract is most commonly seen. In addition, lesions concentrate on the reproductive organs of layers (ovaries, oviduct, and yolk peritonitis).

2.1.5 Lab diagnosis of avian influenza virus

Infections in birds can give rise to a wide variety of clinical signs that may vary according to the host, strain of virus, the host's immune status, presence of any secondary exacerbating organisms and environmental conditions. Virus isolation is the definitive means for the diagnosis of AI virus infections, as the gold standard of diagnosis and virus characterization. At present the conventional isolation and virus characterization techniques for the diagnosis of AI remain the methods of choice, for at least the initial diagnosis of AI infections. However, conventional methods tend to be costly, labor intensive and slow. The past 10 years or so has seen enormous developments and improvements in molecular and other diagnostic techniques, many of these have been applied to the diagnosis of AI infections.

Dependence on reference laboratories, which in the case of many southeast Asian countries affected by avian influenza outbreaks, are situated abroad, potentially results in unacceptable delays and hampers timely recognition of outbreaks and institution of adequate control measures (Hien *et al.*, 2004). However, due to the economical limitations, diagnostic facilities in many affected countries are in shortage and not sufficiently equipped for virological diagnostics. Global efforts to improve diagnostic capacity in resource-poor countries may prove an important step towards the prevention and control of pandemic influenza (Hien *et al.*, 2004).

2.1.5.1 Virus isolation

The majority of AI viruses are primarily limited to replication in the respiratory and enteric epithelium of avian species, and swabs and tissues from these systems are the preferred samples. However, the viremia that occurs during infections with highly pathogenic viruses allows for virus isolation from multiple other tissues as well, such as the kidney, spleen, liver, brain, heart, and blood. Because of variations among avian species in the route and longevity of viral shedding, simultaneous collection of oropharyngeal or tracheal and cloacal swabs is a more sensitive means for isolation of virus than either swab alone. Swabs should be collected into 1.5 to 2 ml of suitable sterile transport media containing antibiotics. Samples can be stored at 4°C for up to 48 hours after collection but should be frozen (-70°C) for longer periods of storage.

Avian viruses can be isolated in embryonated eggs or in cell culture, using permissive cells such as Madin Darby canine kidney (MDCK) cells or rhesus monkey kidney (LLC-MK2) cells. Unlike avirulent avian strains and in accordance with their promiscuity for cellular proteases, highly pathogenic avian viruses do not require the addition of exogenous trypsine for efficient replication in cell culture. For safety purposes, the isolation of highly pathogenic avian influenza virus requires bio-safety level 3 laboratory facilities or higher. Cytopathic effects in cell culture are non-specific. Initial identification of influenza A virus can be performed by immunofluorescent staining with monoclonal antibodies against the nucleoprotein. Further HA and NA subtyping is performed by subtype-specific RT-PCRs of culture

supernatant or haemagglutination inhibition and neuraminidase inhibition assays using a panel of reference antisera against various subtypes.

While in human infections, avian influenza viruses have mostly been isolated from conjunctival swabs and respiratory specimens such as throat or nasal secretions or washings (Fouchier *et al*., 2004; Tran *et al*., 2004). In one reported case of H5N1 infection, virus was also isolated from serum, cerebrospinal fluid, and a rectal swab (de Jong *et al*., 2005).

2.1.5.2 Rapid antigen detection

The commercially available Directigen® Flu A kit (Becton Dickinson Microbiology Systems), which is an antigen-capture enzyme immunoassay system, has been used for detecting the presence of influenza A viruses in poultry (Slemmons, 1998), particularly in the USA. The kit uses a monoclonal antibody against the nucleoprotein and should therefore be able to detect any influenza A virus. Although it was developed to detect virus in mammalian infections, it has been successfully applied to detecting viruses in poultry and other birds, although there may be some variation in the sensitivity for different specimens. The main advantage of the test is that it can demonstrate the presence of AI within 15 minutes. The disadvantages are that it may lack sensitivity, it has not been validated for different species of birds, subtype identification is not achieved and the kits are expensive. The test should only be interpreted as a flock and not an individual bird test.

However, in patients with avian influenza, the usefulness of these assays seems limited due to low sensitivity (Peiris *et al*., 2004). However, developments of H5N1-specific rapid antigen detection tests are ongoing (Xu *et al*., 2005).

2.1.5.3 Reverse transcription-PCR (RT-PCR)

RT-PCR methods allow sensitive and specific detection of viral nucleic acids and have been shown to increase the diagnostic sensitivity for many viral pathogens

when compared to culture or antigen detection methods. During the H5N1 outbreaks in Hong Kong and Southeast Asia, RT-PCR methods for specific detection of H5N1 viral nucleic acids have proven valuable and seem to be the diagnostic methods of choice in case of an outbreak of avian influenza (Chotpitayasunondh *et al.*, 2005; Tran *et al.*, 2004). Especially when using real-time PCR technology, a reliable subtype-specific diagnostic result can be generated within a few hours after specimen collection. A disadvantage of RT PCR methods is its proneness for contamination and the consequent risk of false-positive results, which should be minimized by proper precautions, including physical separation of laboratories for PCR preparation and amplification, and the use of the glycosylase system to prevent contamination by carryover of amplifiers. In addition, the inclusion of an internal control in RT PCR assays is highly desirable to monitor for false-negative results due to inefficient nucleic acid extraction, cDNA synthesis, or transcription.

2.1.5.4 Serological tests

During outbreaks of avian influenza, the detection of subtype-specific antibodies is particularly important for epidemiological investigations. As all influenza A viruses have antigenically similar nucleocapsid and antigenically similar matrix antigens, agar gel immunodiffusion tests are used to detect antibodies to these antigens. Concentrated virus preparations containing either or both type of antigens are used in such tests. Not all birds develop demonstrable precipitating antibodies. Reference antisera to all of the subtype antigen combinations are used to determine the identity of the virus; however, the virulence of a virus cannot be determined by the antigenic subtype.

Haemagglutination inhibition tests have also been employed in routine diagnostic serology, but it is possible that this technique may miss some particular infections because the haemagglutinin is subtype specific. Enzyme-linked immunosorbent assays have been used to detect antibodies to influenza A type-specific antigens.

Haemagglutination inhibition (HI) assays are the gold standard for detection of antibodies against avian influenza viruses. However, their usefulness for detection of antibodies against avian viruses in mammalian species, including humans, seems limited (Beare and Webster, 1991; Hinshaw *et al.*, 1981; Kida *et al.*, 1994). Several studies have shown a failure to detect HI antibodies against avian viruses in mammals, even in cases where infection was confirmed by virus isolation. It has been demonstrated that HI testing with subunit HA, but not with intact virus, could detect antibodies against an avian H2N2 virus (Lu *et al*., 1982).

2.2 Avian influenza as public health issues

Up to the end of 2003, HPAI was considered a rare disease in poultry. Since 1959, only 24 primary outbreaks had been reported world-wide. The majority occurred in Europe and the Americas. Most outbreaks were geographically limited, with only five resulting in significant spread to numerous farms, and only one which spread internationally. None of the outbreaks had ever approached the size of the Asian outbreaks of H5N1 in 2004 (WHO 2004/03/02). To date, all outbreaks of the highly pathogenic form have been caused by influenza A viruses of the subtypes H5 and H7.

In the past outbreaks, illegal trade or movements of infected live birds or their unprocessed products, and unintended mechanical passing-on of virus through human movements (travelers, refugees, etc.) have been the main factors in the spread of HPAIV. A new dimension of HPAI outbreaks became evident late in 2003. From mid-December 2003 through to early February 2004, outbreaks in poultry caused by the Asian lineage HPAI H5N1 virus were reported in the Republic of Korea, Vietnam, Japan, Thailand, Cambodia, Lao People's Democratic Republic, Indonesia, and China. The simultaneous occurrence in several countries of large epidemics of highly pathogenic H5N1 influenza in domestic poultry is unprecedented. All efforts aimed at the containment of the disease have failed so far. Despite the culling and the preemptive destruction of some 150 million birds, H5N1 is now considered endemic in many parts of Indonesia and Vietnam and in some parts of Cambodia, China,

Thailand, and possibly also the Lao People's Democratic Republic.

The original virus, encountered for the first time in 1997, was of a reassortant parentage, including at least a H5N1 virus from domestic geese (A/goose/Guangdong/1/96, donating the HA) and a H6N1 virus, probably from teals (A/teal/Hong Kong/W312/97, donating the NA and the segments for the internal proteins), which underwent many more cycles of reassortation with other unknown avian influenza viruses (Xu, 1999; Hoffmann, 2000; Guan 2002b). Several different genotypes of the H5N1 lineage have been described (Cauthen, 2000; Guan 2002a, 2003). The so-called genotype Z has dominated the outbreaks since December 2003 (Li, 2004).

The ongoing outbreak of H5N1 influenza among birds with occasional transmission to human beings is of major concern because of intriguing parallels between the H5N1 virus and the 1918 influenza strain. Should H5N1 acquire the capability of easy human-to-human transmissibility, even the most conservative scenario anticipates up to several 100 million outpatient visits, more than 25 million hospital admissions and several million deaths globally (WHO Checklist, 2005).

Fortunately at present, H5N1 avian influenza remains largely a disease of birds. The species barrier is significant: despite the infection of tens of millions of poultry over large geographical areas for more than three years, fewer than 400 human cases have been confirmed by a laboratory (WHO, 200709). Human cases, first documented in 1997 (Yuen, 1998), coincided with outbreaks of highly pathogenic H5N1 avian influenza in poultry. Very limited human-to-human transmission of the H5N1 strain was documented in healthcare workers and family members with contact (Katz, 1999; Buxton Bridges, 2000).

However, H5N1 isolated from apparently healthy domestic ducks in mainland China from 1999 to 2002, and in Vietnam since 2003 have become progressively more pathogenic for mammals (Chen, 2004). H5N1 has expanded its host range, naturally infecting and killing mammalian species (cats and tigers) previously considered resistant to infection with avian influenza viruses

(http://www.who.int/csr/don/2004_02_20/en/ index html).

In April 2005, yet another level of the epizootic was reached, when, for the first time, the H5N1 strain obtained access to wild bird populations on a larger scale (Chen, 2005; Liu, 2005), which is a paradigm shift in the epidemiology of HPAI towards endemicity in migratory wild bird populations seems to be imminent. This would have grave consequences for the poultry industry on a transcontinental scale. Exposure risks for humans are directly linked to the increased presence of potentially zooanthroponotic viruses in domestic poultry.

2.3 Wild birds and avian influenza

2.3.1 Wild birds as reservoir

All of the 16 HA and nine NA subtypes of H5N1 have been identified in wild birds from at least nine different orders and the infections are generally asymptomatic. Waterfowl such as ducks, geese and swans are suspected to be the main carriers of the H5N1 viruses. This is because the influenza viruses can be spread by fomites, surviving and spreading easily in water. In Russia and Kazakhstan, the primary source of infection for poultry is considered the contact between domestic poultry and wild waterfowl at open water reservoirs (Food and Agriculture Organization [of the United Nations] [FAO], 2005). It is possible that HPAI can spread along the migration routes of wild birds through the complex flyway paths.

Until 2005, H5N1 isolations had been taken from dead wild birds which were within a flight range of infected poultry farms and were thought to be dead-end hosts of the virus. However, in April 2005 an outbreak was detected at Qinghai Lake in western China which has no poultry farms in the area (Chen *et al*., 2005). This lake is one of the most important breeding locations for migratory birds that over winter in Southeast Asia, Tibet and India (Liu *et al*., 2005). Around 1,500 birds died, about 90% of which were bar-headed geese (*Anser indicus*) with the remainder being

brown-headed gulls (*Larus brunnicephalus*) and great black-headed gulls (*Larus ichthyaetus*) (Chen *et al*., 2005). The two main symptoms were abnormal neurological signs such as tremours and diarrhea (Liu *et al*., 2005). There are some inconsistencies in the literature as to the origins of the 33 different H5N1 viruses isolated. Chen *et al*. (2005) found that the viruses are characterized as H5N1 genotype Z but were clearly distinguishable from genotype Z viruses that had caused human infections in Thailand and Vietnam. They therefore suggested that the outbreak was caused by a single introduction, most probably from poultry in southern China.

Liu *et al*. (2005) found that one of the Qinghai Lake strains (BhGoose/QH/1/05) was highly virulent in chickens which died within 20 hours and in mice which died within 72 hours. This was different from earlier isolates taken from ducks in China which were less virulent in chickens and mice (Chen *et al*., 2004). Liu *et al*. (2005) also found that none of the sequenced genomes were closely related to the GenBank sequence data implying the viruses were reassortants. They concluded from this that the H5N1 viruses might be emerging from reassortants that originate in birds over wintering in Southeast Asia which is in contrast to Chen *et al*., (2005). This evidence also suggests that wild birds are more than simply asymptomatic carriers and that they are reservoirs of highly pathogenic H5N1 viruses. It is likely that H5N1 viruses are being transmitted between migratory birds at the lake (Chen *et al*., 2005). Although it is possible that the virus may kill its hosts before this happens, the large migratory population at Lake Qinghai makes this extremely unlikely. This means that the viruses could be transmitted to other species which could have asymptomatic infection, as is likely in many species of wild ducks.

These carriers would be far more likely to spread the disease further along migratory routes as they would not be hampered by infection. Furthermore, the current H5N1 viruses, like the precursor A/Goose/Guangdong/1/96, could become established in the bar-headed goose. This would lead to the H5N1 viruses spreading along the birds' winter migration routes to densely populated areas in southern Asia and along migratory flyways linked to Europe (Chen *et al*., 2005). In fact, this may have already happened and it could be undetected. Bar-headed geese migrate

southwards from September towards Myanmar and over the Himalayas to India, returning around April. The H5N1 virus was first detected in India in domestic poultry on 18th February 2006 (WHO, 2006b) which may fit in with this theory as it is possible that there are other undetected carriers in the migratory populations.

2.3.2 HPAI H5N1 Transmission in the Wild Bird Populations

There is further evidence to support the theory that wild birds are, in part, responsible for the spread of H5N1. On 23rd July 2005, H5N1 was detected in Russia and spread to Siberia and dead migratory birds were found in the areas of these outbreaks (WHO, 2006b). A few weeks later on 2nd August 2005, H5N1 was confirmed in poultry in Kazakhstan, and again, dead migratory birds were reported in the vicinity (WHO, 2006b). On 12th August 2005, 89 migratory birds were reported dead in Mongolia, at the remote Erhel Lake which again suggested wild bird transmission (WHO, 2006b).

It was predicted that the H5N1 virus would spread from Siberia to the Caspian and Black Sea areas due to waterfowl migration (FAO, 2005). HPAI H5N1 did actually occur in poultry in Turkey and Romania in October 2005 and in the Ukraine in December 2005 (WHO, 2006b). Although no H5N1 was reported in wild birds, these incidences fit well along the East Africa West Asia Flyway.

It is possible that these incidences are simply due to poultry imports and exports and that the dead migratory wild birds found in the areas are coincidences or that H5N1 virus passes to the wild birds from the poultry. However, it is not possible to rule out wild birds as carriers especially when Turkish strains of H5N1 are genetically similar to strains found at both Lake Qinghai and Novosibirsk, Russia (Bethge, 2006). Both the Atlantic Americas Flyway and the Black Sea/Mediterranean flyways pass through Europe, including countries such as France and Germany where H5N1 has been detected (WHO, 2006b).

The spring migration of 2006 seems to be responsible for this because birds

migrating from southern zones will have had contact with birds originating from Russia and Siberia during the winter of 2005-2006 nesting areas (FAO, 2005). Both Nigeria and Niger lie on the East Africa West Asia flyway where H5N1 has been found in poultry in February 2006 (WHO, 2006b) which coincides with the time when the migratory birds from China and India would have been passing through those areas.

During the winter of 2003-2004, when most of the outbreaks in South East Asia occurred, it was also the time when migratory bird densities were at their highest there (FAO, 2006) which indicates an association between the H5N1 disease and migratory birds. The evidence available suggests that wild birds are at least partly responsible for the international spread of H5N1.

Sturm-Ramirez *et al*. (2004) looked at the pathogenicity of the H5N1 viruses isolated from 1997 to 2003 in Hong Kong in mallards. They found that all the viruses replicated in the birds inoculated via the cloacae, trachea, mouth, nares and eyes. The majority of viruses were also transmitted to contact birds (uninoculated ducks kept in direct contact with the inoculated ducks) (Sturm-Ramirez *et al*., 2004).

Importantly, the 2002 H5N1 viruses had higher titers in the drinking water than the earlier viruses. Sturm-Ramirez *et al*. (2004) argue that aerosol transmission and oral contamination may be more important than the faecal-oral route. This has important implications as to the different ways that the virus can be transmitted to poultry and humans, especially if aerosol contamination does occur. This is of particular importance in non-acute infections, as one duck showed aerosol shedding of the virus for 10 days after the infection. This could cause a spread of the disease over a wide geographical area especially during migration of waterfowl (Sturm-Ramirez *et al*., 2004).

The majority of the ducks inoculated with earlier H5N1 viruses showed no symptoms and remained healthy. However the late 2002 viruses were found to be highly pathogenic to ducks with symptoms such as central nervous system (CNS)

dysfunction (tremors, shaking, loss of balance, decreased coordination) in surviving birds and death from 4-6 days post infection (p.i.) (Sturm-Ramirez *et al*., 2004). This is therefore the first study that has changed the opinion that ducks are resistant to influenza viruses that are highly pathogenic to chickens (Alexander *et al*., 1986). Acute pathogenicity was not limited to one genotype and therefore could be a new characteristic of the more recent H5N1 viruses.

This was also the first description of neurological disorders in ducks and Sturm-Ramirez *et al*. (2004) suggest that the virus' new ability to infect the CNS is crucial to the increased pathogenicity of the H5N1 virus. It is possible that the inoculation of the ducks via different routes with large doses of the virus may have affected its tropism and may be the reason for multiple organs being infected with high titers. However these large doses would not be relevant to the contact birds however they also showed systemic dissemination, neurological symptoms and they also died (Sturm-Ramirez *et al*., 2004), which suggested that the viruses were becoming highly pathogenic even for the ducks.

Worryingly, the human isolate from 2003 caused no symptoms in the mallards which actually gained weight. This could be a serious public health concern as this strain, which infects humans, may be transmitted to humans more easily if ducks infected with human isolates show no symptoms of disease.

2.4 Resident birds and avian influenza

Although aquatic birds have been considered as natural reservoirs for avian influenza viruses, H5N1 viruses have also been isolated from wild terrestrial birds. During 2002 to 2004 genotype Z viruses were isolated from a feral pigeon (*Columba livia*), a tree sparrow (*Passer montanus*) and a peregrine falcon (*Falco peregrinus*) (Li *et al*., 2004). Kou *et al*. (2005) found a new genotype of H5N1 in wild tree sparrows and the origins of the virus are unclear. It has been suggested that new genotype

viruses may be created through transmission between the migrating bird gene pool and the resident gene pool (Kou *et al*., 2005). Terrestrial birds may act as natural reservoirs and transmitters of influenza viruses, especially tree sparrows, which are asymptomatic carriers and live in close contact with both aquatic birds and domestic poultry (Kou *et al*., 2005).The distribution of tree sparrows in the UK and Ireland was mainly on agricultural land which again poses a risk to poultry industries. Long-term surveillance of tree sparrows and other wild birds, both terrestrial and aquatic would be necessary to monitor the H5N1 viruses in this country.

The H5N1 viruses isolated from the tree sparrows were highly pathogenic to chicken but not to ducks (Kou *et al*., 2005). These viruses could therefore cause great devastation to chickens resulting in welfare and economical problems. The results also suggest that if the viruses were transmitted to ducks which then display no symptoms, the risk of transmission to other birds, wild and domestic, as well as to mammals including humans is also increased. Mice were also inoculated with these novel genotypes and the viruses were detected in the lungs and brains (Kou *et al*., 2005). Endemicity of HPAIV in passerine birds such as sparrows, starlings or swallows which live in close connection to human settlements would not only impose a huge pressure on local poultry industries but also increase the exposure risks for humans (Nestorowicz, 1987). This poses a further risk to humans which may well be in direct contact with tree sparrows or other wild birds or have indirect contact, for example, through cats.

2.5 City pigeons

2.5.1 Identification

Pigeons and doves comprise the family Columbidae within the order Columbiformes, which include some 300 species of near passerine birds. In general parlance the terms "dove" and "pigeon" are used somewhat interchangeably. In

ornithological practice, there is a tendency for "dove" to be used for smaller species and "pigeon" for larger ones, but this is in no way consistently applied, and historically the common names for these birds involve a great deal of variation between the term "dove" and "pigeon." This family occurs worldwide, but the greatest variety is in the Indomalaya and Australasia ecozones. The young doves and pigeons are called "squabs." Pigeons and doves are stout-bodied birds with short necks and short slender bills with a fleshy cere. The species commonly referred to just as the "pigeon" is the feral Rock Pigeon, common in many cities. Pigeons included in this study mainly refer to these pigeons.

The Rock Pigeon is 32–37 cm long with a 64–72 cm wingspan. The white lower back of the pure Rock Pigeon is its best identification character, but the two black bars on its pale grey wings are also distinctive. The tail is margined with white. It is strong and quick on the wing, dashing out from sea caves, flying low over the water, its lighter grey rump showing well from above. The head and neck of the mature bird are a darker blue-grey than the back and wings; the lower back is white. The green and lilac or purple patch on the side of the neck is larger than that of the Stock Dove, and the tail is more distinctly banded. Young birds show little luster and are duller. Eye color of the pigeon is generally an orange color but a few pigeons may have white-grey eyes. The eyelids are orange in color and are encapsulated in a grey-white eye ring. The feet are red to pink. When pigeons take off, their wing tips touch, making a characteristic clicking sound. When they glide, their wings are raised at an angle. (http://en.wikipedia.org/wiki/Rock_Pigeon).

2.5.2 Habitat and general behavior

The species was first introduced to North America in 1606 at Port Royal, Acadia (now Nova Scotia). The Rock Pigeon has a restricted natural resident range in western and southern Europe, North Africa, and into South Asia. Its habitat is natural cliffs, usually on coasts. Its domesticated form, the feral pigeon, has been widely introduced elsewhere, and is common, especially in cities, over much of the world. In Britain, Ireland and much of its former range, the Rock Pigeon probably only occurs pure in

the most remote areas.

They are in increasing numbers in town centers and where they have adapted well to the artificial cliffs of buildings. Pigeons are highly dependent on humans to provide them with food and sites for roosting, loafing, and nesting. They are commonly found around farm yards, grain elevators, feed mills, parks, city buildings, bridges, and other structures.

Pigeons are primarily grain and seed eaters and will subsist on spilled or improperly stored grain. They also will feed on garbage, livestock manure, insects, or other food materials provided for them intentionally or unintentionally by people. In fact, in some urban areas the feeding of pigeons is considered a form of recreation. They require about 1 ounce (30 ml) of water daily. They rely mostly on free-standing water but they can also use snow to obtain water.

Pigeons inhabit lofts, steeples, attics, caves, and ornate architectural features of buildings where opening grasses clumped together to form a crude platform. Pigeons are monogamous. Eight to 12 days after mating, the females lay 1 or 2 eggs which hatch after 18 days. The male provides nesting material and guards the female and the nest. The young are fed pigeon milk, a liquid solid substance secreted in the crop of the adult (both male and female) that is regurgitated. The young leave the nest at 4 to 6 weeks of age. More eggs are laid before the first clutch leaves the nest. Breeding may occur at all seasons, but peak reproduction occurs in the spring and fall. A population of pigeons usually consists of equal numbers of males and females.

In captivity, pigeons commonly live up to 15 years and sometimes longer. In urban populations, however, pigeons seldom live more than 3 or 4 years. Natural mortality factors, such as predation by mammals and other birds, diseases, and stress due to lack of food and water, reduce pigeon populations by approximately 30% annually.

2.5.3 Problems aroused by city pigeons

Pigeon droppings deface and accelerate the deterioration of buildings and increase the cost of maintenance. Large amounts of droppings may kill vegetation and produce an objectionable odor. Pigeon manure deposited on park benches, statues, cars, and unwary pedestrians is aesthetically displeasing. Around grain handling facilities, pigeons consume and contaminate large quantities of food destined for human or livestock consumption (Judy Loven, 2000).

Pigeons may carry agents and spread diseases to people and livestock through their droppings. They are known to carry or transmit pigeon ornithosis, encephalitis, Newcastle disease, cryptococcosis, toxoplasmosis, salmonella food poisoning, and several other diseases. Additionally, under the right conditions pigeon manure may harbor airborne spores of the causal agent of histoplasmosis, a systemic fungus disease that can infect humans (Judy Loven, 2000).

The ectoparasites of pigeons include various species of fleas, lice, mites, ticks, and other biting insects, some of which readily bite people. Some insects that inhabit the nests of pigeons are also fabric pests and/or pantry pests. The northern fowl mite found on pigeons is an important poultry pest. Pigeons located around airports can also be a threat to human safety because of potential bird-aircraft collisions, and are considered a medium priority hazard to jet aircraft by the US Air Force (Judy Loven, 2000).

2.5.4 Pigeons as resident birds and avian influenza

Avian influenza viruses (AIVs) have been isolated from more than 90 species of free-living birds representing 13 different orders, and the most frequent source of AIVs has been free-flying aquatic birds (Swayne and Halvorson, 2003).

Fowl plague was regarded to be primarily a disease of gallinaceous birds. Domestic waterfowl and pigeons were less susceptible or undergo subclinical

infections (Stubbs, 1965). Although no report were traced in the detectable and accessible literature that report on natural outbreaks of HPAI in domestic pigeons, the worldwide concern about the spread of AI among humans and poultry also has many fanciers interested in the role of pigeons, especially racing pigeons, whereas there are conflicting reports on the susceptibility of pigeons. Can pigeons as a bridge between some susceptible resident birds and migration birds and play a role in the transmission of avian influenza virus?

2.5.4.1 Isolation of influenza virus from pigeons and serological surveys

During the isolation and characterization of ortho- and paramyxoviruses from feral birds in Europe from 1977to 1980, among samples taken from the trachea and cloaca of sick doves and from organ of dead birds and faeces in Munich, influenza A virus were not isolated (Ottis and Bachmann, 1983).

In the outbreak of Avian Influenza (H5N2) earlier (1983-84) in the northeastern USA, scientists conducted a survey of wildlife to determine the potential of wild birds to spread disease locally among farms, or to carry the virus to more distant locations. Included in this survey were 1) wild and free-flying domestic ducks and geese, 2) wild or free-flying domestic birds closely associated with poultry farms, poultry manure, or poultry carcasses, 3) mice and rats found inside and around houses containing infected poultry, and 4) wild birds of any species reported sick or dead within the quarantine zone. Included in this number were 473 pigeons (92.6% of these pigeons were obtained from known infected farms), 81 pigeon feet (all of them from influenza-affected premises), and seven mourning doves. None of the 4,132 samples was positive for influenza virus. Blood samples from 2,147 non-aquatic birds, including 383 pigeons, were negative for antibodies to Avian Influenza an indication that infection by this virus had not occurred in these birds. An additional 313 birds, including 50 pigeons, collected from the quarantine zone, were also negative for influenza virus. It is important to note that experimental attempts to infect pigeons with this strain of Avian Influenza did not result in either multiplication of the virus in these pigeons, or evidence of antibodies in the blood. The results of all of these studies indicated that pigeons were not infected with Avian Influenza and did not

spread it (Nettles *et al.*, 1985).

In the 1993 outbreak in the USA, in the period from February to May, blood samples were collected from 17 flocks of meat varieties of pigeons, mainly White Kings located within the quarantine area, for evidence of antibodies to Avian Influenza. Flock sizes varied from 2000 - 3000 birds, and represented a total of about 34,000 - 51,000 birds. Approximately 10 birds per flock were sampled, for a total of 160 birds. In every instance, all pigeons tested were negative for antibodies to Avian Influenza (http://www.piedmontracingpigeons.org/chalmers_bird_flu.htm).

Likewise, pigeons involved in the H5N2 HPAI outbreak in Italy in 1997 suffered no clinical disease, despite up to 50% mortality in chickens at the same farm (Capua I, *et al.*, 1996). In the detection of avian influenza viruses amongst wild bird populations in Victoria, no avian influenza virus was isolated from the sampled 133 wild pigeons (Peroulis and O'Riley, 2004) .100 pigeons were caught respectively in the city of Stavanger and Oslo, Norway in 2004. All samples from those 200 pigeons tested by inoculation in embryonated eggs and by RT-PCR were negative for avian influenza A viruses (Lillehaug *et al.*, 2005).

In the cross-sectional virology study done in live bird markets (LBM) in Hanoi, Vietnam, in October 2001, Specimens from 189 birds and 18 environmental samples were collected at 10 live bird markets. Four influenza A viruses of the H4N6 (n=1), H5N2 (n =1), and H9N3 (n=2) subtypes were isolated from healthy ducks for an isolation frequency of over 30% from this species. Two H5N1 viruses were isolated from healthy geese. But no AIV were isolated from 39 pigeons included in this study (Doan C. Nguyen, 2005). Several other attempts were made to recover influenza A virus but did not yield (Lipkind and Weisman *et al.*, 1981; Sennel *et al.*, 1983; Weisman *et al.*, 1986; Wood *et al.*, 1986)

From the respiratory mucosa of a seronegative collared dove (*Streptopelia decaocto*) a virus strain having a haemagglutinin related to the Hong Kong virus (H3N2) was isolated (Romvary and Tanyi, 1975). This is the first report on the isolation and infection of influenza virus in wild pigeons. With the isolate, hens were

infected intrapharyngeally and developed antibodies up to a titre between 1:16 and 1: 256. Indirect evidence of the infection of domestic and wild birds by this HongKong strain was emerging from demonstration of specific antibodies in the birds' sera (Romváry *et al*., 1977). H1N1 subtype was ever isolated from pigeons (Halvorson *et al.*, 1983).

Coincidentally, when H7N1 avian influenza outbreak in 2000 became a devastation epidemic in Italy, cloacal swabs or viscera obtained from 103 wild birds were used for a virological survey. Only two samples yielded HPAI of the H7N1 subtype, and one was the sample obtained from collared dove (*Streptopelia decaocto*), which was found 1 km of an infected farm (Capua *et al*., 2000).

Guan *et al.* (2000) studied three H9N2 viruses isolated from pigeons, Japanese quail or chickens sold at a live bird market in China and Hong Kong SAR, China, but did not find signs of illness or internal changes, but did observe the presence of antibodies in inoculated pigeons.

3 different subtypes of H3N3, H3N6 and H9N2 avian influenza virus were isolated from 6 pigeons out of 1,190 sampled pigeons (0.5% isolation frequency) in at a live bird market in Nanchang, South Central China (Liu *et al*., 2003). None of the viruses isolated from pigeons tested were able to uniformly infect chickens, but all could grow in quail.

Outbreaks of highly pathogenic H5N1 avian influenza have occurred in Hong Kong in chickens and other gallinaceous poultry in 1997, 2001, twice in 2002 and 2003. H5N1 avian influenza virus was also isolated from a dead feral pigeon (*Columba livia*) and a dead tree sparrow (*Passer montanus*) at Kowloon Park during the second outbreak. But all other birds sampled around the quarantine area and bird lake and the 233 environment samples were negative for avian influenza virus.

In recent 2-3 years some new strains of H5N1 avian influenza virus have been isolated from Order Columbiformes: A/littlecuckoo-dove/Tak-2-01/2004 isolated

from Little Cuckoo-Dove (*Macropygia ruficeps*) in Thailand, A/feral pigeon/HK/862.7/2002 and A/Pigeon/Hong Kong/SF215/01 from Pigeon (Species not indicated) in China (Hong Kong) and A/pigeon/Samut Prakan/Thailand/CU-202/04 and A/pigeon/Thailand/KU 03/04 ones from Thailand (www.fluwikie.com).

2.5.4.2 Serological investigation of pigeons

In 1969, 100 doves were live-trapped with portable wire bait traps. Within 24 hours of capture, the doves were given an antemortem examination and serological examination. The serological examination of these doves indicated that all of the doves tested lacked humoral antibodies to 3 strains of influenza type A virus (Carpenter, 1972). 40 sera were taken from migrating wild pigeons caught in October 1971 at Echalar, province of Navarrra, Spain. 57 quail sera were obtained between February and October 1970 from quail farms in another province of Spain (Vicente *et al.*, 1973). Those 97 sera from pigeons and quails were negative for human influenza viruses. Pigeons in this study were wild birds, captured while returning to their southern winter habitat from summer nesting areas in northern Europe (Vicente *et al.*, 1973).

Only a few studies detected positive pigeon sera against avian influenza virus. Among them, Guan et al. detected in sera from pigeons of 23/35 consignments a large proportion of HI antibodies against A/Quail/HK/G1/97 (H9N2) virus (Guan *et al.*, 2000).

2.5.4.3 Pathogenicity of avian influenza virus for pigeons

Two 7-month-old pigeons and two old pigeons together with ducks and geese were released in an isolated room. Each bird was given 10^8 EID_{50} of H5N9 isolate (A/ tukey/Ontario/7732/66). 8-egg passage intravenously and a few drops of the virus suspension intranasally.

Three susceptible turkeys were placed in the room as contact controls. All the

birds remained healthy for 21 days. Virus could not be recovered form the blood of the infected birds 24 or 48 hours after inoculation (Narayan *et al.*, 1969). However, Slemons and Easterday (1972) used the same isolate and found minor signs and death by one out of 19 pigoens.

One study published in 1996 on the susceptibility of pigeons to Avian Influenza, found that groups of pigeons inoculated with two strains of highly pathogenic influenza virus (HP CK/PA H5N2 and HP CK/Australia H7N7) and two strains of nonpathogenic virus (NPAIV; NP CK/PA H5N2 and NP emu/TX H7N1) remained healthy during the 21-day trial period, did not shed virus, and did not develop antibodies to this disease. It was concluded that pigeons were resistant or minimally susceptible to infection with HPAIV or NPAIV and provide further evidence that pigeons are not a factor in the spread of this disease (Panigrahy, 1996).

Emus, domestic geese, domestic ducks and pigeons were intranasally inoculated with the A/chicken/Hong Kong/220/97 (H5N1) highly pathogenic avian influenza virus in the study of Perkins *et al.* (2002). The experimental work has shown that pigeons infected experimentally with the highly pathogenic form of the virus (designated H5N1, and of Hong Kong origin) did not develop signs of this disease and did not have detectable changes to this disease in their tissues. As well, virus was not found in their tissues and neither was it re-isolated from swabs of tissues. Compared with emus and domestic geese, ducks and pigeons probably played a minimal epidemiologic role in the perpetuation of the H5N1 Hong Kong-origin influenza viruses. These findings indicated once again that pigeons (along with starlings, rats and rabbits used in these studies), are largely resistant to infection with this virus.

Seventeen avian species and two mammalian species were intranasally inoculated with the zoonotic A/chicken/Hong Kong/220/97 (chicken/HK) (H5N1) avian influenza (AI) virus in order to ascertain a relative range of susceptible hosts and the pathobiology of the resultant disease (Perkins and Swayne, 2003). A direct association was demonstrated between viral replication and the severity of disease,

with four general gradations being observed among these species. These gradations included the following: 1) widespread dissemination with rapid and high mortality, 2) neurological disease relative to viral neurotropism, 3) asymptomatic infection or only mild transient depression associated with minor viral replication, and 4) absence of disease relative to minimal to no viral replication. Pigeons belongs to the fourth grade of infection and disease in that clinical disease were not observed, gross lesions related to viral infection were not observed, and histological lesions and related viral antigen were absent in all tissues examined.

Kaleta and Honicke (2004) conducted a review of scientific literature on influenza viruses in pigeons and concluded that pigeons play only a minor role in the epidemiology of H5 influenza viruses. By contrast, these workers found that H7-infected pigeons can multiply and excrete H7 viruses, and develop circulating antibodies. These authors also stated: The molecular basis for the discrepancies of susceptibility of pigeons to H5 and H7 viruses are presently not well understood. It is also not clear why pigeons are definitely more resistant to infection with highly pathogenic avian influenza viruses as compared to chickens and turkeys.

However, so far only one report demonstrated death of 1/19 pigeons after intranasal administration of HPAIV A/turkey/Ontario/7732/ 66 (H5N9) (Slemmons RD *et al.*, 1972).

Klopfleisch *et al.* (2006) assessed the susceptibility of experimentally infected pigeons to one of the highly pathogenic avian influenza virus (HPAIV) H5N1 strains in Asian outbreak avian influenza in recent years. Three of the pigeons died after a history of depression and severe neurological signs consisting of paresis to paralysis, mild enteric hemorrhage, resulting in a mortality of 21%. Gross lesions in these pigeons were mild and inconsistent. Microscopic lesions and detection of viral antigen were confined to the central nervous system of these pigeons. The remaining nine pigeons showed neither clinical signs nor gross or histological lesions associated with avian influenza, although seroconversion against H5 indicated that they had been infected.

These results confirm that pigeons are susceptible to HPAIV A/chicken/Indonesia /2003 (H5N1) and that the disease is associated with the neurotropism of this virus (Klopfleisch *et al.*, 2006). This was not the case with the 1997 Hong Kong H5N1 HPAI virus where the virus did not grow in pigeons after intranasal inoculation. This would indicate, in geographic areas with new Asian H5N1 HPAI viruses circulating in wild birds or poultry, restricting the flying of pigeons would be prudent for pigeon health and to prevent addition of a new vector of the virus in the specific region. But a worldwide ban on flying pigeons is not supported by scientific information on ecology of LPAI viruses.

2.6 The significance of this study

Despite some of these reassuring findings, fanciers should be aware of the possibility that if a returning race bird, or any wild bird for that matter, drops into a poultry farm on which the chickens are infected with Avian Influenza, it could pick up the influenza virus on its feet or feathers as it walks through droppings from these infected chickens. If this bird were to fly to another poultry farm, in theory it could be a mechanical means of spreading the virus to chickens on the second farm. However, as noted in earlier studies, the feet of pigeons collected from affected poultry farms were examined for influenza virus and all were found to be negative.

Even in late July of 2005, in response to concerns about reports that school children in Thailand had become ill with the H5N1 strain of virus following exposure to pigeons, Dr David Swayne et al. conducted experimental studies in pigeons using viruses isolated from dead pigeons in Thailand. Even direct inoculation of these viruses into nasal cavity of pigeons caused limited infections with between 60%-80% of the pigeons not becoming infected. This suggests the mortality from H5N1 HPAI virus in pigeons may have resulted from synergy between AI infection and some other pathogen. The illness in school children is later to be verified as an unsubstantiated

rumor. No AI virus was isolated from the children and I am unaware of any evidence of infection.

It is still controversial on the VPH topics as following: whether the current developments caused by the avian influenza a threat to our beloved pigeons sport; whether they are the forecast to new way of pigeon keeping under lock and key. After many stories from news networks, magazines and national papers, the public opinion are being influenced in regard to the pigeons. The current bird flu and possible other - new to come viruses – is suspicious to be a threat to our pigeon sport the way we know it today.

Public has confused die-offs in pigeons to avian influenza when paramyxovirus type 1 is a common cause of neurological disease and death in pigeons and not avian influenza. It is unlikely that banning pigeons will have any impact on AI ecology and will not reduce the risk AI infections of poultry or humans. The primary species that have natural infections with AI viruses are wild ducks and shorebird (turnstones, gulls, etc.). Columbiformes and passerines are not reservoirs and they are rarely incidental hosts following spill-over of the viruses from infected domestic poultry. The banning of racing pigeons in some areas such as Calgary, a location that does not have the Asian H5N1, would have no impact of the broad ecology of AI viruses since they are not the natural hosts of wild bird LPAI viruses.

While all pigeon fanciers are in the dark as to what is happening on how this flu epidemic is going to affect them. If there is a mandatory lock up of the pigeons, any pigeon fanciers will slowly loose their motivation to keep racing pigeons. This would be a disaster for the pigeon sport in the future and will surely end racing.

Pigeon flyers are not the experts looking for the solution to the flu problem. The veterinary public health (VPH) measures against the pigeons in the issues of flying and food safety need scientific basis. There has been a gradual development in the China table pigeon industry many years ago. Keeping pigeons for racing and showing has also become a popular hobby in many Chinese cities. But as residence birds, free-

flying pigeons has more chance to contact with wild birds. There are more and more public concerns on pigeon whether it is possible to be a potential threat for the transmission of avian influenza. Rearing pigeons for meat is an accepted industry also among overseas countries, particularly Europe and the United States. Young pigeons bred for meat are known as squabs. Tianjin, Shanghai and Harbin are the major squab-producing state. Pigeons are very close to human beings nowadays. There is no report on the systematic study of the surveillance of the H5N1 HPAI infection status of pigeon in China till now.

The first systematic of infectious status survey of city pigeons in China were done in this study, which will provide the sound base epidemiological data for the pigeon fanciers, the government policy makers and also the public to have a appropriate understanding of the public health issues of city pigeons related to avian influenza.