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Studies on potential risk factors for introduction and spread of avian influenza in domestic poultry of Pakistan

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Tariq Abbas
aus Dera Ghazi Khan, Pakistan

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Erster Gutachter:	PD Dr. Franz J. Conraths
Zweiter Gutachter:	Univ.-Prof. Dr. Hafez Mohammad Hafez
Dritter Gutachter:	Univ.-Prof. Dr. Karl – Hans Zessin

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Abbreviations

ACA	Adaptive Conjoint Analysis
AI	Avian influenza
AID	Asymptomatic Infection Duration
AWC	Asian Waterbird Census
CFIA	Canadian Food Inspection Agency
CI	Confidence interval
DOC	Day-Old-Chicks
DPPAs	Densely Populated Poultry Areas
DRDC	Defense Research and Development Canada
EM	Expectation-Maximization
EPB	Egg Producing Birds
FAO	Food and Agriculture Organization
FATA	Federally Tribal Administrated Areas
GDP	Gross Domestic Product
GIS	Geographic Information System
GOP	Government of Pakistan
HA	Hemagglutinin
HPAI	Highly Pathogenic Avian Influenza
HPNAI	Highly Pathogenic Notifiable Avian Influenza
IP	Infected Premises
IR	Incidence Rate
IVPI	Intravenous Pathogenicity Index
LASAT	Lagrangian Simulation of Aerosol Transport
LBMs	Live bird markets
LPAI	Low Pathogenic Avian Influenza
LPMs	Live poultry markets
LPNAI	Low Pathogenic Notifiable Avian Influenza
MI	Multiple Imputation
MPB	Meat Producing Birds
NA	Neuraminidase
NAI	Notifiable Avian Influenza
NARC	National Agriculture Research Council
ND	Newcastle Disease
NDMA	National Disaster Management Authority
NPCPAI	National Programme for the Control and Prevention of Avian Influenza
NWFP	North West Frontier Province

OIE	Office International des Epizooties
OLS	Ordinary Linear Squares
OR	Odds ratio
PM	Particulate Matter
PPA	Pakistan Poultry Association
RH	Relative Humidity
SMEDA	Small And Medium Enterprise Development Authority
SRO	Statutory Regulatory Order
SRTM	Shuttle Radar Topography Mission
TRIM	Trends and Indices for Monitoring data
WI	Wetlands International
WWF	World Wildlife Fund

1 Introduction

1.1 General information about Pakistan

Pakistan, (Urdu: پاکستان *Pākistān*), officially Islamic Republic of Pakistan, emerged as an independent sovereign state on 14th August 1947, as a result of the division of former British India. It is located in South Asia between latitudes 24° and 37° North and longitudes 61° and 75° East. It is bordered by Iran on the southwest, Afghanistan on the northwest, China on the northeast, India on the east, and the Arabian Sea on the south (Figure 1.1). In the northeast is the disputed territory of Jammu and Kashmir, of which the part occupied by Pakistan is called Azad Kashmir. The country has five provinces: Punjab, Sindh, Baluchistan, Khyber Pakhtunkhwa, Gilgit-Baltistan, plus Federally Administrated Tribal Areas (FATA) (Figure 1.2). The provinces are subdivided into administrative "divisions", which are further subdivided into districts and tehsils.. The total area of the country is 880,254 square kilometers[1].

1.2 Geography and climate

Pakistan has a diverse landscape, covering nine major ecological zones. Its territory encompasses portions of the Himalayan, Hindu Kush, and Karakoram mountain ranges, making it home to some of the world's highest mountains, including K2 (8,611 m), the world's second highest peak. Inter-mountain valleys make up most of the North West Frontier Province (NWFP) and rugged plateaus cover much of Balochistan in the west. In the east, irrigated plains are located that lie along the River Indus and cover much of Punjab and Sindh. Both provinces have desert areas as well: Cholistan and Thal in Punjab and Tharparkar in Sindh. The country's main river is the Indus and its tributaries: the Chenab, Ravi, and Jhelum. The climate is generally dry and most areas receive less than 250 mm of rain per year, although the northern and southern areas have a noticeable climatic difference. The average annual temperature is around 27°C. However, temperatures vary with elevation from 30°C to -10°C during the coldest months in the mountains and northern areas to 50°C in the warmest months in parts of Punjab, Sindh, and Baluchistan. Mid-December to March is dry and cool, April to June is hot with 25-50% humidity, July to

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[1] http://pakistani.pk/pakistan/geographics

September is the wet monsoon season, and October to November is the dry post-monsoon season with high temperatures countrywide (NDMA, 2010). Despite its arid climate, Pakistan supports more than 780,000 ha of wetlands that is 9.7% of the total land area. There are 225 major wetlands, 19 of which have been recognized as being of international importance by the Ramsar Convention[2] (Figure 1.3). The country lies at the cross-road of the Asia Palearctic migration routes. The Indus flyway is one of the migration routes running from Siberia over the Karakorum, Hindu Kush, and Suleiman mountain ranges, along the Indus river and down to its delta near the Arabian Sea. Based on regular counts at different wetlands, it is estimated that between 700,000 to 1,200,000 birds arrive via the Indus flyway every year (Ali and Akhtar, 2006). Under Global 200[3], the earth has been divided into 238 ecoregions, out of which 5 are located in Pakistan. The names of those ecoregions are: Tibetan Plateau, Western Himalayan Temperate Forests, Rann of Kutch, North Arabian Sea, and the Indus. Identified amongst the 40 biologically richest ecoregions in the world, the Indus ecoregion lies completely within the country's boundaries. It covers approximately 65% of the province of Sindh occupying 18 of its districts while a small northwestern part of the ecoregion extends slightly into Balochistan. All other eco-regions are transboundary (Khan et al., 2010).

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[2] The Convention on Wetlands of International Importance, called the Ramsar Convention, is an intergovernmental treaty that provides the framework for national action and international cooperation for the conservation and wise use of wetlands and their resources. The Ramsar Convention is the only global environmental treaty that deals with a particular ecosystem. The treaty was adopted in the Iranian city of Ramsar in 1971 and the Convention's member countries cover all geographic regions of the planet.

[3] The Global 200 is the list of ecoregions identified by the World Wildlife Fund (WWF) as priorities for conservation. According to the WWF, an ecoregion is defined as a "relatively large unit of land or water containing a characteristic set of natural communities that share a large majority of their species, dynamics, and environmental conditions.

Figure 1.1 Pakistan and its adjacent countries in Asia

Figure 1.2 Map of Pakistan showing its parts

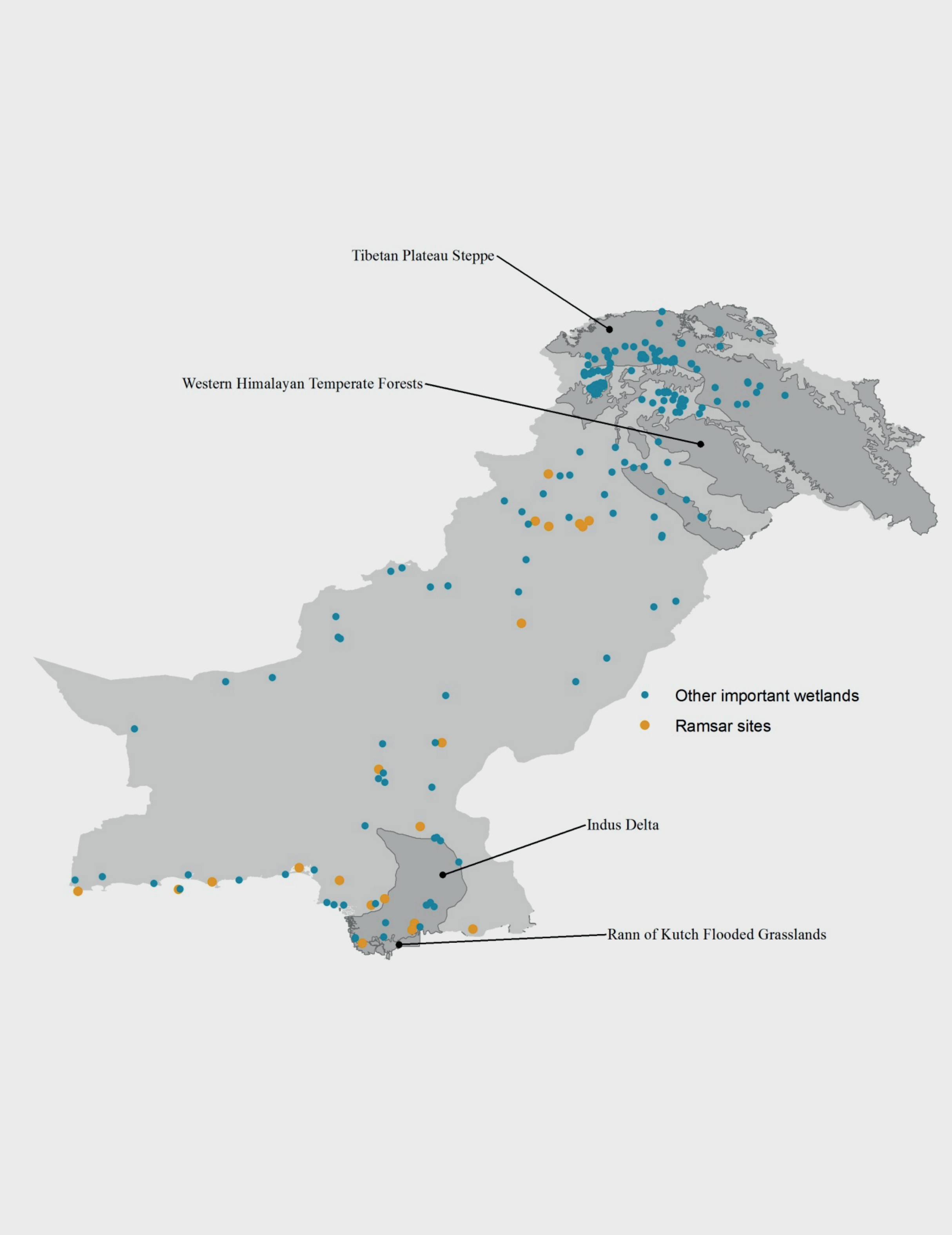

Figure 1.3 Global 200 ecoregions and important wetlands in Pakistan

1.3 Overview of poultry sector

With an estimated total population of about 168.79 million people by the end of 2009 and an average annual growth rate of 1.9%, Pakistan ranks as the sixth most populous nation in the world (Nizami, 2010). The agriculture continues to play a central role in the economy. It accounts for over 21% of Gross domestic product (GDP), and remains by far the largest employer, absorbing 45% of total labor force. Nearly 62% of the population resides in rural areas, and is directly or indirectly linked with agriculture for its livelihood. The poultry sector is one of the vibrant segments of the agriculture industry. It generates employment (direct/indirect) and income for about 1.5 million people. Its contribution to agricultural growth is 4.81% and to livestock growth 9.84%. Poultry meat contributes 19% of the total meat production. The sector has shown a robust growth of 8-10% annually which reflects its inherent potential (Farooq, 2009). It is making a tremendous contribution in bridging the gap between supply and demand of animal protein requirements. With the continuous depletion of supply of red meat, poultry is the cheapest available animal protein for the masses hence an effective check upon the spiraling animal protein prices. Pakistan as a predominantly Muslim country has comparatively high, and rising, levels of meat consumption. According to an estimate of the Pakistan poultry association (PPA)[4], the annual per capita egg and chicken meat consumption ranges between 60-65 eggs and 6-7 kg, respectively.

There are two distinct production systems: the traditional rural system and the commercial poultry system (Khan et al., 2003). Backyard poultry-keeping is a significant livelihood activity for many poor rural families, particularly for women. Native birds, mainly chickens, are reared for home consumption, to supplement income and as a ready source of cash. The meat and eggs from backyard-produced scavenging chickens fetch higher price because their taste and texture are considered superior. This system is characterized by low-input of feeding, housing, and health care, which makes it relatively more profitable. The productivity of village poultry systems tends to be low, with high mortality rates and low hatchability rates. Ducks and geese are reared almost in every village for usually subsistence. According to economic survey of 2008-2009, there were 0.6 million ducks and geese whereas the population of village chickens was 76 million (Farooq, 2009). Pigeons, partridges, and quails are also found all over the country

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[4] http://www.ppapaknorthern.com/index.php?action=memberList

both for hobby and fancy. Flight competitions in pigeons are common. Partridges are mainly kept as pets; singing competitions are also held occasionally. Quails are domesticated in villages as pets and their meat is eaten as a specialty. This is especially true in the rice growing areas where they can be hunted. Peacocks are also kept as pet birds and wild peacock is used as food in Sindh province (Khan et al., 2003).

The commercial poultry includes 28 million layers, 448 million broilers, and 8 million breeding stock (Farooq, 2009). To support these two industries, i. e. the production of chicken meat and table-eggs, there are 122 feed mills, 285 hatcheries, besides companies dealing with poultry medicines, vaccines, equipments etc (Usmani, 2010). The sub-tropical location of Pakistan tends to keep the temperature high, particularly in summer. To avoid heat stress, the poultry houses are naturally ventilated (i. e. open-sided). During the past few years, an environmentally controlled farming technology has been introduced and is becoming very popular among the farmers (SMEDA, 2008).

The Food and Agriculture Organization (FAO) of the United Nations[5] classifies poultry production into four sectors depending on the level of biosecurity. Based on this classification system, a country-specific definition for sectors 1 to 3 is not documented, while sector 4 can be described as traditional backyard (village) poultry production. Small scale market oriented commercial broiler and layer farms were ubiquitous, but there are no data available regarding their distribution and number. These farms operate their own biosecurity standards and are not restricted by movement and transportation regulations except bans imposed on movement during outbreaks. Poultry is normally sold through live bird markets (LBMs) located in cities and villages. Birds are selected live by the consumers and slaughtered and dressed by the retailers. Only a small percentage of commercial broilers are commercially processed, mainly for hotels.

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[5] FAO defined four poultry production sectors based on experiences in Asia as follows:
Sector 1: Industrial Commercial Farms - integrated system with high level biosecurity and birds/products marketed commercially (e. g. farms that are part of an integrated broiler production enterprise with clearly defined and implemented standard operating procedures for biosecurity).
Sector 2: Large Commercial Farms - poultry production system with moderate to high biosecurity and birds/products usually marketed commercially (e. g. farms with birds kept indoors continuously; strictly preventing contact with other poultry or wildlife).
Sector 3: Small Commercial Farms - poultry production system with low to minimal biosecurity and birds/products entering live bird markets (e. g. a caged layer farm with birds in open sheds; a farm with poultry spending time outside the shed; a farm producing chickens and waterfowl).
Sector 4: Village or backyard production with minimal biosecurity and birds/products consumed locally.

The poultry industry is almost in private hands but it has a strong support from the government. Different development projects and incentives have been provided to this sector: *the national program for the control and prevention of avian influenza*, *credit scheme by Zarai Taraqiati Bank Limited, poultry development policy, and reduce input costs policy in poultry production* are notable. Poultry farming, processing, and feed milling were given incentives such as total or partial exemption from import duties, sales tax, and income tax holiday for a number of years.

1.4 Brief history of avian influenza outbreaks

Avian influenza (AI) was never reported in Pakistan during 1963-1993, the period when the commercial poultry sector flourished from a single farm in Karachi to a fully fledged industry (Anonymous, 2009). In December 1994, an outbreak due to AI virus of subtype H7N3 occurred in Salgran, an isolated mountainous poultry rearing region 25 km north of the capital city Islamabad (Naeem and Hussain, 1995). The disease primarily affected broiler breeding stocks and a few commercial broiler flocks. In the case of broiler breeders, the flock age varied between 10-65 weeks, with typical signs of AI, including facial edema, cyanotic combs, and high morbidity. The mortality ranged between 40-80%. Among infected broiler flocks (3-5 week age), the clinical signs included facial swelling and variable mortality between 30-50% (Aamir et al., 2009). The disease was controlled within 4-5 months by mass vaccination with a vaccine prepared from a field isolate (Naeem and Siddique, 2006).

In November 1998, an outbreak of a disease of unknown etiology occurred in Mansehra and Abbottabad districts (Figure 1.4). The disease was reported mostly in breeding flocks of different ages, but flocks over 45 weeks old were mainly affected. The causative agent of this outbreak was later confirmed as H9N2 and oil-based inactivated vaccines were used to control the disease (Naeem et al., 1999).

Another outbreak caused by H7N3 virus occurred in Punjab during 2000-2001. The outbreak was controlled by ring vaccination with an aqueous-based vaccine produced with a local strain, followed by administration of an oil-based vaccine (Abbas et al., 2010).

In the year 2003-2004, a more extensive outbreak of H7N3 struck the southern coastal region of the country, where more than 70% of the total commercial layer flocks were reared. The virus had an intravenous pathogenicity index (IVPI) of 2.8. In several cases co-infection with H9N2 was also detected. The outbreak was controlled by adopting strict biosecurity measures, voluntary depopulation, strategic vaccination, and the implementation of a surveillance program (Naeem et al., 2007).

Abbas et al. (2010) characterized the genomes of the H7N3 type influenza viruses circulating in Pakistan from 1995-2004. Thirteen isolates were selected to represent different times of isolation, sectors of poultry production and geographical origins. The study revealed that there were two introductions of H7 and one introduction of N3. Only one of the H7 introductions became established in poultry, while the other was isolated from two separate outbreaks 6 years apart. The data also showed reassortment between H7N3 and H9N2 viruses in the field, likely during co-infection of poultry. Based on the deduced amino acid sequences for the cleavage site of the HA genes, all isolates were classified as highly pathogenic except 35/Chakwal-01 and 34668/Pak-95. This suggests that first introduction of H7N3 in 1995 was low pathogenic, then, after circulating for a period of 6-8 months in the poultry population, a highly pathogenic virus emerged.

Pakistan reported its first case of highly pathogenic avian influenza (HPAI) H5N1 in February, 2006 in the North-West Frontier Province, now called Khyber Pakhtunkhwa. By June, 2008, 51 outbreaks were reported to the *Office International des Epizooties* (OIE), 39 on commercial farms and 12 among backyard poultry, pet and wild birds. Out of 120 districts[6], outbreaks occurred and re-occurred in eight districts, namely Karachi, Islamabad, Rawalpindi, Charsada, Swabi, Abbottabad, Peshawar, and Mansehra. Most of the outbreaks (29/51) occurred in winter. Between October and November, 2007, three laboratory-confirmed mortalities also occurred in a family in district Peshawar, possibly with a limited human-to-human transmission. The index case was a veterinarian who had a history of culling H5N1-infected poultry (Anonymous, 2008).

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[6] http://www.infopak.gov.pk/districtPK.aspx (accessed on January , 31, 2012)

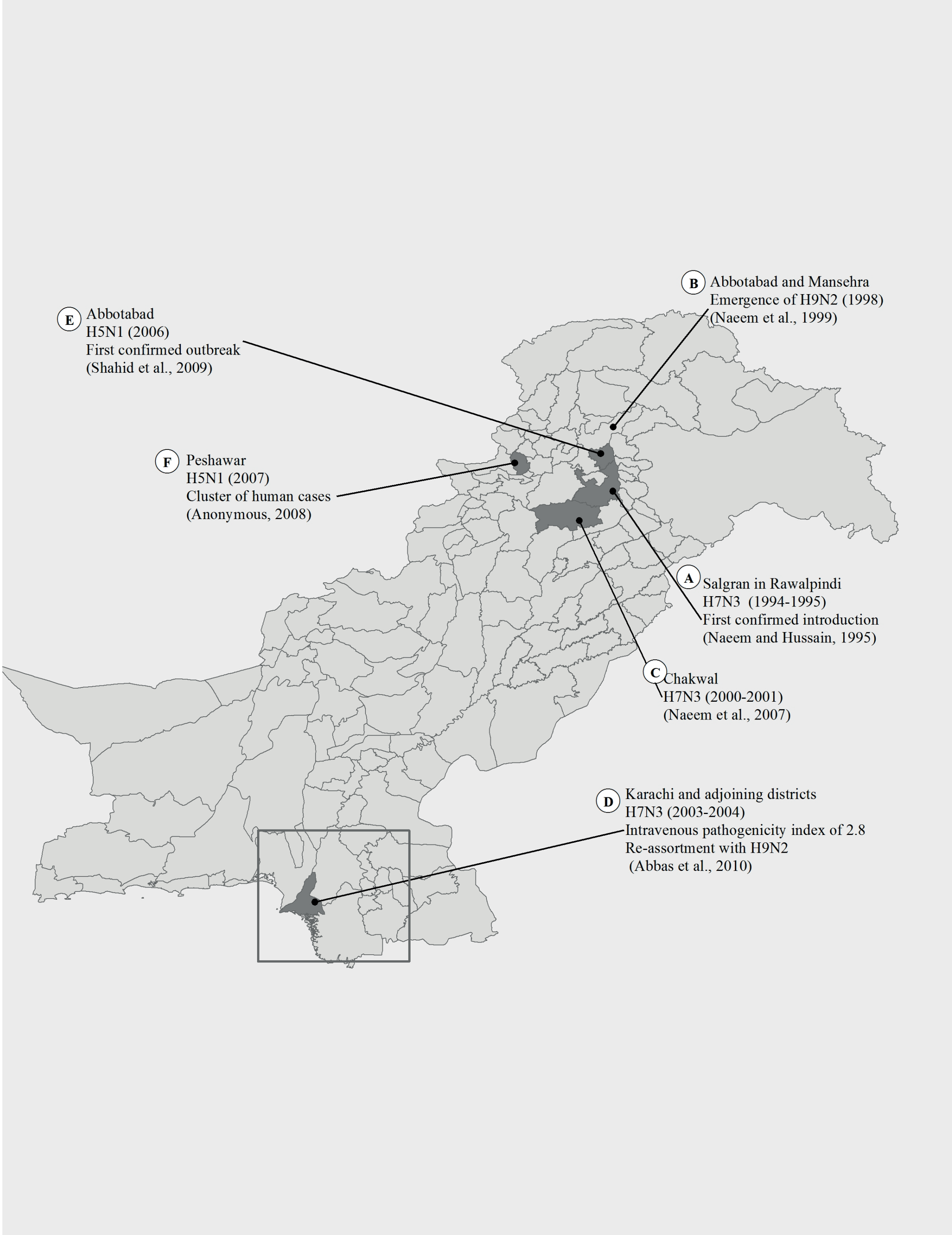

Figure 1.4 Important events in the history of avian influenza of Pakistan

1.5 Disease prevention and control strategy

Considering the socioeconomic and public health impact of AI, the Government of Pakistan (GOP) prepared a national contingency plan. Moreover, a mega project was launched in 2007 under the title "*national programme for the control and prevention of avian influenza (NPCPAI)*." The main objective of the project was to strengthen surveillance, diagnostic capacity, and responsiveness of veterinary services. Under this project, a network of 10 provincial and 40 regional (sub-provincial) laboratories was established. To ensure efficient outbreak management, 66 rapid response units were also set up. In addition, a compensation policy was introduced, to avert the risk of under-reporting by the farmers and sale of infected birds in live bird markets. During its tenure, NPCPAI arranged several workshops to increase disease awareness among various target groups such as farmers, veterinarians etc.

Avian influenza viruses (AIVs), particularly notifiable ones, are one of the biggest risks for Pakistan´s poultry industry as these viruses can disrupt poultry production as evident during the outbreak of 2003-2004. To detect the presence of infection, a comprehensive protocol for sampling and serological testing of commercial flocks, wild resident birds, migratory birds, zoo birds, and backyard poultry has been developed and applied. Preliminary diagnostic work is executed in regional and provincial laboratories whereas confirmatory diagnostic tests are performed in the national reference laboratory for poultry diseases located in Islamabad. In case of confirmation of notifiable avian influenza (NAI), the report is submitted to the animal husbandry commissioner who, after bringing it into the notice of the secretary MINFAL[7], notifies the outbreak nationally and internationally. Soon after receipt of the information on any flock declared positive, the rapid response teams are dispatched to the affected premise for undertaking activities like culling, disinfection, carcass disposal, bio-security measures etc. The surveillance, diagnostic, and flock culling data are stored in the project management unit for analysis, interpretation, and reporting to the concerned authorities. A detailed description of the project is available at www.npcpai.gov.pk.

The HPAI prevention and control policy of Pakistan involves the introduction of environmentally controlled commercial farming, increased biosecurity as well as surveillance. Infection is urgently diagnosed and contained by zoning and selected culling with compensation.

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[7] http://www.npcpai.gov.pk/download.html (accessed on December , 2010)

Strategic vaccination has been adopted preferring autologous vaccines (Usmani, 2010). There is no detailed document on the current situation of AIVs in the country. The following statements have been extracted from an official document [8]

"Since June 2008, no HPAI outbreak in poultry till December 2008 has been recorded. The field surveillance data indicates that poultry reared in areas of Punjab, Khyber Pakhtunkhwa, Sindh Baluchistan, Federally Administered Tribal Areas, and Azad Kashmir sero-converted against the H5, H7, and H9 avian influenza viruses indirectly revealing the circulation of these viruses in flocks reared on commercial basis and those kept as backyard poultry. However, during the period under report, the investigation aimed at isolation of these viruses did not indicate their presence in any of the tissue samples from sick or apparently normal poultry."

1.6 Trade in poultry and poultry products

The following paragraphs provide information on the type of commodities that can be imported according to IMPORT POLICY ORDER 2009 of the Ministry of Commerce, GOP.

"*Poultry and poultry products and other captive live birds (pet/game/wild/exotic/fancy birds) from Vietnam, South Korea, Thailand, Japan, Indonesia, Myanmar, Cambodia, Laos, Taiwan, Hong Kong, Malaysia, South Africa, Russia, Kazakhstan, Mongolia, Turkey, Greece, Romania, Croatia, Iran, Italy Azerbaijan, Ukraine, Iraq, Bulgaria, Slovenia, France, Nigeria, Slovakia, Austria, Bosnia, Herzegovina, Germany, Afghanistan, Scotland-United Kingdom and China on account of avian influenza H5N1 strain, shall not be allowed till further orders. This ban shall however not apply to egg powder from China and to the import of cooked poultry products from South Africa and Malaysia, after certification from designated laboratories in Hong Kong to detect that these are free from avian influenza H5N1 strain:*

Provided that this ban shall also not apply to the import of day-old grandparent stock chicks, day-old parent stock or breeders chicks of layers and broilers and hatching eggs from France, Germany, Iran and United Kingdom subject to certification from the veterinary authorities of the exporting country that these chicks and hatching eggs have not originated from the avian influenza infected zones or areas. Provided further that this ban shall also not apply to the import of fancy/captive/game hobby birds from South Africa subject to certification from their

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[8] http://www.npcpai.gov.pk/download.html

competent veterinary authority that these birds have originated from avian influenza free zones or areas."

According to a notification: S.R.O[9]. 1123 (i) 2010, the order was amended to lift the ban on the import of processed and cooked poultry products from China subject to the certification from competent authority in China to the effect that: *"poultry commodity or product has been processed at not less than 70°C for reasonable time length throughout manufacturing or cooking of the product to ensure the destruction of HPAI H5NI virus and raw material for said processed poultry commodity or product has originated from avian influenza-free zone or area or compartment and processed or cooked in an approved establishment registered with competent authority of exporting country*."

1.7 Rationale and aims of the research

The impact and epidemiology of AI differ widely in different regions of the world. Variation in the opportunity for contact between poultry and wild birds, different biosecurity levels and production systems, and a multitude of other variables all require each region to assess its own specific level of epidemiological risks and to devise prevention, detection, and response strategies accordingly. That is to say, strategies should be devised to suit the specific situation of a country or region as assessed through rigorous epidemiological investigation and a consideration of the epidemiological context. This thesis presents information on possible risk factors for introduction and spread of AI in domestic poultry of Pakistan. The main idea of this research was to design epidemiological tools that may help the veterinary authorities in the control and prevention of the disease. To meet this multi-facet objective, three epidemiological studies were carried out and have been described in detail the relevant chapters of the thesis. The current chapter gives an overview of the poultry sector, brief history of the disease and institutional responses to manage HPAI risk. Published and grey literature, various reports, and official documents were used in the preparation of this section. Chapter 2 provides a review of literature from an epidemiological perspective. In chapter 3, available information on wetlands and waterbird populations has been mapped. Moreover, a framework has been proposed to define priority areas for surveillance during the waterbirds migration season. Chapter 4 introduces the characteristics and contact patterns of small scale broiler and layer farms in a poultry rearing region. In order to generate hypotheses on risk factors for farm-to-farm transmission of infection, a computer-mediated interview of the poultry consultants was also piloted and its findings are given in chapter 5.

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[9] S.R.O. stands for Statutory Regulatory Order

2 Review of literature

2.1 Nature of the disease

Avian influenza is a disease or asymptomatic infection caused by viruses in the family Orthomyxoviridae, genus Influenzavirus A, which contains a genome composed of eight segments of single-stranded negative-sense RNA (Swayne and Halvorson, 2008). Over the last decade, it has emerged one of the most important diseases of the poultry industry around the world (Suarez, 2010). This is because of its ability to cause illness and death in poultry and humans, disrupt poultry trade, threaten the food security of resource-poor countries and the high costs associated with control measures (Lockhart, 2008). The main epidemiological features of avian influenza that contribute to these concerns include the large number of possible virus strains, the presence of a wild bird virus reservoir which represents a constant, uncontrollable source of infection and, the inherent ability of the virus to convert to high virulent strains once it is transmitted to other species as a result of mutation or reassortment. Adding to these complexities, infection with avian influenza viruses produces variable clinical manifestations that are often indistinguishable from endemic poultry diseases (Swayne and Suarez, 2000).

2.2 Virus structure

Influenza A viruses are enveloped with a helical nucleocapsid (Kang et al., 2006). The segmented RNA allows for the easy reassortment. The segments encode at least 10 proteins recognized as: Polymerase Basic (PB1, PB2), Polymerase Acidic (PA), Hemagglutinin (HA), Nucleoprotein (NP), Neuraminidase (NA), Matrix (M1, M2) and Non-structural proteins (NS1, NS2) (Bouvier and Palese, 2008). AIVs can be subtyped by their surface HA and NA glycoproteins, which are major determinants of the pathogenicity, transmission, and adaptation of the virus to other species, but these three traits plus infectivity, are multigenic. The HA is a trimeric rod-shape molecule that binds to the host cell receptor and has a major immunogenic site of the virus. For its full activity, the HA protein must be cleaved into two subunits recognized as HA1 and HA2 subunit molecules (Capua and Alexander, 2007).The HA protein recognizes neuraminic acids on the host cell surface (Yassine et al., 2010). NA is a mushroom-shaped tetramer. Following virus replication, the receptor-destroying enzyme, NA, removes its substrate, sialic acid, from infected cell surfaces so that the newly made viruses are released to infect more cells (Gamblin and Skehel, 2010). So far, 16 HA and 9 NA subtypes have been identified worldwide, making 144 possible combinations between both proteins (Olsen et al., 2006). AIVs have high mutation rates typical of RNA viruses (faulty transcription) resulting in relatively high rates of antigenic drift. In addition, due to their segmented genome (8 segments), genetic reassortment can occur in hosts that are infected with more than one strain, facilitating host adaptation and resulting in high rates of genetic shift.

2.3 Pathotypes

Type A influenza is the only genus of Orthomyxoviridae that has been shown to infect a vast variety of animals including humans, wild and domestic birds, swine, horses, seals, whales, canines, minks and others (Wright et al., 2007). Infection with the influenza A virus results in a wide range of clinical outcomes, depending on virus strain, virus load, host species, host immunity and environmental factors (Yassine et al., 2010). Based on pathogenicity in chickens, influenza A viruses are classified into two main pathotypes: *highly pathogenic avian influenza* (HPAI) and *low pathogenic avian influenza* (LPAI) (Alexander, 2007). Infections with LPAI (include all subtypes) are usually localized, mild to inapparent because the viruses primarily multiply in cells of mucosal surfaces. On the other hand, HPAI viruses infect mucosal surfaces and body systems and cause severe disease with a mortality rate of 75% or greater in infected poultry (Suarez, 2010). LPAI viruses remain in evolutionary stasis in their natural hosts i. e. aquatic wild birds, whereas HPAI may arise unpredictably from their LPAI H5 or H7 progenitors only after transmission to susceptible poultry (Weber and Stilianakis, 2007).

A major molecular determinant for pathogenicity of H5 and H7 viruses is the amino acid sequence specifying the proteolytic-cleavage site of HA. The HA protein of LPAI is characterized by a single arginine (basic amino acid) at the cleavage site and another basic amino acid at position 3 or 4 upstream from the cleavage site (depending on the virus subtype). Thus, the HA protein of LPAI viruses is limited to cleavage by extracellular proteases (trypsin-like) that are secreted by cells or bacteria at the site of infection (e. g. trachea and intestine). On the other hand, HPAI viruses possess multiple basic amino acids at the HA protein cleavage site, making them prone to cleavage by ubiquitous intracellular proteases of the subtilisin type, resulting in severe, systemic infections. In addition, other non-H5/H7 subtypes may also cause serious illness in chickens, but only in combination with other pathogens and factors (Yassine et al., 2010).

2.4 OIE definition for notifiable avian influenza viruses

For the purposes of international trade, avian influenza in its notifiable form (NAI) is defined as [10]an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI):

HPNAI viruses have an IVPI in 6-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in 4-to 8-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other HPNAI isolates, the isolate being tested should be considered as HPNAI.

LPNAI are all influenza A viruses of H5 and H7 subtype that are not HPNAI viruses.

Antibodies to H5 or H7 subtype of NAI virus, which have been detected in poultry and are not a consequence of vaccination, have to be immediately investigated. In the case of isolated serological positive results, NAI infection may be ruled out on the basis of a thorough epidemiological and laboratory investigation that does not demonstrate further evidence of NAI infection.

The following defines the occurrence of infection with NAI virus:

HPNAI virus has been isolated and identified as such or viral RNA specific for HPNAI has been detected in poultry or a product derived from poultry; or

LPNAI virus has been isolated and identified as such or viral RNA specific for LPNAI has been detected in poultry or a product derived from poultry.

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[10] Terrestrial Animal Code, Avian Influenza, Chapter 10.4, Article 10.4.1

2.5 Mechanisms of emergence of highly pathogenic avian influenza viruses

A key event in the genesis of all HPAI viruses is the conversion of a H5 or H7 LPAI virus to a HPAI virus. This has occurred in the past following multiplication of LPAI viruses of these subtypes in chickens, but it is not known whether this is an essential prerequisite (Sims and Narrod, 2009). The emergence of HPAI from LPAI has been proposed to occur by several mechanisms. These include (i) the insertion of basic amino acids at the HA cleavage site, possibly the result of duplication of purine triplets due to a transcription fault of the polymerase complex, (ii) the progressive accumulation of basic amino acids at the cleavage site by a stepwise process involving amino acid substitutions, and (iii) non-homologous recombination resulting in the insertion of a foreign nucleotide sequence adjacent to the HA cleavage site (Pasick et al., 2005).

A HPAI virus has been generated experimentally by repeated passage of a LPAI virus through chickens by air sac and intracerebral inoculation (Ito et al., 2001) but the exact triggers for this change under natural conditions are not known. In some earlier outbreaks of HPAI, it was evident that the change from a LPAI virus to a HPAI virus followed the introduction of LPAI virus to large flocks of commercial poultry. This change apparently occurred within a matter of days in some outbreaks as was the case during the 2004 Canadian outbreak (Bowes et al., 2004). On the other hand, in some Central American countries, low pathogenicity H5N2 strains have circulated in poultry for a number of years without developing into highly pathogenic strains. Even in Mexico, where mutation of a LPAI H5N2 virus to a HPAI virus occurred in 1994 and this HPAI virus strain was subsequently eliminated, H5N2 LPAI viruses continued to circulate (Villarreal, 2006) but did not revert to high pathogenicity.

Conditions that exert selective pressure on circulating viruses at both the host and population level act to increase the rate of mutation of viruses and therefore favor the appearance and establishment of dominant virus strains (Ferguson et al., 2003). Thus, intensive poultry production systems in which a continuous and an easily accessible source of susceptible hosts are present are considered prime conditions under which pathogenicity may emerge. Other cited conditions have been the inadequate use of vaccinations or incomplete vaccination coverage that have allowed field strains to reassort with vaccinal strains (Escorcia et al., 2008). Although it is well accepted that the presence, or absence, of multiple basic amino acids at the HA cleavage site is a key factor in determining virulence, it has been shown that the other 7 genes are also important (Basler and Aguilar, 2008). Studies have shown that the HPAI H5N1 circulating in three continents (from the goose Quandong lineage), carried only the HA gene from its H5N1 Gs/GD/1/96 lineage whilst the remaining 7 genes were acquired from other avian influenza viruses through genetic reassortment (Zhao et al., 2008). This implies that a number of different H5N1 virus strains/clades could potentially co-circulate in a region with possible emergence of new viruses with varying levels of virulence as has been shown in Vietnam (Wan et al., 2008) and Africa (Ducatez et al., 2007).

2.6 The global spread of avian influenza viruses

2.6.1 Wild birds as a natural reservoir

Wild birds in the orders *Anseriformes* and *Charadriiformes* are the natural reservoir for AI viruses. These birds are generally infected asymptomatically, demonstrate no clinical signs or pathological lesions, and shed high concentration of viruses in their faeces (Webster et al., 1978). Worldwide surveillance studies have consistently revealed the occurrence of LPAI viruses in wildfowl, from boreal (Koehler et al., 2008) to tropical latitudes (Gaidet et al., 2007). Phylogenetic relationships and gene reassortment found between AIVs isolated from wildfowl worldwide indicate that intercontinental exchange of viruses via migratory birds does occur (Dugan et al., 2008; Koehler et al., 2008). LPAI viruses with antigenic subtypes H3 and H6, as well as N2, N6, and N8, were the most frequently isolated viruses from wild ducks, whereas H9, H11, and H13 were predominant HA subtypes in shorebirds and gulls (Swayne and Halvorson, 2008).

Prior to 2002, HPAI viruses responsible for severe mortality in domestic birds (i. e. gallinaceous poultry and ostriches) were generally not detected in wild birds (Olsen et al., 2006). The HPAI H5N1 virus that re-emerged in domestic birds in 2002 showed the capacity to infect a large diversity of wild birds, including wildfowl. Since 2002, HPAI H5N1 viruses have been reported in more than 120 species of wild birds, usually found dead or diseased (Liu et al., 2005; Hesterberg et al., 2009). In a few cases however, HPAI H5N1-infection has been found in healthy free-living wildfowl, with no apparent clinical signs (Chen et al., 2006; Saad et al., 2007; Hesterberg et al., 2009), indicating that some healthy carriers may exist in the wild. An increasing number of recent experimental infection studies have revealed that some wild species of ducks, geese and swans can replicate and shed HPAI H5N1 virus asymptomatically for several days without exhibiting any apparent clinical signs or before the onset of illness (Brown et al., 2006b; Brown et al., 2008; Kalthoff et al., 2008; Keawcharoen et al., 2008; Kwon et al., 2010). Although there is heterogeneity amongst species in clinical susceptibility, these findings consistently suggest that some wildfowl could spread HPAI H5N1 virus during a period of asymptomatic infection. For a bird to be a long-distance vector of the disease (i) it must be in contact with the virus, be receptive to infection and shed virus; (ii) infection should be asymptomatic, at least temporally, without hampering bird movements; (iii) it must be able to perform long-distance movements within a timeframe of asymptomatic infection; (iv) timing of asymptomatic infection must coincide with the time when it performs a long distance movement; and (v) it must transmit virus infection to other susceptible hosts through direct contact or a shared environment. There is a potential difference in host response to HPAI H5N1 infection according to species, bird age and virus strain. In addition, the asymptomatic infection duration (AID) was consistent amongst species and strains, ranging on average between 3 and 5 days in 65% of inoculation trials. Environmental constraints, such as adverse climatic conditions, episodic high concentration of birds, resource limitation, predation or hunting pressures, as well as concurrent physiological stress or infections with other pathogens, may increase the impact of HPAI H5N1 virus infection in free-living birds. Prior natural exposure to LPAI viruses may result in partial acquired immunity and could modulate the outcome of an HPAI H5N1 infection. Wildfowl with naturally or experimentally acquired LPAI-specific antibodies showed no or reduced clinical signs and a lower, delayed and shorter period of viral shedding compared to immunologically naive birds. This suggests that pre-existing immunity may increase the proportion of subclinical infections in wildfowl populations. Migratory birds are physiologically well-adapted to demanding long flights, without compromising the capacity of their immune function. Birds were capable of achieving their maximal dispersal distances in a timeframe of 1-4 days, which suggests that wildfowl may disperse the virus over great distances before the effects of infection, if any, would hamper their migration. The delayed effect of infection may impose a longer staging period at a stopover. Finally, for virus dispersal to be effective, it must be shed at a sufficiently high concentration, in a location with appropriate environmental conditions for virus survival, and in a location with suitable density and species assemblages for a successful transmission to another host. Asymptomatically infected birds excrete the virus at lower concentrations than symptomatic birds, although exceptions seem to exist. Several studies have, however, shown that even low concentrations of inoculated virus can produce productive infections in captive wildfowl which subsequently contaminated contact birds Outside the breeding season, wildfowl are generally gregarious, particularly at stopover sites during migration where birds from various species, geographic origins and destinations aggregate in large numbers, offering suitable locations for transmission and dispersion over extensive regions (Gaidet et al., 2010).

2.6.2 Role of trade

Legal and illegal trade of live birds and bird products may play a major role in the spread of AI, even over large distances. During previous epizootics of HPAI of subtypes H5 and H7, it was shown that the expansion of these viruses was due to human activities, in particular, movements of poultry or their products. Although the epidemiology is more complex, the same mechanisms have played a crucial role in the dispersal of the Asian HPAI H5N1 panzootics.

In a recent study, it was attempted to predict the pathways by which H5N1 had and could spread between countries (Kilpatrick et al., 2006). The authors integrated data on phylogenetic relationships of virus isolates, migratory bird movements, and trade in poultry and wild birds to determine the pathway for 52 individual introductory events into various countries, and predict future spread. Assigning relative probabilities to the trade in poultry and wild birds, but also to the natural movements of wild birds, the study suggested that most introductions of H5N1 into Europe probably came through the natural migrations of wild birds, whereas the spread through Asia and Africa involved both migratory birds and the poultry trade. The main indicator used to assess the risk of the poultry trade was the legal trade in live poultry from infected countries, much of which involves day-old chicks (DOC). However, the legal and illegal trade in wild birds was also identified as an important potential pathway, unless all imported birds are quarantined and tested for AI.

The risk of introducing HPAI through trade depends on several factors, including: the ability of the importing country to demonstrate freedom from NAI through adequate surveillance and diagnostics, the types of NAI present (LPAI versus HPAI), the type of products traded and the use of any treatment for virus inactivation (van den Berg, 2009). Swayne and Thomas (2009) have recently proposed a classification from highest to lowest risk, according to the specific product, as follows: (i) live poultry [older than day-old], (ii) live birds other than poultry, (iii) day-old poultry, (iv) hatching eggs, (v) eggs for human consumption ,(vi) egg products; products derived from poultry, such as semen, raw meat and other untreated products , and (vii) products derived from poultry which have been treated to inactivate influenza viruses.

Commercial circuits can also play an important role in the dissemination of AI within a country. In analysis of the environmental factors contributing to the spread of H5N1 in mainland China, Fang et al. (2008) concluded that the transportation of poultry and their products along the highways contributed significantly to the long-distance spread of the disease. When outbreaks of AI occur in new places, analysing the possible route of entry is often complicated by: the limited capacity of some countries to investigate the disease, the scarcity of information on illegal movements of poultry or poultry products, and delays in reporting outbreaks when they first occur. Even when the disease has been recognized early, and full investigations are undertaken, it is often not possible to determine how the virus entered the country and was disseminated to poultry flocks. As a consequence, in many countries, the index case is not the first case of infection. In addition, HPAI can occur in smallholder or village poultry without being diagnosed because mortality in village flocks occurs regularly from other causes, such as Newcastle disease (ND), which is endemic in many parts of the world. ND is particularly endemic in Africa, Asia, where H5N1 is presently entrenched (van den Berg, 2009). These deaths are not always reported and, even if local authorities have been informed, there is no guarantee that all cases will be investigated or that further reporting will occur. This under-reporting is most evident in areas where human cases have occurred in the absence of reported avian infections (Smith et al., 2006). Indeed, sick poultry is often sold by farmers and villagers as soon as it begins to show signs of disease. However, delays in notification can also occur on large commercial chicken farms, even though it is usually difficult to hide such cases for extended periods, because the number of infected birds increases rapidly.

Live bird markets are found in many parts of the world. They serve as a source of poultry meat for local populations who prefer to buy fresh poultry to eat. In Asia, live bird markets were the source of the HPAI H5N1 that was transmitted to 18 people in Hong Kong, and killed six (Sims, 2007). This central role has also been shown in Hanoi, Vietnam, in 2001, where HPAI virus H5N1 was detected in domestic birds in a live bird market (Nguyen et al., 2005), and in China (Webster, 1999). These so-called "wet markets" are widespread in Asian countries and countries to which Asian people have migrated, and they are recognized as important reservoirs of H5N1 viruses, if the markets use a continuous flow system and especially if poultry are allowed to remain for longer than 24 hours on site (Sims, 2007). Mixing different species of domestic poultry (terrestrial poultry and waterfowl) in live bird markets is a common practice in most South Asian countries. This facilitates virus dissemination and evolution. Finally, these markets also represent a perfect interface where domestic and wild-caught birds are kept in close proximity, posing a high risk of cross-contamination. Live bird markets are therefore blamed for maintaining and spreading AIVs and pose major challenges to veterinary and public health authorities. This is exemplified by recurrent findings of HPAI H5N1 in bird markets, such as the Hong Kong market in June 2008, where the first outbreak of H5N1 in five years was declared, despite the implementation of "down periods", when the bird markets are closed, and extensive controls (Kung et al., 2003; Sims, 2007). Similarly, although the last outbreak of H5N1 in Nigeria occurred in October 2007, at least four new incursions were recorded in the markets of four different Nigerian states in July 2008 (Salzberg et al., 2007).

Wild bird trade is a globally important phenomenon which carries the risk of introducing several pathogens including influenza, West Nile virus, and others. The risks associated with these animals are highly variable, depending on whether the animals are captive-bred or wild-caught, and, whether they are moved legally or illegally. Illegally traded animals bypass any testing and quarantine requirements that may be in place, and thus are a significant threat. Indeed, on two separate occasions, illegally traded wildlife with active H5N1 infections entered Europe; however, they were intercepted before mixing with, and potentially spreading the virus to local birds (Vandegrift et al., 2010).

2.7 Important AIVs of veterinary and public health importance

2.7.1 Subtype H7

Several NA combinations with H7 HA subtypes have been reported for LPAI (N1-4, N7, and N9) and HPAI (N1, N3, N4, and N7) viruses in birds and/or humans. Since 1995, infection of poultry with H7 AI viruses has greatly increased, as well as its geographic spread. Outbreaks of H7N1 LPAI occurred in Italy (1999-2001) and in Canada (2000). H7N2 LPAI outbreaks occurred in the Unites States (1996-1998, 2002, 2003, and 2004) and the United Kingdom (2007). H7N3 LPAI outbreaks occurred in Italy (2002-2003), Chile (2002), Canada (2004), the United Kingdom (2006), and Pakistan (2001-2004), whereas H7N7 LPAI outbreaks occurred in Australia (1976, 1979), the United Kingdom (1996), Ireland (1995, 1998), and Germany (2001, 2003). Outbreaks of H7N1 HPAI have occurred in Italy (1999–2000), whereas outbreaks of H7N3 HPAI occurred in the United Kingdom (1963), Australia (1992 and 1994), Pakistan (1995-2004), Chile (2002), and Canada (2004). In Australia, there were outbreaks of H7N4 HPAI (1997) and H7N7 HPAI (1976, 1985, and 1996), but the most severe outbreak of H7N7 HPAI occurred in the Netherlands (2003) (Chemielewski and Swayne, 2010). The H7N7 HPAI virus in the Netherlands resulted in the culling of 30 million birds. Since 1995, at least 75 million heads of poultry (chickens/turkeys) have been culled or depopulated worldwide because of H7 HPAI epizootics (Capua and Alexander, 2004; Belser et al., 2009). For LPAI viruses in the United States, the live poultry market (LPM) system has various frequencies of poultry infected with various LPAI viruses. H7N2 LPAI viruses have been circulating in LPM from 1994 to 2006, whereas H5N2 LPAI viruses have been sporadically isolated from LPM in the northeast United States since 1983 (Suarez et al., 2002; Senne et al., 2003; Senne, 2010). Phylogenetic analysis of H7N2 LPAI isolates from commercial poultry outbreaks in Pennsylvania (1997–1998, and 2001–2002); Virginia, West Virginia, and North Carolina (2002); and in Connecticut (2003) were linked to H7N2 LPAI viruses circulating in the LPM in the northeastern United States (Akey, 2003; Spackman et al., 2003). The fact that H5 and H7 LPAI viruses can mutate to HPAI viruses after circulating in the poultry population (Horimoto et al., 1995; Dusek et al., 2009) has prompted surveillance studies to track the genetic changes of the H5 and H7 subtypes circulating in LPM in the United States over a seven-year period (Horimoto et al., 1995; Spackman et al., 2003). These researchers noted specific substitution changes at the hemagglutinin cleavage site of H7 with the addition of basic amino acids.

2.7.2 Subtype H9N2

Influenza A viruses of the H9N2 subtype have become highly prevalent in poultry in many countries, and although these viruses generally cause only mild to moderate disease, they have been associated with severe morbidity and mortality in poultry as a result of co-infection with other pathogens (Nili and Asasi, 2002; Brown et al., 2006a). Antigenic and genetic analyses of H9N2 viruses isolated during the last two decades indicate that these viruses are extensively evolving and have reassorted with other avian influenza viruses to generate multiple novel genotypes (Li et al., 2003; Xu et al., 2004; Li et al., 2005; Xu et al., 2007a; Xu et al., 2007b). Prior to 1990, H9N2 viruses were mainly detected in avian species in North America and "healthy" ducks during surveillance in Southeast China (Brown et al., 2006a). In 1988, the isolation of a H9N2 virus from Japanese quail in Southern China was the first recorded land-based poultry case of H9N2 in Asia (Perez et al., 2003a; Perez et al., 2003b). By 1997, H9N2 viruses had been isolated in multiple avian species throughout Asia, the Middle East, Europe, and Africa (Naeem et al., 1999; Alexander, 2000; Lee et al., 2000; Perk et al., 2006). Vaccines have been used to control the disease; nevertheless, H9N2 infections appear to have become endemic in commercial poultry in a significant number of Asian countries. A significant proportion of H9N2 field isolates have acquired human virus-like receptor specificity, preferentially binding α2-6 linked sialic acid (SAα2-6) receptors, in contrast to the classic avian virus-like receptor specificity that preferentially binds α2-3 linked sialic acid (SAα2-3) receptors (Matrosovich et al., 2001; Choi et al., 2004; Wan and Perez, 2006). Interestingly, a few of the H9N2 viruses that recognize SAα2-6 receptors have transmitted directly to humans, causing mild flu-like illness and the consequent fear that they may become pandemic (Guo et al., 1999; Peiris et al., 1999; Lin et al., 2000; Butt et al., 2005). In addition, some investigations suggest that H9N2 viruses may have contributed to the genetic and geographic diversity of H5N1 viruses (Guan et al., 2000; Lin et al., 2000).

2.7.3 Subtype H5N1

Many neuraminidase subtypes have been linked with H5 subtypes associated with poultry in both high pathogenic (N1-3 and N8-9) and low pathogenic (N1-9) forms. The largest outbreak of HPAI in the past 50 years has been the HPAI H5N1 epizootic in Asia, Africa, and Europe (1996-present). This epizootic has affected at least 62 countries and has been isolated from infected poultry flocks in Asia, Middle East, Africa, and Europe, as well as, but less frequently, from waterfowl, shorebirds, passerine birds, pigeons, and falcons (Stallknecht and Brown, 2007). HPAI H5N1 virus was first reported in 1996 in China (HPAI virus type strain

A/goose/Guangdong/1/1996) followed by reports of outbreaks in live bird markets of Hong Kong and in humans in 1997, and spread in 2003-2004 through eastern and southeastern Asia, affecting poultry, captive birds, and the human population. Genetic characterization of the Hong Kong viruses revealed that these H5 viruses had the H5 HA gene from A/goose/Guangdong/1/96, the NA gene from H6N1 LPAI virus related to A/teal/HK/W312/97, and the internal genes of H9N2 LPAI virus associated with Japanese quail or H6N1 viruses (Xu et al., 1999; Guan et al., 2002; Kim et al., 2009). Surveillance and phylogenetic analysis revealed that in Hong Kong between 1999 and 2002 there was transmission of HPAI H5N1 virus from domestic poultry to domestic ducks. In general, the ducks were less susceptible to AI virus infection than chickens and remain asymptomatic when infected. However, in 2002 a HPAI H5N1 virus appeared in Hong Kong that infected and killed captive-reared ducks in a wildlife park.

LPAI viruses usually replicate in the intestines of the ducks. However, the HPAI H5N1 virus in ducks produces primary infection with higher titers in the respiratory tract than in the intestinal tract. Recent studies revealed that some species of ducks have high susceptibility to the H5N1 virus. Experimental evidence with wild-type mallard and Muscovy ducks shows that mallards may be more resistant to the H5N1 virus than Muscovy ducks (Hulse-Post et al., 2005; Kim et al., 2009). However, when mallards become immunocompromised due to another infection, the birds have increased susceptibility to H5N1 infection (Ramirez-Nieto et al., 2010). Since appearing in 1996, the HPAI H5N1 virus has changed genetically, by drifting, to have 10 distinguishable clades (clade 0-9) with at least six subclades. A few of these subclades (2.1, 2.2, 2.3, 2.5) have been responsible for the human H5N1 infections and deaths, with clade 2.2 being the most frequently reported in humans in Asia, Europe, Middle East, and Africa (Webster et al., 2007). Other aspects of concern are the transmission of H5N1 from domestic birds to migratory aquatic birds, as occurred in Qinghai Lake, China in 2005 (Chen et al., 2006).

The persistence of H5N2 LPAI virus in the poultry population of Mexico for over a year eventually led to critical mutations that resulted in the 1994 outbreak of H5N2 HPAI. Phylogenetic analysis indicated the H5N2 LPAI virus that circulated in the poultry population in Mexico in 1993 was derived from a North American lineage circulating in migratory aquatic birds, which mutated by adding an insert of two basic amino acids and substitution of a nonbasic with a basic amino acid in the HA cleavage site, and consequently caused an outbreak of HPAI in 1994-1995 (Horimoto et al., 1995; Perdue et al., 1996). Mexico initiated a vaccination program in January 1995, and the last HPAI virus was isolated in June 1995. However, H5N2 LPAI virus has continued to circulate.

2.8 Control and Prevention

Control programs for AI are designed to achieve one of three broad goals or outcomes: (i) prevention, (ii) management, or (iii) eradication (Swayne, 2004; Swayne and Akey, 2005). The individual goal or outcome is achieved through incorporating various essential components including inclusion and exclusion biosecurity practices, diagnostics and surveillance, elimination of infected animals, increasing host resistance, and education of personnel in AI control strategies. The level of incorporation and practice of these five components will determine whether the control strategy will prevent, manage, or eradicate AI. Vaccines can be used as a single tool in a comprehensive strategy by increasing host resistance to AI virus infection and decreasing environmental contamination, but other components are essential in order to achieve success within any of these three goals. Practice of AI vaccination varies around the world with infrequent use of vaccines in the developed world to some developing countries routinely using AI vaccines for control of H9N2 (Asia and Middle East) and H5N2 (Central America) LPAI, and HPAI H5N1 (Asia and Africa). However, vaccines have not been a universal solution in the control of AI in the field. Concerns have been raised about inconsistencies in field protection with quality of some vaccines and inadequate administration. Based on experimental studies and field usage, influenza A virus vaccines can be categorized into four broad technological groups: (i) inactivated whole influenza viruses, (ii) in vitro expressed HA protein, and (iii) in vivo expressed HA protein, and (iv) nucleic acid vaccines (Swayne, 2009).

Using vaccination to reduce the transmission rate might provide an alternative to mass culling, by reducing both the susceptibility of healthy birds and the infectiousness of infected birds. However, incomplete protection at the bird level can cause the silent spread of the virus (Savill et al., 2006). Furthermore, vaccines might provide immunological pressure on the circulating strains, which might engender the emergence of drifted or shifted variants. Therefore, although vaccination programs have been recommended recently, some field evidence indicates that vaccination alone will not achieve eradication. Moreover, if not used appropriately, vaccination might result in the infection becoming endemic (Iwami et al., 2009).

A nationwide vaccination programme of backyard and commercial poultry could not prevent circulation of HPAI H5N1 in Egypt. The virus was detected in 35/3,610 (0.97%) and 27/8,682 (0.31%) of examined commercial poultry farms and 246/816 (30%) and 89/1,723 (5.2%) of backyard flocks in 2007 and 2008, respectively. Positive flocks were identified throughout the year, with the highest frequencies occurring during the winter months. Anti-H5 serum antibody titers in selected commercial poultry ranged from <2 (negative) to 9.6 $\log_2$ when determined in the hemagglutination inhibition test using a H5 antigen (Hafez et al., 2010). Kim et al. (2010) investigated the inefficiency of H5N1 influenza vaccines in Egyptian poultry. They described that the failure of commercially available H5 poultry vaccines in Egypt might be caused in part by the passive transfer of maternal H5N1 antibodies to chicks, inhibiting their immune response to vaccination. They propose that the induction of a protective immune response to H5N1 is suppressed for an extended period in young chickens.

Experimentally, amantadine, an M2 ion channel blocker, has been shown to be effective in reducing mortality in HPAI-infected poultry, but the drug is not approved for food animals, and its use rapidly gives rise to amantadine-resistant viruses. The detection of H5N1 and H9N2 amantadine-resistant AI virus strains in poultry from China was purported to be associated with the addition of amantadine in the feed by some farmers. Currently, anti-influenza A virus drug therapy is not recommended for poultry and such anti-viral drugs should be used only in humans in order to minimize the development of resistant influenza A virus strains. Supportive care and antibiotic treatment have been employed to reduce the effects of concurrent bacterial infections as an aid in recover of poultry and other birds from LPAI (Swayne, 2009).

2.9 Special topics

2.9.1 Likelihood / risk mapping

Miller et al. (2007) identified areas and populations of importance for surveillance of HPAI in the United States. The analysis focused on waterfowl groups thought to be responsible for large-scale movements of HPAI H5N1. Banding and recovery data from dabbling ducks, light geese, dark geese, and swans from 1991 through 2006 (n = 241,619 recoveries) were used to map areas within the mainland United States where higher proportions of migrant waterfowl originating from northeastern Asia, Alaska, and Canada stop or overwinter. In addition, national agricultural statistics service data on the number and size of poultry farms were used in conjunction with the bird banding data to identify areas important for enhanced surveillance in domestic poultry flocks. National wildlife refuges and state wildlife areas were also prioritized. The spatial analysis ranked 483 counties (15% of total) as very high, high, and medium priority for domestic poultry surveillance. Ranking of national wildlife refuge system lands and state wildlife areas identified 9% (918) of national refuge system lands and state wild life areas lands as important to sample. The analysis provided foundation for understanding the geographic distribution and overlap between high risk waterfowl and the commercial poultry.

Snow et al. (2007) developed a methodology for risk-based surveillance for H5N1 avian influenza virus in wild birds in Great Britain (GB). Extensive monitoring data on the 24 wild bird species considered most likely to introduce the virus into GB, and analyses of local poultry populations, were used to develop a risk profile to identify the areas where H5N1 is most likely to enter and spread to commercial poultry. The results indicated that surveillance would be best focused on the areas of Norfolk, Suffolk, Lancashire, Lincolnshire, south-west England and the Welsh borders, with areas of lower priority in Anglesey, southwest Wales, north-east Aberdeenshire, and the Firth of Forth area of Scotland. Those areas had significant poultry populations including a large number of free-range flocks, and a high abundance of the 24 wild bird species.

Grabkowsky (2008) brought about a regional risk assessment for the introduction of avian influenza in poultry producing farms in Europe. The data on poultry production were collected from the statistical departments of 25 European member states. The database described the poultry production in the European Union on different NUTS levels (Nomenclature of territorial units for statistics) for the years 1999-2006. The data were integrated and visualized in Geographical Information System (GIS). Within a pilot study, a risk factor analysis was carried out exemplarily for Lower Saxony in Germany. In this example risk areas were supposed to be regions with a high density of poultry population, regions with a high density of holdings with free range housing systems and a high concentration of wetlands which were often frequented by migratory and wild birds. The result of the analysis presented a map with different risk levels identifying regions with low, medium, and higher risk for virus introduction.

East et al. (2008) conducted a spatial analysis to identify areas of Australia at risk of H5N1 avian influenza infection from exposure to migratory birds. The study compared the distributions of migratory shorebirds and native waterfowl to recognize six regions where the likelihood of exotic HPAI incursion and establishment in native waterfowl was highest. Analysis of bird banding records showed that native waterfowl did not move further than 10 km during the spring breeding season when migratory shorebirds arrived in Australia. Therefore, poultry farms within 10 km of significant shorebird habitats in these six regions of highest comparative risk were identified. The final analysis showed that the estimated risk to Australia was low with only two poultry farms, one at Broome, and one at Carnarvon, located in the regions of highest risk.

Fang et al. (2008) evaluated environmental factors contributing to the spread of H5N1 avian influenza in mainland China. Database including incident dates and locations was developed for 128 confirmed HPAI H5N1 outbreaks in poultry and wild birds, as well as 21 human cases in mainland China during 2004-2006. These data, together with information on wild bird migration, poultry densities, and environmental variables (water bodies, wetlands, transportation routes, main cities, precipitation and elevation), were integrated into a GIS. A case-control design was used to identify environmental factors associated with the incidence of the disease. Multivariate logistic regression analysis indicated that minimal distance to the nearest national highway, annual precipitation and the interaction between minimal distance to the nearest lake and wetland, were important predictive environmental variables for the risk of HPAI. A risk map was constructed based on these factors. The study revealed that environmental factors contribute to the spread of the disease. The risk map was proposed to be used to target countermeasures to stop further spread of the HPAI H5N1 at its source.

Gilbert et al. (2008) mapped the H5N1 risk in Southeast Asia. Statistical association was determined between HPAI H5N1 virus presence and a set of five key environmental variables comprising elevation, human population, chicken numbers, duck numbers, and rice cropping intensity for three synchronous epidemic waves in Thailand and Vietnam. A consistent pattern emerged suggesting risk to be associated with duck abundance, human population, and rice cropping intensity in contrast to a relatively low association with chicken numbers. A statistical risk model based on the second epidemic wave data in Thailand was found to maintain its predictive power when extrapolated to Vietnam, which supported its application to other countries with similar agro-ecological conditions such as Laos or Cambodia.

Glanville et al. (2009) mapped the risk of spread of HPAI H5N1 in Indonesia using multicriteria decision modeling. A range of risk factors for the spread of HPAI H5N1 in Indonesia were identified. Risk factors considered, were: rice paddies, poultry density, main roads, presence of poultry markets, seaports, wetlands and water bodies, diversity of migratory bird populations and density of cultivation production system. The relative importance of the risk factors was established through expert opinion. Presence of rice paddies was rated as the most important risk factor for spread of disease. The importance of wild birds was considered to be low. The risk of disease spread in Indonesia was heterogeneous, with large areas with low potential for the spread of disease (Papua, Kalimantan) interspersed with fewer areas with high to very high potential for spread (Java, Banka-Belitung). The frequency distribution of scores for the country was right skewed, indicating that in general, there is a low potential for the disease to spread. Risk estimates were highly robust; altering parameter weights by 25% had a relatively minor average effect on the overall risk estimate (average change in risk score ranged from 0.46 to 4.19 on the 1 to 255 scale). Limitations of the technique were subjectivity in assigning weights to the risk factors and inability to include all risk factors in the model.

Ward et al. (2009) conducted a study to determine if the occurrence of HPAI H5N1 outbreaks in village poultry in Romania, 2005–2006, was associated with proximity to populations of migratory waterfowl. Reported outbreaks (which could be grouped into three epidemic phases) and migratory waterfowl sites were mapped. The migratory waterfowl site closest to each outbreak was identified. The distances between outbreaks occurring in phase 1 and 2 of the epidemic and the closest migratory waterfowl site were significantly ($P < 0.001$) less than in phase 3, but these distances were only useful in predicting when outbreaks occurred during phase 1 (October–December, 2005) of the epidemic. A spatial lag ($q = 0.408$, $P = 0.041$) model best fit the data, using distance and [distance]*[distance] as predictors ($R^2 = 0.425$). The correlation between when outbreaks were predicted to occur and when they were observed to occur was 0.55 ($P = 0.006$). The results supported the hypothesis that HPAI virus subtype H5N1 infections of village poultry in Romania during the autumn of 2005 might have occurred via exposure to migratory populations of waterfowl.

Pfeiffer et al. (2009) prepared risk maps for HPAI H5N1 in Africa using multi criteria decision modeling. The objectives of multi criteria decision model were (i) to identify areas in Africa with a high likelihood for the introduction of H5N1, (ii) to identify areas in Africa with a high potential for H5N1 to spread, once introduced. The risk factors considered were (i) places where poultry is imported, traded (legally or illegally), produced, and consumed [main roads major markets and major metropolitan areas, ports, airports], (ii) major global flyways for migratory birds (wetlands and irrigated fields) . The relative importance of the risk factors was established through expert opinion. Areas identified as highly vulnerable to introduction were the Nile Delta, the coastline of Northern Africa, Western Africa, and parts of South Africa. Areas identified as having the lowest likelihood included Northern Africa, Somalia, Ethiopia, and Botswana. Most of sub-Saharan Africa was identified as having the highest risk for the spread of H5N1. The regions with the lowest risk of spread included Northern Africa, Somalia, Angola, Namibia, and the south-west parts of South Africa.

Iglesias et al. (2010) assessed environmental characteristics of European Ramsar wetlands that could have contributed as risk factors for HPAI H5N1 in waterbirds (2006-2009). Ramsar wetlands in which H5N1 outbreaks were reported were considered infected (positive) and a case-control study was conducted using a logistic regression model to identify environmental risk factors associated with disease. Forestry (OR = 6.90) and areas important for waterbirds with mixosaline water (OR = 6.31) as well as the distance to the nearest positive wetland (OR = 0.66) which was included into the model to adjust for spatial dependence, were associated with status of the wetlands. The model was used to estimate the risk for HPAI H5N1 on each European Ramsar wetland.

2.9.2 Farm-to-farm transmission

An epidemic of HPAI occurred in the Netherlands in 2003. A survey of 173 infected and 401 uninfected commercial poultry farms was carried out to identify factors associated with the introduction of the HPAI virus into poultry farms. Data on farm size, production characteristics, type of housing, presence of cattle and pigs were gathered by the national inspection service for livestock and meat from all farms included in this study. The available data were analyzed by Thomas et al. (2005) to explore and quantify risk factors for the introduction of HPAI into poultry farms. For each risk factor available for analysis, the Mantel-Haenszel odds ratio was calculated (stratified by farm size and housing type). The investigators found an increased risk of HPAI virus introduction in layer finisher type poultry with an odds ratio (OR) of 2.05 (95% confidence interval [CI] = 1.29-3.27). An explanation for this increased risk was the high number of contacts between these farms, especially via cardboard egg trays used for removal of eggs during the epidemic. The analysis did not indicate significant differences between the infected and uninfected farms with regard to housing type, presence of cattle or pigs.

McQuiston et al. (2005) evaluated risk factors associated with the spread of LPAI H7N2 among commercial poultry farms in western Virginia during an outbreak in 2002. During a case-control study, questionnaires were used to collect information about farm characteristics, biosecurity measures, and husbandry practices on 151 infected premises (128 turkey and 23 chicken farms) and 199 non-infected premises (167 turkey and 32 chicken farms). The most significant risk factor for AI infection was disposal of dead birds by rendering (OR = 7.3). In addition, age ≥ 10 weeks (OR) for birds aged 10 to 19 weeks, 4.9; OR for birds aged ≥ 20 weeks, 4.3) was a significant risk factor regardless of the poultry species involved. Other significant risk factors included the use of nonfamily caretakers and the presence of mammalian wildlife on the farm. Factors that were not significantly associated with infection included the use of various routine biosecurity measures, food and litter sources, types of domestic animals on the premises, and the presence of wild birds on the premises. The results suggested that an important factor contributing to rapid early spread of AI virus infection among commercial poultry farms during the outbreak was the disposal of dead birds via rendering off-farm.

In the year 2005, a serological survey was carried out in response to an outbreak of H5N2 avian influenza in ostriches in the Eastern Cape Province, South Africa. 16.3% of ostrich farms were found seropositive. Thompson et al. (2008) subsequently performed a questionnaire-based census survey on all available registered Western Cape ostrich farms that existed at the end of 2005 (367 farms, of which 82 were seropositive). The purpose of the survey was to identify risk factors associated with farm-level seropositivity. A farm was classified as seropositive if one or more birds tested positive (HI titer >1:16) in the 2005 survey, which had been designed to detect a minimum within-group seroprevalence of 10%. For each farm, risk factor information was collected using a questionnaire administered during a face-to-face interview with each farm owner or manager. Information was obtained on the ostrich population, movements of birds, environmental factors, management practices, and frequency of contact between ostriches and various wild bird species. Multiple logistic regression models were developed for the whole Western Cape Province and for the two largest ostrich farming regions, ‘‘Klein Karoo’’ and ‘‘Southern Cape’’. Seroprevalence differed between regions, being highest in Klein Karoo (31.6%). In all three models, an increased risk of farm-level H5 AI virus seropositivity was associated with increasing numbers of ostriches, excluding chicks, present on the farm. Increased risk of seropositivity was also associated with reduced frequency of cleaning of feed troughs (<1/week vs. >1/week), both overall (OR = 4.5; 95% CI: 1.5, 13.3) and in the Southern Cape (OR = 53.6; 95% CI: 3.3, 864), and with failure to clean and disinfect transport vehicles, both overall (OR = 2.3; 95% CI: 1.1, 4.8) and in Klein Karoo (OR = 2.6; 95% CI: 1.1, 6.5). Increased risk of seropositivity was also associated with increasing frequency of contact of ostriches with certain wild bird species: overall with white storks (*Ciconia ciconia*), in the Southern Cape with gulls (*Larus* spp.), and in Klein Karoo with Egyptian geese (*Alopochen aegyptiaca*).

Kung et al. (2007) used epidemiological evaluation, molecular epidemiology, and a case-control study to identify possible risk factors for the spread of HPAI H5N1 in chicken farms during the first quarter of 2002 in Hong Kong. Farm profiles, including stock sources, farm management, and biosecurity measures, were collected from 16 case and 46 control chicken farms by using a pretested questionnaire and personal interviews. The risk for influenza A (H5N1) infection was assessed by using adjusted odds ratios based on multivariate logistic regression analysis. Retail marketing of live poultry was implicated as the main source of exposure to infection on chicken farms.

Avian influenza outbreaks caused by a low-pathogenic H5N2 virus occurred in Japan from June to December 2005. All 41 affected farms housed layer chickens. Nishiguchi et al. (2007) conducted a case-control study targeting all commercial layer chicken farms within the movement restriction areas in Ibaraki prefecture, where most outbreaks were detected, to investigate the risk factors for the introduction of virus. Four variables were identified as potential risk factors: (i) introduction of end-of-lay chickens (OR = 36.6) (ii) sharing of farm equipment among farms (OR = 29.4) (iii) incomplete hygiene measures of farm visitors on shoes, clothes and hands (OR = 7.0) and (iv) direct distance to the nearest case farm [0-500 m, OR = 8.6; 500-1000 m, OR = 0.8; 1000-1500 m, OR = 20.1; referenced more than 1500 m].

Pfeiffer et al. (2007) described the spatio-temporal pattern of an epidemic of HPAI in Vietnam and identified potential risk factors for the introduction and maintenance of infection within the poultry population. The results indicated that during the time period 2004-early 2006, a sequence of three epidemic waves occurred in Vietnam as distinct spatial and temporal clusters. The risk of outbreak occurrence increased with a greater percentage of rice paddy fields, increasing domestic water bird and chicken density. It increased with reducing distance to higher population density aggregations, and in the third epidemic wave with increasing percentage of aquaculture. The findings indicated that agri-livestock farming systems involving domestic waterbirds and rice production in river delta areas are important for the maintenance and spread of infection.

Hamilton et al. (2009) assessed the vulnerability of the Australian poultry industries to large outbreaks of HPAI. Data on 1,594 commercial Australian chicken meat, chicken egg, duck, and turkey farms were collected by a telephone questionnaire of farm managers. The risk factors considered, were: high densities of poultry farms, frequent contacts between farms by service providers, the supply of live poultry markets (LPM) and the presence of free-range duck flocks. The survey revealed that five regions of Australia had farm densities comparable to overseas regions that experienced widespread HPAI. Common service providers routinely contacted different classes and types of farms over wide geographic areas. However, no responding farms supplied LPM and the majority of duck farms did not produce free-range ducks. It was concluded that outbreaks of HPAI have the potential to cause serious impacts on the Australian poultry industry. The risk posed by LPM and free-range ducks is limited, but the movement of genetic stock and common service providers could spread infection between companies, industries, or geographical regions.

A matched case-control study was carried out by Biswas et al. (2009) to identify risk factors for HPAI H5N1 infection in commercial chickens in Bangladesh. A total of 33 commercial farms diagnosed with H5N1 before September 9, 2007, were enrolled as cases, and 99 geographically matched unaffected farms were enrolled as control farms. Farm level data were collected using a pretested questionnaire, and analyzed by matched-pair analysis and multivariate conditional logistic regression. Two factors independently and positively associated with H5N1 infection remained in the final model. They were (i) farm accessible to feral and wild animals (OR=5·71, 95 % CI 1·81 to 18·0, P=0·003) (ii) footbath at entry to farm/shed (OR = 4·93, 95% CI 1·61 to 15·1, P=0·005). The use of a designated vehicle for sending eggs to a vendor or market appeared to be a protective factor (OR=0·14, 95% CI 0·02 to 0·88, P=0·036).

After 11 consecutive months of control, the Mekong Delta in Vietnam experienced a wave of HPAI H5N1 outbreaks on small holder poultry farms from December 2006 to January 2007. Henning et al. (2009) conducted a retrospective matched case-control study to investigate farm- and flock-level risk factors for outbreak occurrence during this period. Twenty-two case farms were selected from those where clinical signs consistent with HPAI H5N1 had been present and HPAI H5N1 had been confirmed with a positive real-time PCR test from samples obtained from affected birds. For every case farm enrolled, two control farms were selected which matched on the time of outbreak occurrence, farm location and species. Veterinarians conducted interviews with farmers, to collect information on household demographics, farm characteristics, husbandry practices, trading practices, poultry health, vaccination, and biosecurity. Exact stratified logistic regression models were used to assess putative risk factors associated with a flock having or not having a HPAI outbreak. Nested analyses were also performed, restricted to subsets of farms using scavenging, confinement, or supplementary feeding practices. Risk of an outbreak of HPAI H5N1 was increased in flocks that had received no vaccination (OR=20.2; 95% CI: 1.0, + infinity) or only one vaccination (OR = 85.2, 95% CI: 6.5, + infinity) of flocks compared to two vaccinations, and in flocks on farms that had family and friends visiting (OR = 8.2; 95% CI: 1.0, +infinity) and geese present (OR = 11.5; 95% CI: 1.1, + infinity). The subset analysis using only flocks that scavenged showed that sharing of scavenging areas with flocks from other farms was associated with increased risk of an outbreak (OR = 10.9; 95% CI: 1.4, 492.9). The authors concluded that none or only one vaccination, visitors to farms, the presence of geese on farms and sharing of scavenging areas with ducks from other farms increased the risk of HPAI H5N1 outbreaks in poultry flocks in Vietnam.

Models of between-farm transmission of pathogens have identified service vehicles and social groups as risk factors mediating the spread of infection. Because of high levels of economic organization in much of the poultry industry, Leibler et al. (2010) examined the importance of company affiliation, as distinct from social contacts, in a model of the potential spread of avian influenza among broiler poultry farms in a poultry-dense region in the United States. They obtained data on the nature and frequency of business and social contacts through a national survey of broiler poultry growers. Daily rates of contact were estimated using Monte Carlo analysis. Stochastic modeling techniques were used to estimate the exposure risk posed by a single infectious farm to other farms in the region and relative risk of exposure for farms under different scenarios. The mean daily rate of vehicular contact was 0.82 vehicles per day. The magnitude of exposure risk ranged from <1% to 25% under varying parameters. Risk of between-farm transmission was largely driven by company affiliation, with farms in the same company group as the index farm facing as much as a 5-fold increase in risk compared to farms contracted with different companies. Employment of part-time workers contributed to significant increases in risk in most scenarios, notably for farms who hired day-laborers. Social visits were significantly less important in determining risk.

Zheng et al. (2010) carried out a cross-sectional survey of influenza A infection and management practices in small rural backyard poultry flocks in New Zealand. During August-October 2006 a questionnaire was sent to 105 farms in the Bay of Plenty and Wairarapa with poultry flocks comprising fewer than 50 chickens, located near wetlands where AI virus had been detected previously in wild ducks. Information was collected on the number and species of poultry reared, opportunities for interaction between wild birds and poultry, farm biosecurity measures, and health status of poultry. Between September and November 2006, blood and tracheal/cloacal swabs were collected from poultry on a subset of 12 high-risk farms in each location. Influenza A-specific antibodies in sera were assayed using ELISA, and positive sera were further tested for the presence of H5 and H7 subtype-specific antibodies, using haemagglutination inhibition (HI) assay. The presence of influenza A virus in swabs was detected using real-time reverse transcriptase-PCR (RRT-PCR). Completed questionnaires were received from 54 farms. Overall, 80% had only chickens, 13% chickens and ducks, and 7% had chickens and other galliform species. Nearly all (96%) kept backyard chickens for personal consumption of eggs, with a small proportion (19%) preparing birds for the table. On surveyed farms wild waterfowl were seen on pastures (70%) and/or farm waterways (46%). Waterfowl were recorded as visiting areas where domestic birds were kept on 31% of farms. Bird litter and manure were composted (94%) or buried (6%) on-farm, as were most (82%) dead birds. During the targeted cross-sectional survey of 24 farms, clinical disease was not recorded in any poultry flock. Of 309 chicken sera tested, 11 (3.6%) from five farms across both regions tested positive for influenza A antibodies. In contrast, 16/54 (30%) duck sera from three farms in the Wairarapa were positive. Avian influenza H5 and H7 subtype-specific antibodies were excluded in ELISA positive sera using the HI testing, and influenza A virus was not detected using RRT-PCR. The study confirmed that small backyard poultry flocks located near waterfowl habitats were exposed to non-notifiable low-pathogenic AI viruses. Findings indicated a number of potential risk pathways for the transmission of AI viruses between wild birds and non-commercial poultry, and hence the need for continued surveillance for AI in backyard flocks and wild birds in New Zealand.

2.9.3 Possibility of short distance windborne spread

An outbreak of AI occurred during March-April 2004 in the Abbotsford area of British Columbia. There was a need to determine if infectious viral particles were being spread via an aerosol route. It was anticipated that such particles, should they be found, would occur as rare events. By coincidence, Defense Research and Development Canada (DRDC), Suffield, has been developing aerosol samplers that can be used to detect such rare events. Schofield et al. (2005) deployed two sampling systems, consisting of a slit sampler array and a large column air sampler (XMX virtual impactor). The sampling scheme was designed to capture four specific events requested by the Canadian Food Inspection Agency (CFIA). These were (i) up and down wind sampling of an acutely infected barn prior to euthanasia, (ii) up and down wind sampling of a barn being euthanized to capture the displacement effect of carbon dioxide pushing air and possibly virus out the barn, (iii) the effect of clearing the barn of carbon dioxide by starting up the ventilation fans after euthanasia, (iv) the possible re-aerosolization of virus from a barn being actively composted or depopulated by sampling the up and down wind positions. In addition to these specific events, XMX samples were taken in 10 random positions in and around Abbotsford. The first two were in acutely infected barns with high morbidity/mortality while the remaining eight were randomly taken in the outbreak area. Liquid samples from an XMX device were analyzed using PCR as the initial method of identification followed by virus culture. Quantification was to be carried out by plaque assay. The results showed that all slit samples were PCR negative while three of the XMX samples were unmistakable positives. One sample collected from 250 m southeast of the CFIA emergency operation centre (EOC) in Abbotsford was also positive. Estimated viral load yielded a value of 292 viral doses/m^3 of barn air.

Mannelli et al. (2006) evaluated the effects of risk factors and control policies following the HPAI epidemic that struck northern Italy's poultry industry in the winter of 1999-2000. The epidemic was caused by influenza A virus of the H7N1 subtype, which originated from a low-pathogenic AI virus which spread among poultry farms in northeastern Italy in 1999 and eventually became virulent by mutation. Most infected premises (IP) were located in the regions of Lombardy and Veneto (382 out of 413, 92.5%), and the eradication measures provided for in the European legislation were enforced. In Veneto, where flock density was highest, infection-control was also accomplished by means of depopulation of susceptible flocks through a ban on restocking and pre-emptive slaughter of flocks that were in the vicinities of or that had dangerous contacts with IPs. In Lombardy, such control measures were applied to a lesser extent. The infection incidence rate (IR) was 2.6 cases per 1000 flocks per day in Lombardy and 1.1 in Veneto. After the implementation of infection-control measures, the at-risk population, the percentage of flocks < or =1.5 km from IPs, and the HPAI-IR underwent a greater reduction in Veneto than in Lombardy. Although the proximity (< or = 1.5 km) to IPs in the temporal risk window was a major risk factor for HPAI at the individual flock level, its effect at the population level (population-attributable fraction) did not exceed 31.3%. Viral transmission therefore also occurred among relatively distant flocks. Turkey flocks were characterized by greater IR of HPAI compared with other bird species such as layer hens, broilers, gamebirds, and waterfowl, even when located at distances >1.5 km from IPs. In Lombardy, IR for species other than turkeys was also relatively high.

Using the guinea pig as a model host, Lowen et al. (2007) showed that aerosol spread of influenza virus is dependent upon both ambient relative humidity and temperature. Twenty experiments performed at relative humidities from 20% to 80% and 5°C, 20°C, or 30°C indicated that both cold and dry conditions favor transmission. The relationship between transmission via aerosols and relative humidity at 20°C was similar to that previously reported for the stability of influenza viruses (except at high relative humidity, 80%), implying that the effects of humidity act largely at the level of the virus particle. For infected guinea pigs housed at 5°C the duration of peak shedding was approximately 40 hours longer than that of animals housed at 20°C; this increased shedding likely accounted for the enhanced transmission seen at 5°C. To investigate the mechanism permitting prolonged viral growth, expression levels in the upper respiratory tract of several innate immune mediators were determined. Innate responses proved were comparable between animals housed at 5°C and 20°C, suggesting that cold temperature (5°C) did not impair the innate immune response. Although the seasonal epidemiology of influenza is well characterized, the underlying reasons for outbreaks occurring predominantly during wintertime are not clear. The authors provided direct, experimental evidence to support the role of weather conditions in the dynamics of influenza and thereby addressed a long-standing question fundamental to the understanding of influenza epidemiology and evolution.

In 1999-2000, Italian poultry production was disrupted by an H7N1 virus subtype epidemic of highly pathogenic avian influenza (HPAI). Busani et al. (2009) studied risk factors for infection on poultry farms located in regions that had the highest number of outbreaks (Veneto and Lombardia) and the impact of pre-emptive culling as a complementary measure for eradicating infection. A Cox regression model that included spatial factors, such as the G index, was used. The results confirmed the relationship between risk of infection and poultry species, production type and size of farms. The effectiveness of pre-emptive culling was confirmed. An increased risk of infection was observed for poultry farms located near an infected farm and those at altitudes less than 150 m above sea level. It was suggested that the measures for the control and eradication of infection need to consider species differences in susceptibility, the types of production and the density of poultry farms in the affected areas.

Sedlmaier et al. (2009) conducted a risk assessment to evaluate the possibility of windborne spread of avian influenza between neighboring farms. As a model for aerosol transmission, chicken feces were spiked with AI virus of subtype H10N7 and used to generate a fine particulate matter aerosol. For this purpose, an innovative aerosol chamber was developed, that collected $PM_{2.5}$ on quartz microfiber filters. Virus contaminated fecal $PM_{2.5}$ deposited on filters was exposed to various combinations of incubation periods (0, 15, 48. 96 h), temperature (-70, -20, +4, +20, +37 and +50˚C) and humidity (water saturated, normal atmosphere and exsiccated dry). ‘‘Water-saturated’’ means that dust-coated filters were exposed to water-saturated air [relative humidity (RH) 60%]; ‘‘normal atmosphere’’ means that dust coated filters were exposed to unchanged indoor air (RH 30%) and ‘‘exsiccated dry’’ means that dust-coated filters were exposed in silica gel dried air in an airtight jar (RH 19%). Embryonic death in inoculated hen's eggs with filter elute was the virus infectivity read out. The virus remained remarkably stable for a period of even 4 days at +20˚C exsiccated dry air and under normal indoor humidity conditions. The filter elutes contained viral genome as well as viable virus whereby +20˚C indicated a borderline temperature for infectious virus stability. In addition, high relative humidity was critical for the viability of virus in $PM_{2.5}$. It was concluded that under suitable weather conditions, virus laden dust particles can expose susceptible poultry in nearby farms.

3 Priority areas for surveillance and prevention of avian influenza during the waterbird migration season in Pakistan

3.1 Introduction

Wetlands are aggregation sites for migratory and resident wild birds and therefore constitute potentially higher risk areas for the introduction and transmission of avian influenza viruses (AIVs) (Hlinak et al., 2006; Jourdain et al., 2007; Martinez et al., 2009; Iglesias et al., 2010). Wetland habitats, both natural and man-made, cover approximately 7,800 km^2 of Pakistan which is 9.7% of the total area of the country (Sheikh and Kashif, 2006). Natural wetlands, whether permanent or seasonal exist as peat lands, rivers, stream, lake marshes, estuaries, mudflats, and inter-tidal areas whereas lakes, canals, dams, and lagoons being part of Pakistan's extensive Indus basin irrigation system are classified as man-made wetlands. These occur in a broad variety of ecological zones including arid, semi-arid, alpine, and coastal areas (Ahmed and Ishaque, 2011). From the northern mountains to the southern coast, wetland areas provide wintering grounds for a large number of waterbirds coming from Siberia and central Asian states. The arrival of these birds starts in early September and continues until the end of October or mid-November. The birds follow Indus flyway to reach various wetlands distributed all over the country. They choose the wetlands which suit them with respect to their number and time of arrival. After spending their winter in relatively warm wetlands, these birds migrate back to their breeding habitats during February to March. Their breeding activity continues throughout summer and the southward migration starts in autumn when temperature falls and food availability becomes difficult. This migration cycle is continuous since generations (Malik, 2010).

Aquatic wild birds are the natural reservoir of AIVs. These birds are generally infected asymptomatically, demonstrate no clinical signs or pathological lesions, and shed high concentration of viruses in their faeces. Prior to 2002, HPAI H5N1 viruses responsible for severe mortality in domestic birds were generally not detected in wild birds. Since 2002, these viruses have been reported in more than 120 species of wild birds usually found dead or diseased (Liu et al., 2005; Hesterberg et al., 2009). In a few cases, however, H5N1-infection has been found in healthy free-living wildfowl with no apparent clinical signs (Chen et al., 2006; Saad et al., 2007). Experimental infection studies reveal that some wild ducks, geese, and swans shed this virus asymptomatically and hence have the potential to spread it as they move (Gaidet et al., 2010). The exact role of migratory wild birds in the epidemiology of H5N1 and other AIVs in Pakistan is still unknown. Also there is not much information on the routes of virus transmission between wild birds and domestic poultry. Backyard poultry are usually free-range and therefore more vulnerable to contact wild birds or their carcasses while foraging in the fields. In rural households the drinkers and feeders of chickens are readily accessible to wild birds. A recent study has serologically confirmed the presence of AIVs of subtype H9 and H5 in backyard poultry in Lahore (Chaudhry et al., 2010). The spread of these viruses was attributed to low biosecurity or mixing of wild and migratory birds with backyard poultry. Possible mechanisms by which virus from infected waterfowl may enter a commercial poultry holding could be through the use of untreated surface water, entry of bridge species into poultry sheds, or access of wild birds to drinking water, feed or bedding material. The interface between wild birds and poultry is a critical factor that can be controlled through biosecurity measures. Creating awareness and the involvement of farmers in the surveillance and reporting of unusual bird mortalities should enhance early detection of the virus. The objective of this study was to evaluate and map available information on wetlands and waterbird populations of the country. In addition, a framework has been provided to define priority areas for surveillance and prevention AIVs during the migratory season.

3.2 Materials and Methods

3.2.1 Description of the Datasets

The Asian waterbird census (AWC) is a regional programme to promote public participation to monitor the distribution and populations of waterbirds and status of wetlands. Since 1987, the AWC covers the region of Asia, from Afghanistan eastwards to Japan, Southeast Asia, and Australasia. AWC runs parallel to other international census of waterbirds in Africa, Europe, West Asia, and the Neotropics under the umbrella of the International waterbird census. Through a large network of volunteers working through national coordinators, the census is normally organized on the second and third weeks of January. The AWC sites represent all types of natural and man-made wetlands, including rivers, lakes, reservoirs, ponds, freshwater swamps, mangroves and tidal mudflats, coral reefs, rice fields, sewage farms, etc. that are covered by the Ramsar convention. All waterbird groups encountered in the region as recognized by the Ramsar convention are covered by the census. Pakistan has participated in the AWC since 1987. During 1987-1991, and from 2006 onwards, the census was carried out by the national council for the conservation of wildlife. Other organizations involved in the coordination at the national and provincial level included the zoological survey department (1987-2001), the Sindh wildlife management board (1988-2004), and the Punjab wildlife department (1989-2004) (Li et al., 2009).

The AWC data relevant to Pakistan were obtained from Wetlands International (www.wetlands.org). The data contained the local names of 535 sites and for each site, the annual count of waterbirds from 1987-2007. The excel file also contained a spreadsheet providing a list of 128 species and their yearly count at country level.

The poultry density raster used in the study was derived from the "Gridded Livestock of the World "database, which is freely available on FAO website (www.fao.org/geonetwork). The map represents the predicted poultry density at a resolution of 3 minutes of arc (approximately 5 km). Each pixel contains an estimated value for the number of poultry per km$^{2.}$ The layer was developed by Robinson et al.(2007). In short, available national agriculture statistics [11]on livestock populations were converted into densities and adjusted to account for the area of land deemed suitable for livestock production based on environmental, land-cover and land-use criteria. For example, deserts, lakes and high mountains are unsuitable for either arable or livestock production. Cultivation and animal husbandry are also not usually allowed in national parks or game reserves. Robust statistical relationships between livestock densities and predictor variables were established. The modeling approach predicted livestock densities in areas for which no livestock data were available. Because predicted densities were produced at the resolution of the raster imagery, the models generated heterogeneous densities within polygons that had only one single observed value, thus disaggregating the original data. Since the predictors of animal density are unlikely to be consistent from region to region, therefore, models were developed separately for different geographical regions and ecological zones. A part of this study involved use of shuttle radar topography mission (SRTM) water body (vector shapefile)

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[11] For Pakistan , the input data source was poultry population according to agriculture statistics of 2009

data representing lakes and rivers. These data were extracted from DVD of ESRI data and maps (2004). Districts are the second order administrative divisions of Pakistan. For analyses and interpretation of the findings, the shapefile of districts was downloaded from www. pakresponse.info, a website dealing with relief activities after flooding and other natural disasters in the country.

3.2.2 Data analyses

The AWC data were managed in IBM® SPSS 19 (SPSS Inc., Chicago, IL, USA). The mapping was done in ArcGIS 10 (ESRI Inc., Redlands, CA, USA). Screenshots of various operations completed in these two softwares are given in appendix A. The majority of the sites were not counted regularly leading to gaps in the sites-by-years data matrix (here called "missing values"). At first, the patterns of missing values in the data were analyzed. Moreover, Little's MCAR test was run to assess whether values were missing completely at random. For 239 sites, information about the coordinates was not available. Those sites were excluded from further analyses. For each site, the north, and east coordinates were separated as they were provided in the data as a single string. The coordinates were transformed into decimal degree format using the formula

$$Decimal\ value = Degrees\ + \left({}^{Minutes}\!/_{60}\right) + \left({}^{Seconds}\!/_{3600}\right)$$

The subset of data (535-239 = 296 points/sites) was displayed by XY coordinates. Sites with coordinates falling outside the boundaries of the country were also excluded (n = 21). For creating the map of maximum reported count, the sites were divided into five classes using natural breaks (Jenks) classification (Jenks and Caspall, 1971). The natural breaks classification algorithm was used because the data were skewed and values were distributed in multiple clusters. This ensured internal homogeneity with-in classes while maintaining heterogeneity among the classes. The same classification scheme was used for reclassifying the poultry density layer. The reclassification process transformed raw cell values into values from 0 to 5 in order of low to high density.

It may be interesting for the national coordinators to identify regions (sub-national) where sites with high missing values tend to cluster. To locate such areas, Anselin's local Moran's I statistic was applied using the cluster and outliers analysis tool in the spatial statistics toolbox. Anselin's local Moran's I statistic was first described in 1995 (Anselin, 1995). The purpose of this technique is to identify clusters of features with values similar in magnitude and to identify outliers by comparison to neighboring features and the mean of the entire population (Sugumaran et al., 2009). As projected data is required for the ArcGIS spatial statistics tools, the analysis was carried out with a projected version of the data. The projection used was Asia South Lambert Conformal Conic (Cima and Urbano, 2010). The variable of interest was the number of missing values per site. A fixed elucidation distance band of 178 km was selected to ensure that each site has at least one neighbor. In order to alleviate the effect of unequal numbers of neighbors, row standardization was also applied.

For surveillance and prevention of H5N1 and other AIVs during the migratory season, priority may be given to the poultry rearing areas closer to the wetlands. To map such areas, buffer zones of 3, 6, and 9 km radius were created around the SRTM waterbodies. For each category of buffer zones (i) the overlapping polygons were dissolved, (ii) the output layer was spatially joined to the AWC data, (iii) polygons containing AWC coordinates (type A) were separated, (iv) circular polygons (type B) of the size same as buffer were drawn around the coordinates falling out of the defined buffer range. Again, the overlapping polygons were dissolved, (vi) “A” and “B” types of polygons were combined through “union “followed by “dissolve”, and (vii) the final output layer was used as a mask to extract (detect) the pixels of predicted poultry density (Figure 3.1).

The list of wild bird species obtained from Wetlands International was compared with the list of H5N1 affected species from the U. S. Geological Survey[12]. Moreover, a retrospective case-series analysis was performed. Outbreaks reported to OIE during year 2006 to 2008 were used as cases. The first reported outbreak in a given area was treated as the index case and included in the study. Outbreaks occurring within 3 km radius of the index case in a temporal window of 21 days after detection of the index case were considered as secondary outbreaks and excluded. The proportion of outbreaks between during the migratory and the non-migratory period was compared using the one-way chi-square test.

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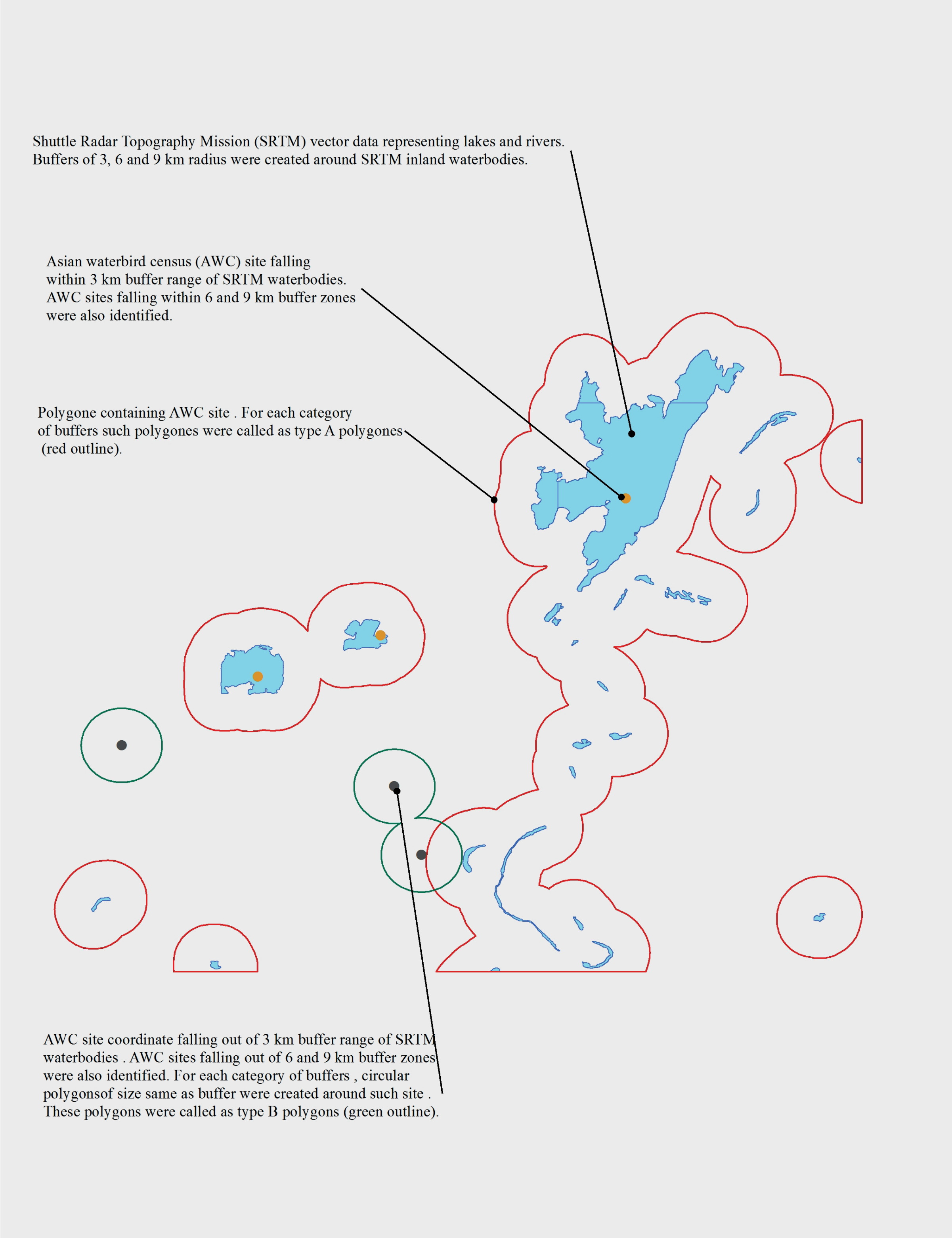
[12] http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp



Shuttle Radar Topography Mission (SRTM) vector data representing lakes and rivers.
Buffers of 3, 6 and 9 km radius were created around SRTM inland waterbodies.

Asian waterbird census (AWC) site falling
within 3 km buffer range of SRTM waterbodies.
AWC sites falling within 6 and 9 km buffer zones
were also identified.

Polygone containing AWC site . For each category
of buffers such polygones were called as type A polygones
(red outline).

AWC site coordinate falling out of 3 km buffer range of SRTM
waterbodies . AWC sites falling out of 6 and 9 km buffer zones
were also identified. For each category of buffers , circular
polygonsof size same as buffer were created around such site .
These polygons were called as type B polygons (green outline).

Figure 3.1 Geo-processing of AWC and SRTM data

3.3 Results

The AWC data contained a substantial amount of missing values. Of 535*21 site-year combinations, 1730 were observed (15%) and 9505 (85%) were missing. The pattern of missing values was completely at random (Little's MCAR test: Chi-square = 911.97, DF = 920, Sig. = 0.57) and monotone. In Figure 3.2, the years have been ordered from left to right in increasing order of missing values. A dataset can potentially have 2^n patterns of missing values where n is the number of variables (SPSS, 2010). In the data under investigation, the number of variables (i. e. years) was 21, and 151 patterns were observed. The most frequent pattern was 151 which represents the sites for which a count was reported only once during 1993.

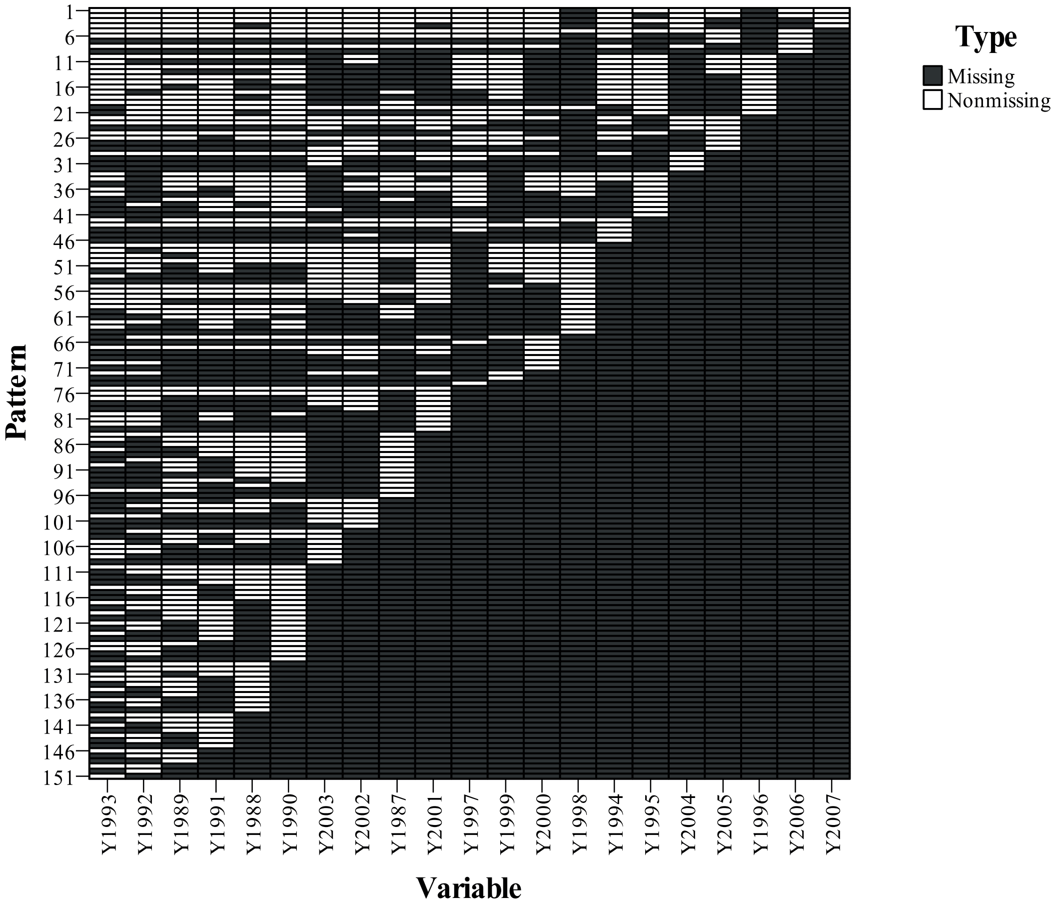


Figure 3.2 Patterns of missing values in Asian waterbird census data relevant to Pakistan (1987-2007)

The results of the analysis of missing values using local Morans I statistics are shown in Figure 3.3. The map illustrates statistically significant (0.05 level) clusters of high values (HH), clusters of low values (LL), outliers in which a high value is surrounded primarily by low values (HL), and outliers in which a low value is surrounded primarily by high values (LH). HH clusters and most LH outliers (except one) were found in Sindh province. Clusters of sites with low missing values (LL) and HL type outliers were detected throughout the country, albeit with low frequency. A total of 81 sites were classified as HH, whereas the number of sites included in the LL category of clusters was 25. Among the spatial outliers, 10 were of HL and 12 of LH type. The mean number of missing values of sites categorized as HH and LL was 19.28 and 7.68, respectively. The average of missing values of outliers was also computed. It was 19.90 for the HL and 10.33 for the LH category.

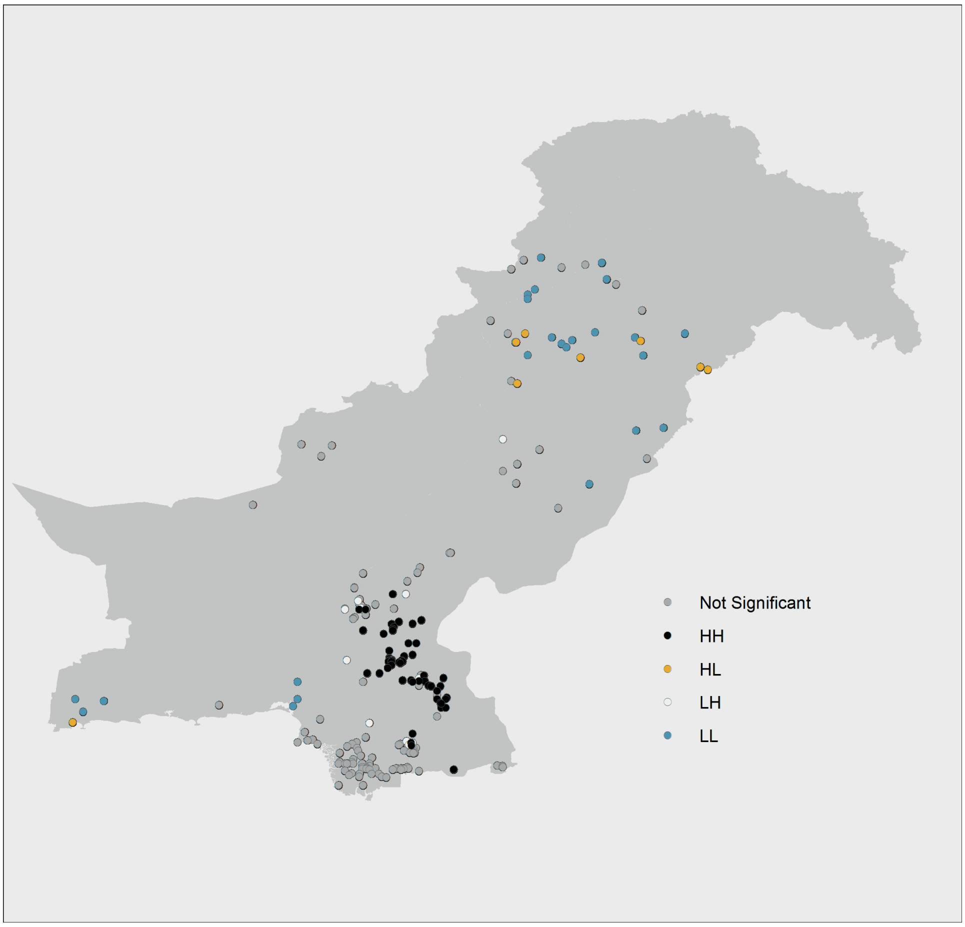


Figure 3.3 Spatial clusters and outliers of missing values revealed by local Moran's I method

The AWC sites (coordinates) were found in 58 districts. The districts containing higher numbers of coordinates were Badin, Thatta, and Sanghar, all of which are in Sindh. The coordinates are assumed to provide a crude approximation of the distribution of waterbirds during the migratory season (Figure 3.4).

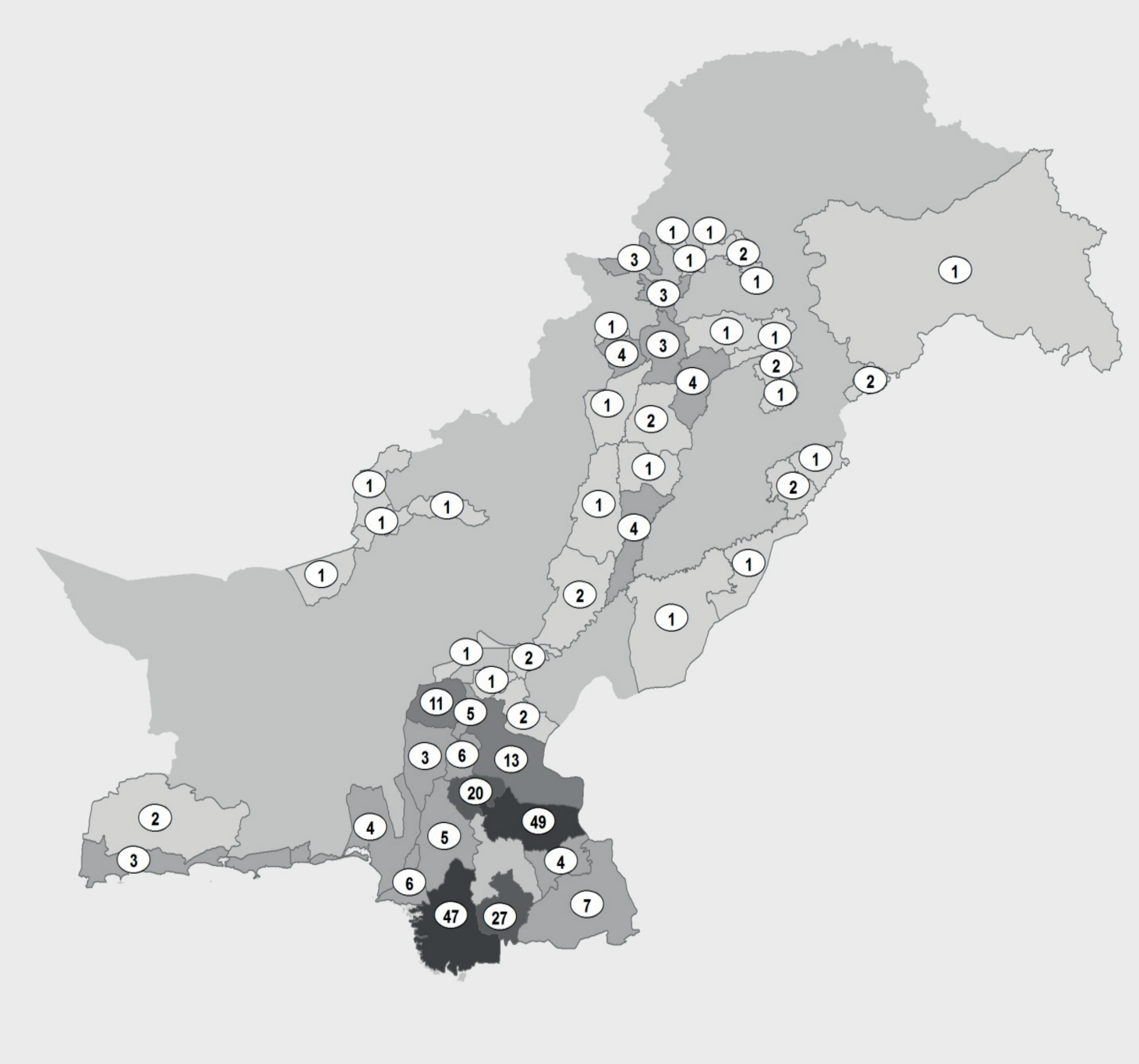


Figure 3.4 Number of Asian waterbird census sites (coordinates) in various districts. The map was created using data from 270 (out of 535) sites. Those were the sites whose coordinates were known and completely fell into the administrative boundary of Pakistan.

There were 42 sites where the count was ≥ 20,000. The coordinates of these sites were situated in Thatta, Shahdad Kot, Sanghar, Okara, Mushki, Mianwali, Layyah, Larkana, Lakki Marwat, Khairpur, Kashmore, Karachi, Jhelum, Jamshoro, and Badin (Figure 3.5).

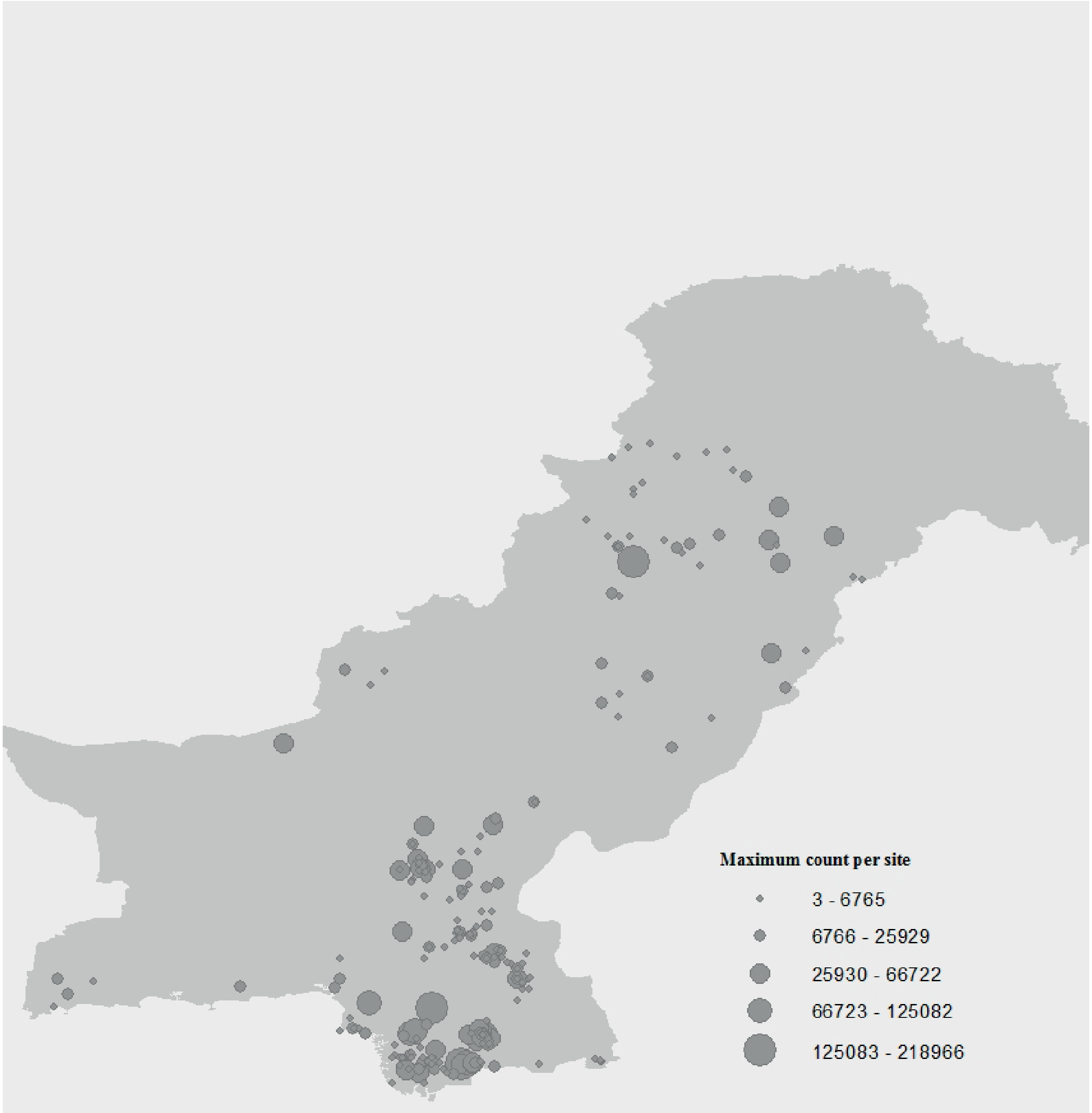
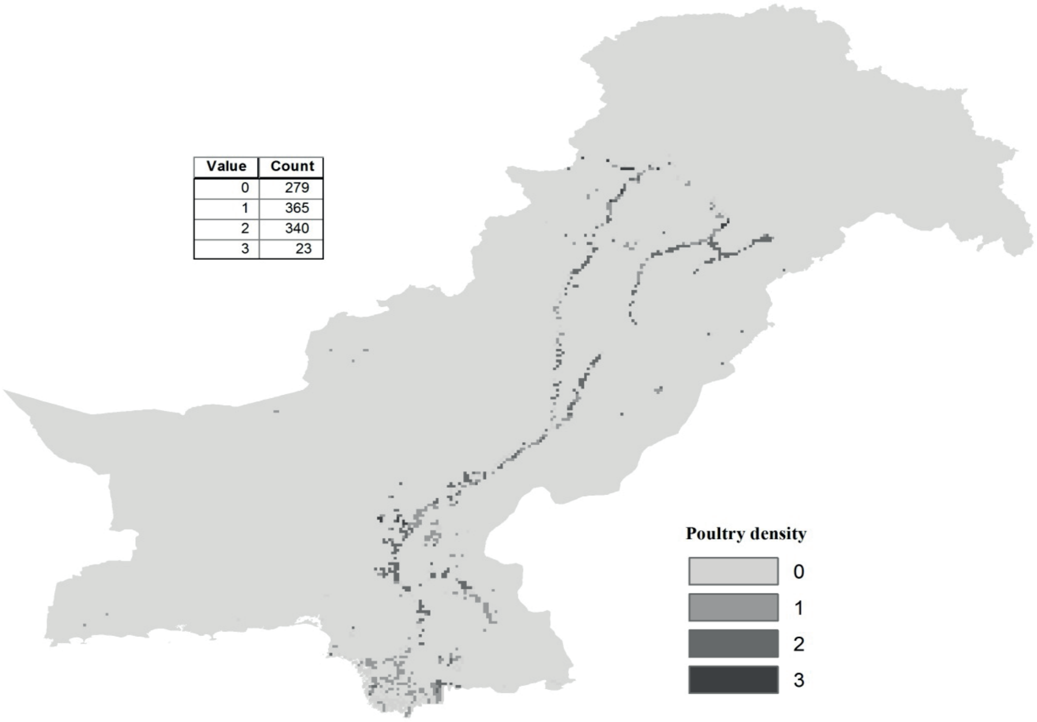


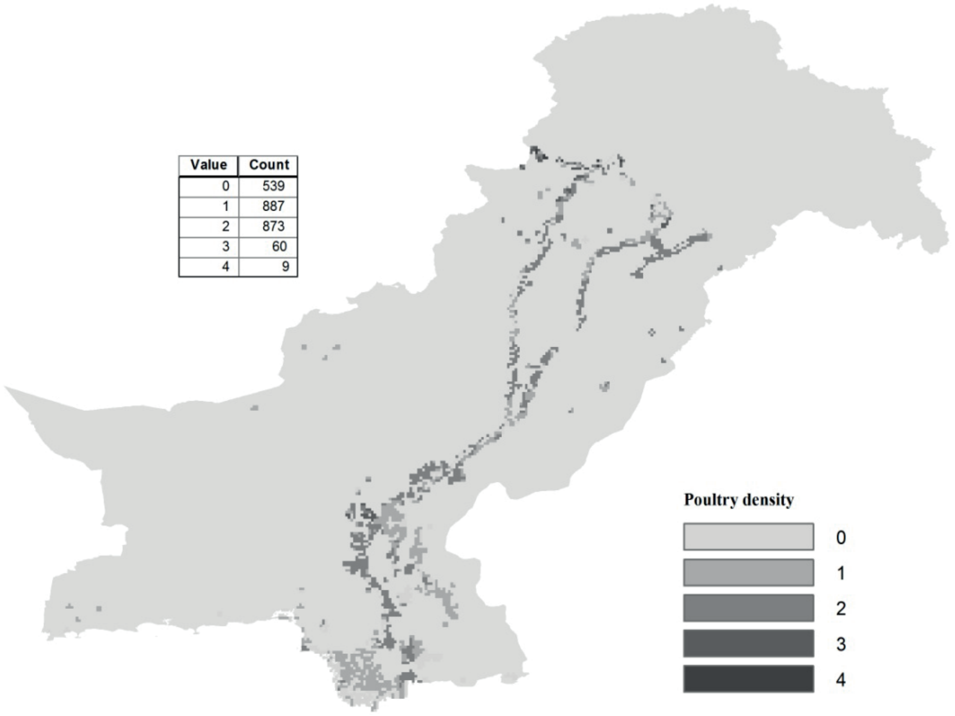
Figure 3.5 Maximum counts of waterbirds reported during 1987-2007. The map was created using data from 270 (out of 535 sites).Those were the sites whose coordinates were known and completely fell into the administrative boundary of Pakistan.

A total of 137 out of 270 coordinates (50%) fell within the 3 km range of SRTM waterbodies. The number of coordinates within the 6 and 9 km zones was 181 and 209, respectively. With a radius of 3 km, the output layer (produced by combining type A and type B polygons), enclosed 1,007 pixels of predicted poultry density with a reclassified values ranging from 0 to 3. There were only 23 pixels (2.23 %) having a value of 3. Those were found in the districts Charsada, Haripur, Jhelum, Gujrat, Kohat, Larkana, Nowshera, and Shahdadkot. The values of pixels within the 6 and 9 km buffers ranged from 0 to 4. There was no pixel with a value equal to 5. As expected, the number of pixels increased with the size of the buffer, but the distribution of their values remained right skewed (Figure 3.6, Figure 3.7, and Figure 3.8).



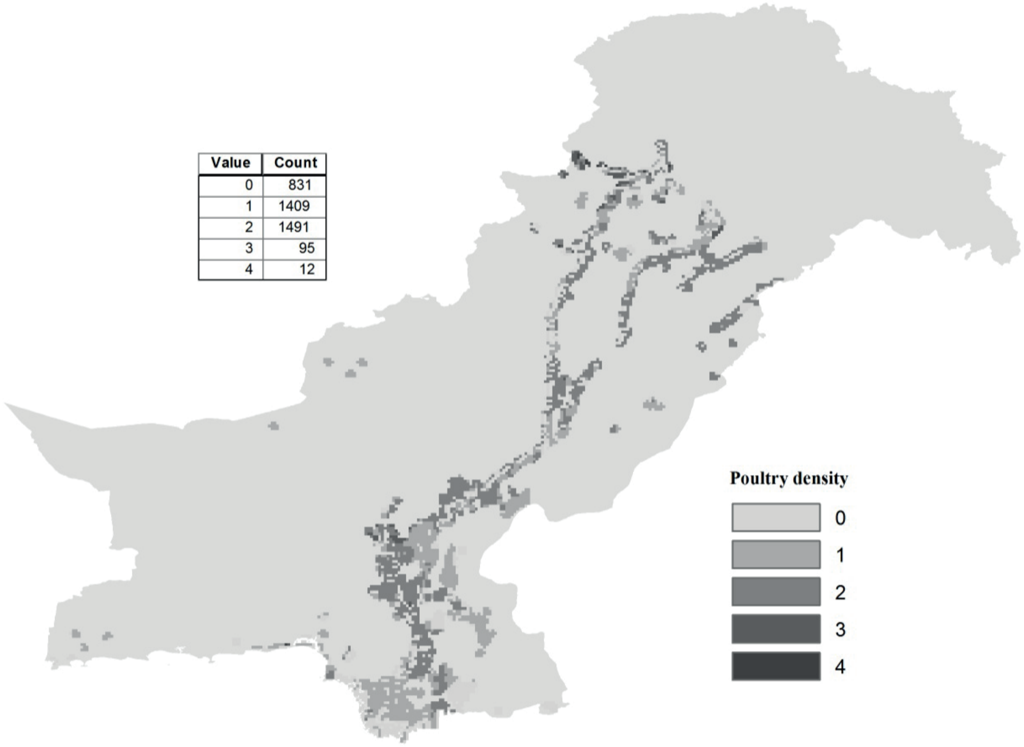
Value	Count
0	279
1	365
2	340
3	23

Figure 3.6 Distribution of pixels of poultry density within 3 km buffer zones. The values of the pixels have been reclassified. The map contains pixels having a value from 0 to 3 in order of low to high poultry density. The embedded table shows the number of pixels (count) within each class (value).



Value	Count
0	539
1	887
2	873
3	60
4	9

Figure 3.7 Distribution of pixels of poultry density within 6 km buffer zones. The values of the pixels have been reclassified. The map contains pixels having a value from 0 to 4 in order of low to high poultry density. The embedded table shows the number of pixels (count) within each class (value).



Value	Count
0	831
1	1409
2	1491
3	95
4	12

Figure 3.8 Distribution of pixels of poultry density within 9 km buffer zones. The values of the pixels have been reclassified. The map contains pixels having a value from 0 to 4 in order of low to high poultry density. The embedded table shows the number of pixels (count) within each class (value).

It was evident that from 1987 to 2007, Pakistan hosted 33 species of migratory birds from which H5N1 virus has been isolated in other parts of the world (Table 3.1).

Table 3.1 H5N1 affected avian species occurring in Pakistan

Common name	**Scientific name**
Northern Pintail	*Anas acuta*
Common Teal	*Anas crecca*
Eurasian Wigeon	*Anas penelope*
Mallard	*Anas platyrhynchos*
Gadwall	*Anas strepera*
Greylag Goose	*Anser anser*
Bar-headed Goose	*Anser indicus*
Common Pochard	*Aythya ferina*
Tufted Duck	*Aythya fuligula*
Greater Scaup	*Aythya marila*
Common Buzzard	*Buteo buteo*
Common Kestrel	*Falco tinnunculus*
Common Coot	*Fulica atra*
Watercock	*Gallicrex cinerea*
Common Moorhen	*Gallinula chloropus*
Brown-headed Gull	*Larus brunnicephalus*
Great Black-headed (Palla's) Gull	*Larus ichthyaetus*
Common Black-headed Gull	*Larus ridibundus*
Goosander	*Mergus merganser*
Black Kite	*Milvus migrans*
Red-crested Pochard	*Netta rufina*
Dalmatian Pelican	*Pelecanus crispus*
Great White Pelican	*Pelecanus onocrotalus*
Unidentified Pelicans	*Pelecanus spp.*
Great Cormorant	*Phalacrocorax carbo*
Little Cormorant	*Phalacrocorax niger*
Greater Flamingo	*Phoenicopterus ruber roseus*
Great Crested Grebe	*Podiceps cristatus*
Black-necked Grebe	*Podiceps nigricollis*
Purple Swamphen	*Porphyrio porphyrio*
Little Grebe	*Tachybaptus ruficollis*
Ruddy Shelduck	*Tadorna ferruginea*
Green Sandpiper	*Tringa ochropus*

The retrospective case-series analysis of the H5N1 data revealed that 33 out of 51 outbreaks (64%) had occurred during the migratory season, 21 of which were primary outbreaks according to the defined study criteria. There was a statistically significant difference in the number of outbreaks during the migratory and the non-migratory period (Chi-squared value = 4.00, DF =1, Asymp. sig. 0.04). Most (32/33) outbreaks occurred in districts containing either at least one major wetland or located in an ecoregion. These districts were Abbottabad, Rawalpindi, Islamabad, Karachi, and Peshawar.

3.4 Discussion

The role of migratory birds in long distance transmission of H5N1 has been considered in several studies but still remains controversial due to many knowledge gaps. Wild birds (especially wild ducks) were identified as potential long distance vectors for the virus in some studies (Kilpatrick et al., 2006; Gaidet et al., 2008; Keawcharoen et al., 2008) while other authors considered it unlikely (Feare, 2007; Saad et al., 2007; Weber and Stilianakis, 2007). Gaidet et al. (2010) evaluated the dispersive potential of HPAI H5N1 viruses by wildfowl. The authors analyzed the movement range and movement rate of birds monitored by satellite telemetry in relation to the apparent asymptomatic infection duration measured in experimental studies. It was estimated that in migratory birds there are, on average, only 5-15 days per year during which infection could result in a dispersal of virus over 500 km. Overall migration, which is commonly 4000-6000 km, follows a sequential rather than a continuous process. Migration is performed in a series of a few rapid long flights, generally undertaken in 1-4 days, interrupted by staging periods longer than the period of infection and viral shedding. The authors further explained that intercontinental virus dispersal by wildfowl requires a relay transmission amongst a series of birds successively infected. The large abundance and species diversity of wildfowl congregating at stopover sites along a migratory flyway, as well as the asynchronous timing of their arrival and departure, may facilitate such relay transmission.

The country lies across the Central Asian Flyway of migratory birds and toward its intersection with West Asian - East African Flyway. To mitigate the risk of incursion during migratory season, there is a need to create awareness among the farmers and target surveillance on areas and populations at risk. In this study, a subset AWC data was mapped and its attributes were identified, which may be helpful in surveillance and prevention of H5N1 and other important AIVs of domestic poultry.

The analysis of AWC data revealed a high proportion of missing values. One of the reasons for this has been described as inconsistencies in annual coverage of many sites (Li et al., 2009). An attempt was made to impute the missing values using the TRIM software, version 5.35 (http://www.ebcc.info). TRIM stands for **Tr**ends and **I**ndices for **M**onitoring data and is currently the standard programme to analyse count data obtained from bird monitoring schemes (Pannekoek and Van Strien, 2001). It analyses time-series of counts, using Poisson regression (or loglinear regression) and produces estimates of yearly indices and trends (Ter Braak et al., 1994). The imputed values were not mapped because the model fitness was poor, partially due to site heterogeneity (i. e. within each district trends differed between sites). Many of the missing values in the data were consecutive and could therefore not be replaced by moving average, mean (or median) of nearby points, interpolation or last observed carried forward. A linear trend model assumes a constant annual increase or decrease in waterbird count. Such a model may be adequate for short time series but usually becomes unrealistic if the time series is longer. The long term (secular) trend in the data could not be confirmed; therefore we did not substitute the missing values by series mean. Over the last couple of decades, several methods have evolved for handling missing data. Some approaches such as those evaluated in this study are considered "basic" because they are conceptually straightforward and require minimal computations. Relatively new and complex techniques such as multiple imputation (MI) and expectation-maximization (EM) are also available in statistical software, but their validity with this dataset would need further investigation.

Clusters of sites with high missing values were concentrated in the Sindh province. One possible explanation for this finding is that Sindh has a large number of wetlands and at some locations, groups of small lakes are present, e. g. there are 200 small lakes in Nara canal area. It is likely that such sites were included in the AWC, but later on wild birds could not be counted regularly there. Other factors influencing the missing value rate of a site may be its accessibility and perceived importance. The counting of birds is subjected to observer bias and may have a long term trend. These sources of error must be considered while interpreting the map of maximum reported counts. For creating the maps, 275 sites had to be excluded because their coordinates were either unknown or fell outside the boundaries of the country. The average missing value rate of these sites was 95% which indicates rare waterbird activity events. Some known to contain important wetlands but not presented in these maps include : Chiral, Ghizer, Ghanche, Gilgit, Mansehra, Karak, Kashmore, Lakki Marwat, Killa Saifullah, Poonch, Swat, and Zhob (www.pakistanwetland.org).

Mapping the geographic distribution of disease risk is a tool that may help decision makers to define high risk areas and allocate resources accordingly. Priority areas for surveillance of HPAI H5N1 have been mapped in the United States (Miller et al., 2007), United Kingdom (Snow et al., 2007) and Australia (East et al., 2008). For Pakistan, such areas may be divided into two main categories:

(i) The poultry rearing areas located closer to major wetlands. Rawal Lake, for example, is an artificial reservoir that provides the water needs for the cities of Rawalpindi and Islamabad. There are approximately 170 poultry farms within the catchment area of the lake (Anonymous, 2004)

(ii) The poultry rearing areas where surface water is used for drinking purpose. A major source of drinking water for the human, animal and poultry population of Karachi originates from water reservoirs including Haleji Lake, Hub dam, and Kinjhar Lake. These water reserves are internationally well known for the breeding, staging and wintering of migratory waterfowl (Anjum, 2004).

There are no clear guidelines for defining risk areas around waterbodies. East et al., (East et al., 2008) used a buffer of 10 km where as FAO recommends a minimum distance of 2 km (Anonymous, 2009). In this study, we compared the distribution of values of pixels of predicted poultry density at varying distances from AWC sites or nearby waterbodies. The choice of the buffer radii was partially based on the recommendations of the FAO and can be further modified, e. g. based on expert opinion.

It is important to note that the poultry density layer used in this study was created using low-resolution data and may have omission and commission errors. Omission errors occur when a pixel is not assigned a value, when in fact, it should be assigned. Commission errors occur when a pixel is assigned a value other than its true value (Baker, 2008). A high poultry density pixel should not be confused with increased likelihood of infection. The characteristics of the migratory birds population can also affect the likelihood of exposure such as gregariousness, degree of mixing with other species, contact risk with poultry, feeding habits (scavenging/predation) and the percentage of juveniles in the population (Caron et al., 2010). The current raster is limited by the fact that it does not show commercial and backyard poultry separately. The government has formulated a plan to register all poultry farms in the country; therefore in future the current poultry density map can be improved or a layer of poultry farm density can be created at the level of union councils.

The coordinates of 66 out of 270 eligible sites (25%) fell at a distance of more than 9 km from SRTM waterbodies. The following considerations may offer possible explanations: (i) the coordinates of AWC sites may be inaccurate, (ii) wetlands may have been degraded over the period of time, and (iii) there could be errors in SRTM data, e. g. misclassification of land into water body.

Retrospective case series analysis of H5N1 outbreaks confirmed significantly high number of primary outbreaks during the migratory season. Proesser et al. (Prosser et al., 2011) studied the movements of bar-headed geese marked with satellite transmitters at Qinghai Lake in China. H5N1 outbreaks in domestic birds were found to spike in frequency when up to 50% of the global population of bar-headed geese winter in sheltered river valleys surrounding Lhasa region. Reperant et al., (Reperant et al., 2010) studied spatial and temporal association of outbreaks of H5N1 in wild birds. The analysis concluded that waterbird movements associated with cold weather, and congregation of waterbirds along the 0°C isotherm likely contributed to the spread and geographical distribution of outbreaks of H5N1 infection in wild birds in Europe during the winter of 2005–2006. Putative seasonal stimuli drive seasonal influenza incidence in humans through three primary mediating mechanisms: seasonal variations in host contact rate, virus survival, and host immunity (Tamerius et al., 2011).

From this study, it is apparent that the coordinates contained in the AWC database can be helpful to identify poultry rearing areas within buffer zones of AWC sites and nearby waterbodies. As the scheme is already running, therefore the volunteers and other associated professionals may be involved in the active and passive surveillance of wild birds. The methodology of cluster analysis can be useful for the local authorities to redirect limited resources and investigate the characteristics of sites neglected from AWC. It will be interesting to aggregate missing values and map the spatial clustering at the district level. Participatory surveillance is a choice for backyard poultry and small scale commercial chicken farms near major wetlands. The study has highlighted the importance and limitations of the AWC and SRTM datasets. Viral transmission between migratory waterfowl and domestic bird populations, in either direction, can occur through several mechanisms, including direct contact in areas where the two groups share environments, where scavenging on H5N1 virus-infected carcasses may occur, and where ‘‘bridge’’ species exist that can transmit the virus between domestic poultry and migratory waterfowl populations (Brown et al., 2009a). Therefore, the proposed methodology may also be applied to identify hot zones for the exchange of AIVs between wild birds and domestic poultry in endemically infected countries, and where biosecurity in poultry holdings should be augmented.

4 Contact structure and potential risk factors for avian influenza transmission among open-sided chicken farms in Kamalia, an important poultry rearing area of Pakistan

4.1 Introduction

Avian influenza (AI) infections have caused heavy economic losses to the poultry industry in Pakistan as well as numerous other regions worldwide. The first introduction of H7N3 AI virus to Pakistan occurred during 1994, since then H7N3, H9N2, and H5N1 viruses have been sporadically isolated (Abbas et al., 2010). It was evident from the outbreak of 2003-2004 that notifiable avian influenza can have substantial impact on the poultry industry due to disease-related morbidity and mortality, costs associated with control measures; and market and consumer reaction affecting demand for poultry and poultry products[13]. As with many other developing countries, the threat of AI entry into Pakistan may be related to legal and illegal trade and wild migratory birds. Once the virus establishes itself (i. e. an index case occurs), the outbreak may propagate depending on factors such as time to confirm and eradicate infection foci, and on horizontal contacts and level of biosecurity within the production and marketing chain. At the moment, many aspects of the epidemiology of the disease are unknown. To mitigate the risk of an extensive outbreak, it is necessary to devise an evidence-based prevention programme for various sectors of the poultry industry. Epidemiological tracing and analytical investigations have revealed several factors for secondary transmission of AI in different countries of the world including Japan (Nishiguchi et al., 2007), the People's Republic of Bangladesh (Biswas et al., 2009), the Netherlands (Thomas et al., 2005), Italy (Busani et al., 2009), South Africa (Thompson et al., 2008), USA (McQuiston et al., 2005), Hong Kong (Kung et al., 2007), the Republic of Korea (Yoon et al., 2005), and Vietnam (Henning et al., 2009). The aim of this survey was to collect baseline data on contact structure and the prevalence of selected risk factors for AI transmission between open-sided table egg layer and broiler (grow-out) farms in a poultry rearing region of the country.

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[13] http://www.pakissan.com/english/allabout/livestock/poultry/activities.of.ppa.punjab.zone.shtml

4.2 Materials and Methods

4.2.1 Survey design

Kamalia is an administrative division (tehsil) of the district Toba Tek Singh in the Punjab province. It is an area of high poultry density containing 224 broiler and 304 layer farms[14]. Farming is mainly non-integrated, small-scale (≤ 5,000) and market-oriented. Chickens of single breed and age are usually managed in purpose-built open-sided sheds. Such traditional farming is common in Kamalia and was a reason for selecting this tehsil. The survey was cross-sectional and the unit of interest was the individual farm. A sample size of 52 broiler and 56 layer farms specified as the target which would allow estimating the prevalence of the putative risk factors at the 90% confidence level with 10% precision assuming frequencies of 50% for binary (yes/no) variables. Due to logistics, the data were collected using a non-probability sampling technique.

4.2.2 Data collection and analyses

A questionnaire was prepared which contained 16 closed and 22 open-ended questions. A summary of the questions asked and their response coding is given in the appendix B. The variables considered were : (i) husbandry system being practiced, (ii) straight-line distance to the nearest poultry farm, (iii) method for disposal of carcasses of dead birds, (iv) entry of wild birds into poultry sheds, (v) presence of boundary wall, (vi) biosecurity applied on high risk visitors i. e. those entering the poultry sheds, (vii) cleaning and disinfection of the essential vehicles, (viii) ensuring disinfection of the vaccination equipment, (ix) biosecurity measures adopted by the farmer after visiting a high-risk place, (x) frequency of various intermediaries and service providers, (xi) visits of the farmer to potential cross-contamination places, (xii) transport of diseased or dead birds for diagnostic purpose, and (xiii) re-use or sharing of egg trays among layer farms at production. The questionnaire was developed in English, but administered in Urdu, the national language, to ensure that the farmers understood all the questions. The questionnaire was pre-tested with 5 farmers and refined before implementation. All the respondents were assured for anonymity and personal information was neither asked nor intended. The data were collected from April to July, 2009, with the help of local veterinarians. The data were entered into a database which had been custom built in Microsoft Access 2007

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[14] The list was retrieved from http://www.agrilive.com.pk/

and later exported to IBM® SPSS® Statistics 19 (SPSS Inc., Chicago, IL, USA). The farms were divided into two groups on flock size. For this purpose, a cut off value of 5, 000 was chosen as this was the estimated median of the study population. The normality of the numeric variables was confirmed using the Shapiro-Wilk test. The raw data on the frequency of visits to the farm and visits of the owner to potential cross-infection sites were transformed into monthly contact rates to permit comparisons between broiler and layer enterprises. Associations between farm characteristics and categorical variables were investigated with the Fisher exact test, whereas the t-test for independent samples was used for scale variables. The 95% confidence interval (CI) of proportions and means was determined through bootstrapping.

4.2.3 Dispersion calculation

Particulate matter (PM) emissions from poultry sheds consist of feather fragments, faecal material, dander (skin debris), feed particles, biological materials (mould, bacteria, fungi) and litter material (Dunlop, 2009). These particles are identified according to their aerodynamic diameter as either PM_{10} (particles with an aerodynamic diameter smaller than 10 μm) or $PM_{2.5}$ (aerodynamic diameter smaller than 2.5 μm) (Lopez, 2010). On AI affected poultry farms, culling actions and subsequent cleaning of the sheds can cause very high dust production and the generation of bioaerosol including fine particulate matter (Sedlmaier et al., 2009). Considering the flock size and structure of open-sided chicken farms, the extent of dispersion of PM_{10} dispersion was modeled. The dispersion calculation was done by Andreas Falb (Bavarian Environment Agency, 2010) using the dispersion model LASAT (Lagrangian simulation of aerosol transport) (Janicke, 2003). LASAT computes the dispersion of trace substances in the boundary layer (the lowest layer in troposphere, about 500 m to 2000 m thick). It simulates the transport and the turbulent diffusion of a representative sample of tracer particles using a random walk process (Lagrangian simulation). Table 4.1 shows the assumptions made for the dispersion calculation. Due to lack of adequate meteorological data and because the outcome was supposed to give information of particle density depending on distance, dispersion situations were simulated with different wind velocities and stability classes at a fixed wind direction. A combination of high wind velocity and stable conditions is unrealistic at meteorological aspects and therefore excluded.

Table 4.1 Assumptions made for PM_{10} dispersion calculation

Category	Open-sided chicken farm
Number of sheds	Single
Flock size	Layer : 2500 , Broiler : 2200
PM_{10} emission rate per bird	Layer : 2.15 mg /h , Broiler : 6.73 mg/h (TÜV Süddeutschland, 2000)
PM_{10} sedimentation rate	0 m/s (PM_{10} represented by the model as gas)
PM_{10} deposition rate	0.01m/s
Building area	$240m^2$ (A. Qadir, Personal communication , July 24, 2010)
Emission height (source represented as field)	0.15 m
Calculation time	24 hours
x-direction	-50 m to +3500 m (buffer distance between farms less than 3 km)
y-direction	-400 m to +400 m
z-direction	16 levels up to 500 m (results are given for the ground level, 0 m to 3 m)
Horizontal resolution	10 m
Wind direction	270 ° (straight towards next adjacent settlement) (www.windfinder.com)
Combinations of wind velocity (v) and stability class (SC)	v =1 m/s , with SC = unstable, indifferent or stable v = 3 m/s , with SC = unstable or indifferent

4.3 Results

In total, data were collected from 42 broiler and 36 layer farms. None of the farmers declined to participate in the survey. The high response rate was due to the involvement of local veterinarians in the data collection process. For all the variables, the missing value ratio was less than 20%. Tables 4.2 and 4.3 show results of the descriptive analysis for the categorical and numeric variables. The mean flock size was 3,702 (95% CI, 3,098-4,306) for broiler and 6,930 (95% CI, 2,972-10,888) for layer farms. Most of the farms (89.7%, 95% CI 82.1-99.2) were found to practice an all-in-all-out husbandry system. The all-in-gradual-out system was rare (10.3%, 95% CI 3.8-17.9) and exclusively reported by broiler holdings. The majority of the respondents reported the presence of another commercial poultry farm within 3 km radius of their farms. The straight-line distance to the nearest poultry farm was 494.4 m (95% CI, 368.7-630.7). Malpractice of throwing dead birds into nearby fields was as high as 84.0% (95% CI 72.0-94.0). Other methods of disposal of dead chicken were off-site burial (12.0%, 95% CI 4.0-

22.0) and on-site burial (4.0%, 95% CI 0-10.0). On 63.9% of farms (95% CI 52.8-75.0%), wild birds had excess into the poultry sheds whereas 76.3% (95% CI 65.8-85.5) either had a damaged or no boundary wall.

The majority of the farms (92.1%, 95% CI 85.5-97.4) had no foot bath with disinfectant at the entrance to poultry sheds. There were a few farmers who provided clean footwear (33.3%, 95% CI 21.9-44.9) or clothes (17.9%, 95% CI 10.3-26.9) for the visitors who enter the poultry sheds. A requirement to wash hands before handling the birds was stated by 53.8% (95% CI 42.3-65.4) respondents. Biosecurity implemented on essential vehicles was generally poor. Only a small proportion of farmers, 25.7% (95% CI 17.1-37.1), reported to clean vehicles with a hose or wet brush. Spaying disinfectant on the wheels of vehicles was also not a very common practice (28.8%, 95% CI 18.2-40.9).

More than half of the farmers (59.2%, 95% CI 47.9-70.4) claimed to ensure disinfection of the equipment used by the vaccinators. As many as 53.1% (95% CI 42.2-64.1) of the respondents had a history of transporting diseased or dead birds to a local diagnostic laboratories. Almost all growers confirmed their visits to the offices of feed distributors, other poultry farms, and live bird markets. Biosecurity measures adopted by the owners after visiting such places were either incomplete or totally absent. Only 29.7% farmers (95% CI, 20.3-40.5) asserted to shower after visiting a high risk place. Those who declared changing to clean clothes were 40.0% (95% 27.2-51.4). 64.9% (95% 52.1-75.7) of the farmers reported changing their shoes following a visit to a site where they may have become contaminated.

Social contacts among the farmers were common besides visits by intermediaries and service providers. The mean number and various types of contacts are presented in Table 4.2. Transport of eggs accounted for the highest mean number of contact followed by feed delivery. Among layer farms, 83.0% were at production at which some contacts may make them more vulnerable to exposure, notably by re-use of egg trays, cake out[15] , waste haul[16], and egg transport. In addition, the number of visits of the commercial vaccinators was high on layer farms due to its vaccination schedule (4-5 shots per flock) and the fact that vaccination crews were reported to be invited for debeaking as well.

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[15] Removal of poultry wastes from the poultry houses

[16] Removal of poultry wastes from the property

Between broiler and layer holdings, there was a significant difference in the type of husbandry system used (p-value = 0.01), ensuring disinfection of equipment used by vaccinators (p-value = 0.04), number of vaccines administered per flock (p-value < 0.001), routine visits (per month) of the veterinarian (p-value = 0.04), visits (per month) of the farmer to the office of the feed distributor (p-value = 0.01), and visits to other poultry farms (p-value < 0.001), and live bird markets (p-value < 0.001). We could not find a statistically significant difference between small and large flocks (cutoff value = 5,000) except for provision of shoes to visitors (p-value = 0.01), spraying disinfectant on vehicles (p-value = 0.03), presence of a boundary wall (p-value = 0.01), and biosecurity implemented on vaccination apparatuses (p-value = 0.01).

Table 4.2 Survey of small scale commercial chicken farms in Kamalia (2009): Summary of categorical variables included in the questionnaire

Variable	**Response category**	**All operations** ****RR (95%CI)**	**Broiler** **RR (95%CI)**	**Layer** **RR (95%CI)**
*Type of husbandry system	AIAO[1]	89.7 (82.1-99.2)	81.0 (69.0-92.9)	100
	AIGO[2]	10.3 (3.8-17.9)	19.0 (7.1-31.0)	0
Method for disposal of dead birds	Off-site burial	12.0 (4.0-22.0)	6.7 (0-16.7)	20 (5.0-40.0)
	On-site burial	4.0 (0-10.0)	6.7 (0-16.7)	4.2 (1.8-23.5)
	DIS[3]	84.0 (72.0-94.0)	86.7 (73.3-96.7)	80.0 (60.0-95.0)
Entry of wild birds into poultry sheds	Yes	63.9 (52.8-75.0)	70 (55.0-82.5)	56.3 (37.6-71.9)
Boundary wall	Damaged or absent	76.3 (65.8-85.5)	76.2 (61.9-88.1)	76.5 (61.8-88.2)
Foot dip with active disinfectant at entrance to poultry sheds	Absent	92.1 (85.5-97.4)	95.2 (88.1-100)	88.2 (76.5-97.1)
Provision of clean footwear to visitors or scrubbing of shoes in use	Yes	33.3 (21.8-44.9)	31.0 (19.0-45.2)	36.1 (22.2-52.8)
Provision of clean clothes to the visitors	Yes	17.9 (10.3-26.9)	16.7 (7.1-28.6)	19.4 (8.3-33.3)
Require visitors to wash hands before handling the birds	Yes	53.8 (42.3-65.4)	52.4 (38.1-69.0)	55.6 (38.9-72.2)
Cleaning of vehicles with a hose or wet brush	Yes	25.7 (17.1-37.1)	26.3 (13.2-42.1)	30.0 (13.3-46.7)
Spraying disinfectant on the vehicles	Yes	28.8 (18.2-40.9)	27.8 (13.9-41.7)	30.0 (13.3-46.7)
*Ensure disinfection of equipment used for vaccination	Yes	59.2 (47.9-70.4)	45.0 (30.0-60.0)	77.4 (61.3-90.3)
Transport of dead or diseased birds to laboratory	Yes	53.1 (42.2-64.1)	44.4 (27.8-61.1)	64.3 (46.4-82.1)
***Shower in	Yes	29.7 (20.3-40.5)	23.8 (11.9-38.1)	37.5 (21.9-53.1)
***Change to clean shoes	Yes	64.9 (52.1-75.7)	60.0 (45.0-75.0)	70.6 (55.9-85.3)
***Change to clean clothes	Yes	40.0 (27.2-51.4)	40 .0 (25.0-55.3)	40.0 (23.3-60.0)
Sharing or reuse of egg trays	Yes	- (- --)	- (--)	83.3 (69.4-94.4)

Difference statistically significant (p value ≤ 0.05), **RR= Number of responses obtained /Number of respondents x100, * by owner after visiting a high risk place, 1 = all in all out, 2 = All in gradual out, 3= Disposal of dead birds direct into the environment.*

Table 4.3 Survey of small scale commercial chicken farms in Kamalia (2009): Summary of numeric variables included in the questionnaire

Variable	**All operations** **Mean (95% CI)**	**Broiler** **Mean (95% CI)**	**Layer** **Mean (95% CI)**
Flock size	3651 (3429-14344)	3702 (3098-4306)	9630 (2972-10888)
Distance to nearest poultry farm	494.4 (368.7-630.7)	389.1 (230.7-572.3)	605.9 (430.9-801.3)
Frequency of visitors			
*Veterinarian from feed company[1]	2.4[a](2.1-2.6)	2.7[a] (2.3-3.1)	*1.9[a] (1.7-2.2)*
Feed delivery[1]	2.9[a] (2.7-3.2)	3.2[a] (3.0-3.4)	2.7[a] (2.2-3.2)
Persons from other poultry farms[1]	2.2[a] (1.8-2.6)	2.3[a] (1.7-2.9)	2.1[a] (1.6-2.7)
Egg transporters[2]	- (-)	- (-)	15.6[a] (14.1-16.7)
*Commercial vaccinators	- (-)	1[b] (1.0 -1.0)	4.2[b] (4.1-4.3)
Crew for beak trimming	- (-)	- (-)	1 [b](1.0-1.0)
Waste haul[2]	- (-)	- (-)	1.2 [c](1.1-1.3)
Cake haul [2]	- (-)	- (-)	2.7[c] (2.5-3.0)
Visits of the owner to cross contamination sites			
*Office of the distributor [1]	2.7[a] (2.3-3.1)	3.1[a] (2.6-3.4)	2.3[a] (1.8-2.9)
*Other poultry farms [1]	2.2[a] (1.8-2.6)	2.5[a] (2.0-3.1)	1.7[a] (1.3-2.2)
*Live bird markets[1]	2.3 [a] (1.9-2.7)	3.2[a] (2.9-3.5)	1.2[a] (0.7-1.8)

** Difference statistically significant (p value ≤ 0.05), a = mean number of visits per month, b= mean number of visits per flock, c = mean number of visits during laying period, 1 = the variable was computed from survey data, 2 = the variable is about layer farms at production.*

Figure 4.1 shows the dispersion of PM_{10} (concentration [$\mu g/m^3$], 24h-mean) for various combinations of wind speed, stability class, and type of poultry farms. The widest spread of particles was not necessarily simulated with higher velocities. For a velocity of 1 m/s, stable conditions and higher emissions (broilers), concentrations of more than 0.05 $\mu g/m^3$ were simulated at a distance of 3 km. Compared to velocities of 3 m/s and indifferent conditions, similar concentrations were detected up to a distance of 0.75 km. The lowest dispersion near the ground was evident for instable conditions, where high turbulence delimited expanding horizontal transport.

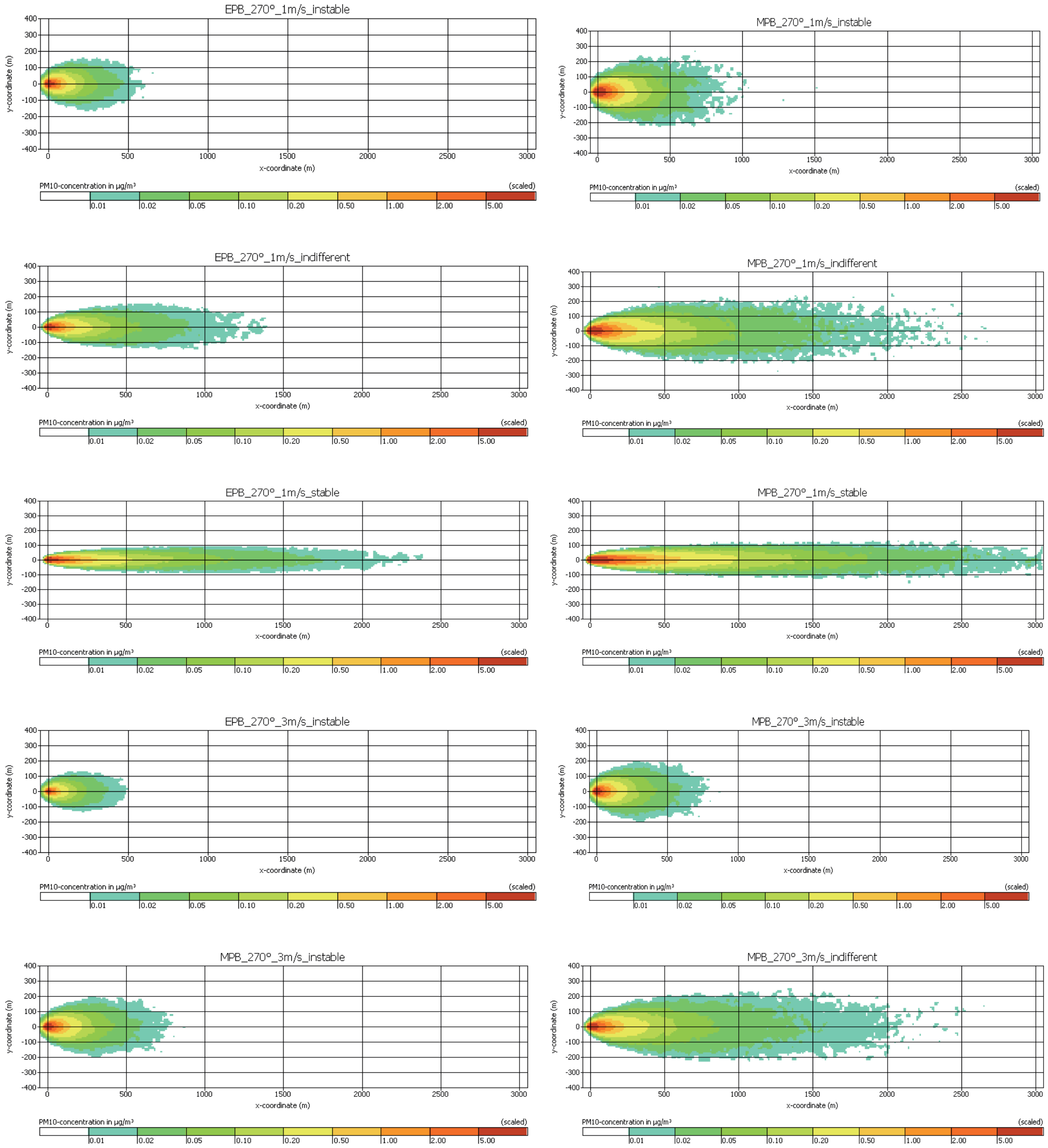


Figure 4.1 Dispersion calculation with different stability classes and wind velocities at wind direction 270°

Header info for each plot: <kind of farm*>_<direction>_<velocity>_<stability class>; *E: egg, M: meat, PB: producing birds

4.4 Discussion

Vehicles, containers, and catching teams from live bird markets are potentially contaminated and may therefore introduce pathogens such as AIVs into farms (Kung et al., 2007; Biswas et al., 2009). In Kamalia, the impact of this route appears low for those farms which practice the all in-all-out system. In Indonesia, the likelihood of virus introduction through bird collectors was concluded as moderate to extreme for multi-age layers or small broiler sheds with extended collection (Toribio et al., 2010).

A factor which makes Kamalia vulnerable to an extensive outbreak is the short buffer distance between farms. A high density of poultry farms was concluded as a risk factor for the spread of HPAI in Italy (Marangon et al., 2004), the Netherlands (Elbers et al., 2004) and Canada (Power, 2005). Poultry farm densities in these regions ranged from 0.05 to 4 per km^2 (Hamilton et al., 2009). During 2003-2004, Pakistan was affected by a devastating outbreak of HPAI H7N3. The outbreak occurred in Karachi region which is an area of poultry density (Naeem et al., 2007). A significant association with medium poultry density was apparent also in another study carried out in Vietnam (Henning et al., 2009). Conversely, Fang et al. (2008) did not find an association between poultry density and risk of HPAI in China. The authors explained this result with a greater proportion of industrialized chicken production sites in areas of higher poultry densities, with associated higher biosecurity standards, and with effective vaccination protocols. In poultry-dense areas, short buffer distances and/or high stocking densities may act as stepping stones in rapid HPAI transmission (Trampel et al., 2009). In addition to dust, wind-blown feathers from poultry infected with HPAI virus are potentially infectious because of viral replication within the feathers (Yamamoto et al., 2008) and their contamination with fecal material from infected birds. In densely populated poultry areas, transmission by flies and vermin is also possible, given the fact that virus has been isolated from blow flies in Japan (Sawabe et al., 2006) and that these viruses can multiply in a range of mammalian species, including mice, without prior adaptation. Disposal of dead birds directly into the environment as practiced by farmers in Kamalia can be a serious biosecurity threat. The dead birds are eaten by feral and wild animals which may serve as mechanical vectors for transmission from neighboring affected areas (McQuiston et al., 2005). A matched case-control study conducted in Bangladesh revealed a positive association between H5N1 incidence and access of feral and wild animals to farms (Biswas et al., 2009).

During the survey more than half of the farmers stated that wild birds had access into their poultry sheds. Entry of wild birds into poultry buildings is a mechanism by which HPAI can be transmitted (Swayne and Halvorson, 2003; Swayne, 2008). Wild birds may mechanically transfer contaminated faeces from infected poultry to premises with susceptible domestic birds or become infected and disseminate the virus through their own faeces and respiratory tract secretions (Stallknecht and Brown, 2008). Sparrows, feral pigeons, crows, and magpies have been found infected with H5N1 virus (Feare, 2007). A subclinical infection in tree sparrows was detected in China. Brown et al. (2009b) inoculated house sparrows (*Passer domesticus*) with HPAI (H5N1) virus of the strain influenza A/whooper swan/Mongolia/244/05. The birds were evaluated for morbidity, mortality, viral shedding, and seroconversion over a 14-day trial. The house sparrows were highly susceptible to the H5N1 virus as evidenced by low infectious and lethal viral doses. In addition, house sparrows excreted virus via the oropharynx and cloaca for several days prior to the onset of clinical signs. Based upon all these previous studies, the findings of the survey suggest that access of wild birds to the commercial poultry houses is a potential risk for the disease transmission.

Incomplete biosecurity on visitors and absence of a footbath at the entrance to a farm/shed have been proven as risk factors for outbreaks of AI (Nishiguchi et al., 2007; Biswas et al., 2009). Frequent contacts among farms by intermediaries and service providers are reliable source of pathogen transmission (FAO, 2008). In Kamalia, the intermediaries and service providers are veterinarians, egg transporters, feed suppliers and distributors, traders of poultry products, manure haulers, representatives of companies dealing in poultry medicines, service crews for procedures such as vaccination, beak trimming , and bird catching. Leibler et al. (2010) found that the company affiliation was a major driver of the farm-based exposure risk to an infection like AI in a region with high-density food animal production. Farms within the same integrator group as the index farm were concluded to face 5-fold increase in the exposure risk compared to farms affiliated with a different integrator. The authors estimated that a single infectious farm within the context of a dense, broiler producing region could result in a quantifiable AI exposure risk to other farms as a result of vehicular business contacts. The authors stated that in a realworld setting, where it might take up to 2 weeks to detect a LPAI outbreak in an industrial flock and where a virus could persist for long periods of time in manure, farms associated with the same integrator as the index farm might face a 25% higher risk of exposure to a vehicle that had serviced an index farm during its period of infectiousness. Dorea et al. (2010) modeled off-farm spread of HPAI stochastically. The spread was more frequently associated with feed trucks (highest daily probability and number of farm visits) and with company personnel or hired help (highest level of bird contact).

Two important contacts identified in this survey are distributors and veterinarians from feed companies which make up the industry-based health delivery system. The veterinarians are ambulatory and are usually contacted in case of progressive or high mortality. In the case of an outbreak, a single veterinarian has to pay multiple visits per day sometimes over a large geographic area. In each region, different feed mills have their designated distributors who coordinate visits of the veterinarians and act as middle men. These distributors also have business links with hatcheries and companies dealing in poultry medicines. From the distributors, the farmers can purchase day old chicks, feed, medicines at normal or extended prices depending on the availability of cash. At offices of the feed distributors, farmers from distant locations and destination come into contact (e. g. exchange of currency, hand shaking, and use of common floor), therefore such places could be potential cross-contamination points. The same applies to the veterinary diagnostic laboratories and live bird markets. Commercial vaccinators are invited to a farm for injection of inactivated vaccines and drugs. They can be a very potent route of virus dissemination because they enter the sheds, contact the birds, perform invasive procedures, and visit multiple farms per day. Some contacts were either more frequent or unique to layer farms and may make them more prone to exposure. Examples of such contacts are (i) sharing of egg trays, (ii) waste haul and in some cases cake out by personnel from companies dealing in manure and, (iii) visits of egg transporters during production. This partially explains the higher number of outbreaks of HPAI H7N3 in layer farms in Karachi during 2003-2004 (Arshad and Qureshi, 2004). Thomas et al. (2005) evaluated risk factors for the introduction of HPAI virus into poultry farms during the epidemic of 2003 in the Netherlands. An increased risk of HPAI virus introduction in layer finisher farms was explained with the use of cardboard egg trays used for the removal of eggs during the epidemic. In Bangladesh, HPAI outbreaks have been mainly reported in commercial layer flocks (Loth et al., 2010). Sharing of manure disposal equipment and reuse of egg trays have been reported as risk factors for AI outbreaks in other epidemiological studies (Wee et al., 2006; Nishiguchi et al., 2007).

A limitation of this survey was the use of convenience sampling, which is a non-probability technique for data collection. It may lead to ascertainment bias if the sample differs from the study population with respect to the variables under investigation. We compared demographics (flock size and farm capacity) of the sample and the study population and did not find any significant difference, which suggest that this concern is less germane. Another type of systematic error which may be expected is reporting bias which occurs when participants give answers in the direction they perceive are of interest to the researcher or under-report a particular variable. The likelihood of this bias was partially reduced by involving local veterinarians in the data collection process and blinding the farmers about the name of the disease. For putative risk factors with binary responses, a high prevalence (≥ 70.33) justifies to assume adequacy of our sample size. The exact absolute error calculated on the basis of "actual "sample size, was 10.45% for broiler and 11.76% for layer type of farms. The farmers usually modulate biosecurity needs (and therefore practices) as the threat increases or decreases. The survey was carried during a "no outbreak "period; which may lead to overestimation of some variables. A repetition of the same survey during the course of an epidemic in future would be valuable in this regard. There may be regional differences in knowledge, attitude, and practices of the farmers due to experience with previous outbreaks and effects of prevention programmes being run by the government and the industry. This constrains extrapolation of the findings beyond the boundaries of Kamalia.

From this survey it was apparent that there is a definite need to improve biosecurity on open-sided chicken farms in Kamalia. Given the structure of the farms, it seemed difficult to implement any biosecurity at the farm gate and between the farm boundary and poultry sheds. The reasons for poor compliance with biosecurity are not clear and may be complicated. Environmental contamination with some endemic pathogens (e. g. infectious bursal disease virus) may require maintaining a high level of biosecurity which may not be a cost-effective choice for small scale farmers. Open sheds are usually rented from the distributors and the farmers may not be willing to invest in the property they do not own. High cost and poor quality of inputs, extraction of profit by middlemen, fluctuations in prices of outputs may be the other factors. It is also possible that the farmers are not convinced about the effectiveness of biosecurity. A case-control study in Indonesia indicated that biosecurity had little influence on the occurrence of HPAI, as only very few farmers applied the biosecurity measures correctly (Bambang and Bustanul, 2008). Sarini et al. (2010) evaluated whether there is any correlation between mortality and weight gain with both status and level of biosecurity implemented by farmers. Neither farm biosecurity status nor the level of biosecurity implemented showed any correlation with mortality or broiler weight gain. In our survey, we could not find a significant difference between small and large flocks for most variables. Farmers' characteristics such as education, experience in poultry and age can influence the adoption of biosecurity measures. These variables may be considered in further studies to confirm whether poor biosecurity is a general problem.

This study does not prove short distance wind borne transmission of AI; rather it has assessed its possibility and suggests further research on this topic. The results of the dispersion calculations can have some uncertainties (i) meteorological details, notably stability class, were not available for the study area, therefore Andreas Falb (Bavarian Environment Agency) calculated dispersion following conventional assumptions [most conservative case: very stable boundary layer, low and constant wind velocity as 1 m/s over 24 hours and a wind direction straight towards the next adjacent settlement] (ii) PM_{10} emission rates used as model input were adopted from Germany as there were not similar data collected locally. A prerequisite for wind borne spread is the persistence of the virus in the aerosols. Low temperature is accompanied with low relative humidity in winter. Such conditions favor aerosol transmission of the virus by impairing the respiratory mucociliary clearance system of the host (Lowen et al., 2007), and by forming droplet nuclei which remain suspended in the air for an extended period of time and have more penetration into the low respiratory tract (Tellier, 2009). The environmental stability of AIVs depends on several other factors as well i. e. the level of ultraviolet radiation (Tang, 2009), the strain of the virus (Mitchell et al., 1968; Mitchell and Guerin, 1972), salinity in the air (Power, 2005), the nature of surfaces (Tiwari et al., 2006), protective coating of organic materials i. e. mucous or saliva (Thomas et al., 2008), and pH (Stallknecht and Brown, 2009). It is emphasized that thermostability of AIVs depends upon the subtype (Suzuki et al., 2010), and can even vary between isolates (Negovetich and Webster, 2010). Other factors which can influence the airborne transmission include flock immunity, physical, and biological variables affecting formation, concentration and emission of PM from poultry holdings (Lopez, 2010).

This paper is profile of Kamalia at the time of survey. The numbers of farms and flock sizes were calculated from the source mentioned in the survey design. The information obtained through this survey can be used to design a biosecurity plan, education material for the farmers, prospective analytical studies and an outbreak tracing questionnaire. The veterinary health delivery system, dust emissions from poultry sheds, and company affiliations are areas which should be given priority in future research and the planning of protective measures. Although open-house poultry farming is decreasing rapidly, these farms still have a considerable potential to propagate several pathogens. Improving biosecurity in this high-risk sector is crucial for the welfare of the whole poultry production and marketing chain in the country and may even help to prevent human infection.

5 Pilot study on the use of adaptive conjoint analysis to assess risk factors for high pathogenic avian influenza outbreaks on commercial poultry holdings of Pakistan

5.1 Introduction

For many developing countries, a little is known about the relative importance of risk factors determining introduction, spread, and maintenance of HPAI in commercial poultry production units. Quantification of those risk factors through classical epidemiological studies is often difficult for several reasons including data protection, logistics, poor record keeping by farmers, lack of cooperation, inability to control confounders under field situations, and potential selection and misclassification bias. In this context, the systematic collection and analysis of opinions and experiences of indigenous experts may be highly valuable to fill the knowledge gaps. Expert opinion has been used to get insight into the epidemiology of various epidemic diseases of livestock (Garabed et al., 2009). Adaptive Conjoint Analysis (ACA) is one of the techniques available for elicitation of expert opinion. At first, it was used for marketing research but later applied in a variety of fields like nuclear power industry, engineering, human medicine and to some extent also in veterinary medicine. Conjoint analysis has been used to evaluate the comparative risk and relevance of disease control options (Staerk et al., 1997; van Schaik et al., 1998b; Horst, 1999; Fels-Klerx et al., 2000; Nissen, 2001; Sorensen et al., 2002; Peddie et al., 2003; Milne et al., 2005; Valeeva et al., 2005; Cross et al., 2009; Huijps et al., 2009). The survey technique has some advantages over traditional paper-based or personal interviews. First, ACA is administered via computer. This minimizes interviewer bias and facilitates data collection and management. The computer interface provides respondents a greater degree of anonymity (Philips et al., 2009) and prevents socio-psychological processes that influence a person's opinion in a group situation (Staerk et al., 1997). The data may be collected over the internet which further adds speed, ease, economy in the survey process. Second, ACA focuses on the attributes that are most relevant to the respondent and avoids information overload by focusing on just a few attributes at a time. Moreover, its interactive format captures and holds the participants' attention in a more powerful way. Thirdly, immediately upon the completion of the interview, the results are available for discussion and analysis. It is also possible to detect and exclude respondents with inconsistent answers (van Schaik et al., 1998a).

To the best of my knowledge, the technique was never applied in the context of animal diseases in any developing country. This paper describes findings of an ACA study conducted in Pakistan. The main research question addressed was *"which risk factors may be important in determining the incidence of HPAI on open-sided commercial (broiler and layer) chicken farms if the virus enters and establishes itself in the country?"*

5.2 Materials and Methods

The conjoint model is a multi-attribute model, which assumes that consumers purchase products (e. g. an apple) based on the characteristics, or attributes, of the product (e. g. flavor), and that each attribute may have two or more levels (e. g. sweet, tart). The individual's utility for a product concept can be expressed in a simple way as the sum of the utilities of its attributes (Churchill and Iacobucci, 1999). An epidemic in an animal population also represents a multi-attribute phenomenon. Multiple risk factors "attributes" may have a variable impact in determining the incidence of any disease (Staerk et al., 1997; Horst, 1999). For example, the type of husbandry can be a risk factor for introduction of virus into a poultry holding (Toribio et al., 2010). In this example, the attribute "type of husbandry" has three levels, i. e. all-in-all-out (all birds enter together and leave together), all-in, gradual-out (all birds enter together but leave in separate batches over a period of time) and non-specific production system (new birds are introduced into the exiting flocks during production cycle). Bird collectors enter the farm once and at the end of production, therefore all-in-all-out husbandry is the preferred method to reduce the likelihood of infection from live bird markets.

A total of 21 risk factors were included in this survey and are given in Table 5.1. The list of risk factors was created based on available literature and personal communication with local poultry consultants. At the farm level, the source of virus may be related to the area (i. e. location), pests, people, organic and inorganic items, therefore the risk factors were divided into four categories. Each risk factor was assigned two mutually exclusive levels named as level 1 and level 2 indicating its presence in two extreme scenarios, e. g. "location of farm close to a live bird market" versus "location of farm away from live bird market." For the area-related risk factors, minimum distance standards were obtained from the literature (Ahsan-ul-Haq, 2004; Anonymous, 2009)

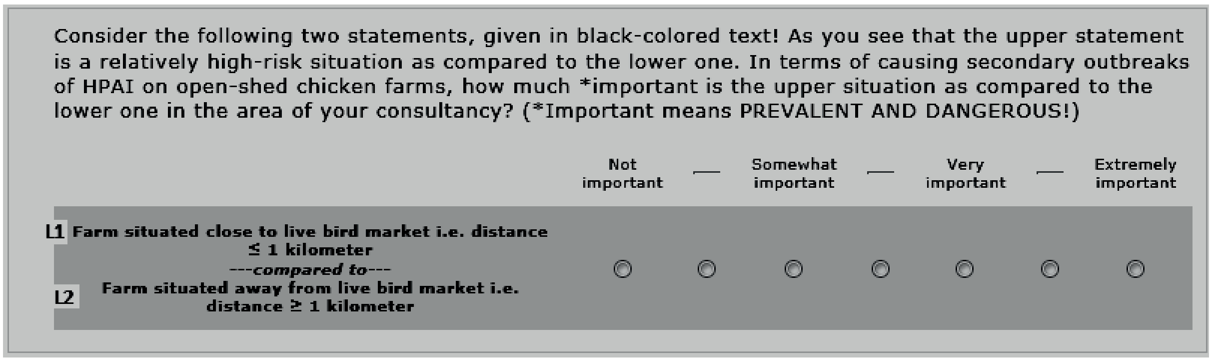
A separate ACA questionnaire was created for each category using ACA/web system version 6.4 (Sawtooth Software, Inc, Sequim, USA). Each questionnaire contained three sets of questions called ranking, paired-comparison, and calibration tasks. We did not use the software option "rating task" as the hierarchy of the levels was already known. Number, scale, and format of the questions were set according to the instructions given in the documentation of the software.

Ranking questions were placed first in the interview and their intent was to assign a score to each risk factor on a seven-point Likert scale. Figure 5.1 illustrates a prototype ACA ranking question. A single question was asked for each attribute in the response of which the respondents had to compare a high risk level (L1) with a low risk level (L2) on the basis of its prevalence and ability to cause an outbreak. The ranking questions were followed by a series of customized paired-comparison questions (conjoint task). In each paired question, the respondents had to trade-off between combinations of levels from two different risk factors as shown in Figure 5.2. ACA is interactive in that it uses the information obtained from each new paired comparison to update utility estimates and to select the next pair of options. Utility measures become more precise as the interview proceeds. The software continues presenting the subject with paired comparisons until enough data have been collected to estimate utilities for each level of each attribute (Fraenkel, 2010). Mathematical details of these calculations are available at http://www.sawtoothsoftware.com/technicaldownloads.shtml#acatech and have been summarized in appendix C. The third and last type of questions asked in the ACA interview was calibration questions. The purpose of the questions was to determine the correlation coefficient in order to assess the level of consistency in the responses. A screen shot of ACA calibration question is given in Figure 5.3. In each question, the respondents had to type a number between 0 and 10 (inclusive) to indicate the risk of HPAI outbreak on a farm with a set of features.

The respondents for this ACA survey were local veterinarians from Pakistan with at least five years experience in control and prevention of poultry diseases. An a priori list of potential respondents was not available. University teachers, field veterinarians from public and private sectors and animal health research workers were consulted to compile a list of 33 potential respondents for this survey. Most of the respondents were contacted directly. In a face-to-face discussion, the respondents were informed about the purpose of the survey and were made familiar with the format of ACA questions. This was followed by an email invitation which a link to the questionnaires. The answers to the questions were analyzed by Ordinary Linear Squares (OLS) regression using the ACA Sawtooth software. For each section, the respondents with correlation coefficients equal to or less than 0.8 were excluded from the analysis.

Table 5.1 List of potential risk factors included as attributes in the adaptive conjoint analysis survey, and their corresponding sources

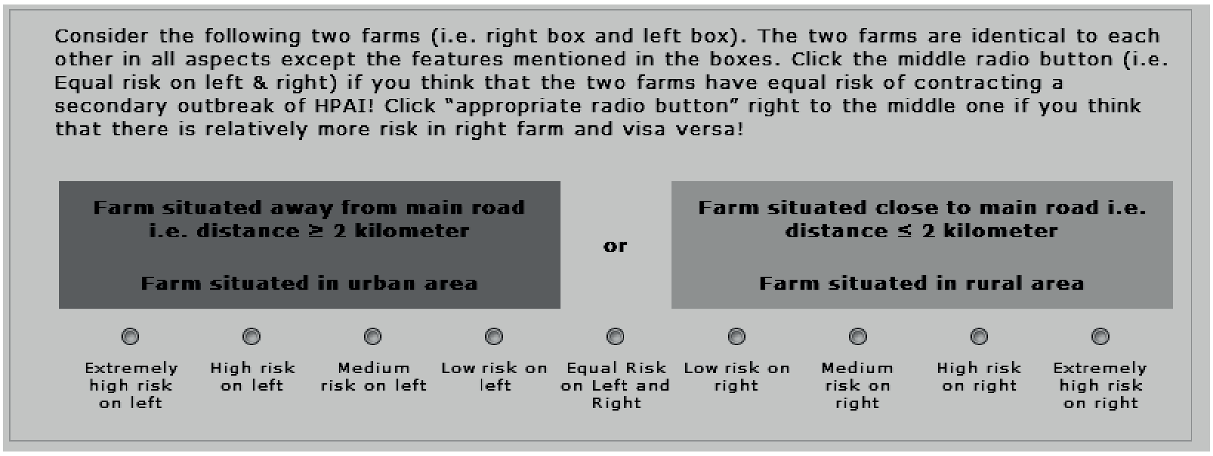
Category	Attribute
Area	Proximity to surface water body (≤ 10 km) (Cecchi et al., 2008; Fang et al., 2008; Gilbert et al., 2008)
	Short buffer distance among the farms (≤ 3km) (Elbers et al., 2004; Marangon et al., 2004)
	Location of farm close to main road (≤ 2) (Fang et al., 2008)
	Place near the farm where organic wastes (e. g. dropped feathers, droppings) from other poultry farms are disposed (≤ 1km) (Yamamoto et al., 2008)
	Distance to live bird market (≤ 1km) (Bulaga et al., 2003; Choi et al., 2005)
	Location of farm in urban area (Pfeiffer et al., 2007)
Pests	High prevalence of rodent infestation (ProMed-mail, 2007)
	Access of feral and wild animals into the farm (Biswas et al., 2009)
	Keeping backyard poultry or pet birds at farm (Terregino et al., 2007)
	Purchase of replacement stock (e. g. D) from a source with poor biosecurity (Kasemsuwan et al., 2008)
	Entry of wild birds into poultry sheds (Kung et al., 2007)
People	Visits of intermediaries and service providers (Nishiguchi et al., 2007)
	Involvement of relatives of worker with poultry production and /or marketing chain (Kung et al., 2007)
	Contact of workers with other farmers (S. Sharif, personal communication , 18 June , 2009)
	Visits of the owner to potential cross-contamination points e. g. poultry diagnostic laboratory, other farms, live bird market, office of the feed distributors (S. Sharif, personal communication , 18 June , 2009)
	Keeping at home of backyard poultry or pet birds by workers (and/or owner) (FAO, 2008)
Organic and organic things	Use of feed contaminated before and /during delivery (FAO, 2008)
	Sharing equipment with other farms e. g. manure (Nishiguchi et al., 2007)
	Purchasing poultry products from live bird market (for use on farm) (S. Sharif, personal communication , 18 June , 2009)
	Using water from untreated sources or surface water bodies without proper sanitization (Anjum, 2004)
	Admission of vehicles without cleaning and disinfection (FAO, 2008)



Consider the following two statements, given in black-colored text! As you see that the upper statement is a relatively high-risk situation as compared to the lower one. In terms of causing secondary outbreaks of HPAI on open-shed chicken farms, how much *important is the upper situation as compared to the lower one in the area of your consultancy? (*Important means PREVALENT AND DANGEROUS!)

	Not important	___	Somewhat important	___	Very important	___	Extremely important
L1 Farm situated close to live bird market i.e. distance ≤ 1 kilometer ---compared to--- L2 Farm situated away from live bird market i.e. distance ≥ 1 kilometer	○	○	○	○	○	○	○

Figure 5.1 ACA ranking question. Level 1 and level 2 are two extreme scenarios in which the attribute “location of farm close to live bird market” may occur. Clicking the extreme right radio button indicates that the respondent considers “location of farm close to live bird market” as an extremely important attribute for HPAI outbreak and vice versa. The respondent may check any one radio button to express his or her opinion.

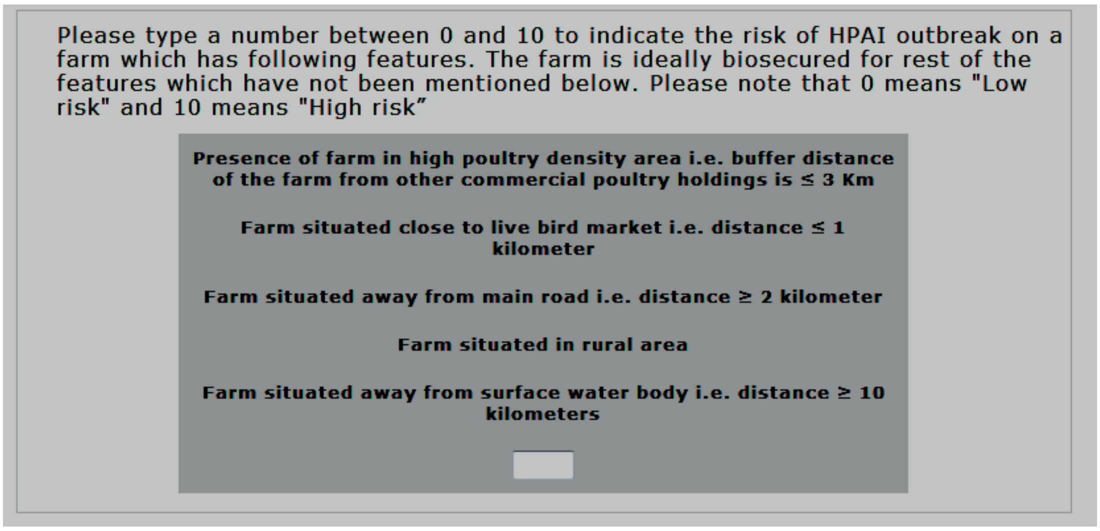


Consider the following two farms (i.e. right box and left box). The two farms are identical to each other in all aspects except the features mentioned in the boxes. Click the middle radio button (i.e. Equal risk on left & right) if you think that the two farms have equal risk of contracting a secondary outbreak of HPAI! Click "appropriate radio button" right to the middle one if you think that there is relatively more risk in right farm and visa versa!

Farm situated away from main road i.e. distance ≥ 2 kilometer Farm situated in urban area	or	Farm situated close to main road i.e. distance ≤ 2 kilometer Farm situated in rural area

○	○	○	○	○	○	○	○	○
Extremely high risk on left	High risk on left	Medium risk on left	Low risk on left	Equal Risk on Left and Right	Low risk on right	Medium risk on right	High risk on right	Extremely high risk on right

Figure 5.2 ACA paired comparison question. Combinations of levels from two different attributes are presented side by side. The software automatically selects those on the basis of similarities in utility (risk) score. The respondent has to trade off which combination is relatively more important.



Please type a number between 0 and 10 to indicate the risk of HPAI outbreak on a farm which has following features. The farm is ideally biosecured for rest of the features which have not been mentioned below. Please note that 0 means "Low risk" and 10 means "High risk”

Presence of farm in high poultry density area i.e. buffer distance of the farm from other commercial poultry holdings is ≤ 3 Km

Farm situated close to live bird market i.e. distance ≤ 1 kilometer

Farm situated away from main road i.e. distance ≥ 2 kilometer

Farm situated in rural area

Farm situated away from surface water body i.e. distance ≥ 10 kilometers

Figure 5.3 ACA calibration question. Each question contains levels of up to 5 attributes. On a numeric scale from 0-10, the respondent has to give the combined importance of the combination of levels.” 0 “means low where as “10” means extremely high risk.

5.3 Results

Among the various sections of the interview, the response rate ranged between 24% and 39%. In total, 13 respondents participated in this survey. The respondents were university teachers, animal health researchers, as well as public sector and private veterinarians. Since the number of respondents in each category was quite low, we did not stratify them in the analysis. The median experience of the respondents was 20 years. The median time to complete various sections ranged from 10 to 17 minutes. Three respondents from two different sections (animals, organic and inorganic items) had to be excluded for low level of consistency in their answers. Overall, the level of consistency among the respondents was more than 90%.

Table 5.2 shows the relative importance of the risk factors ranked as first, second and third in each risk category. Risk factors with the highest mean relative importance were: short buffer distance among the farms (23.9% ± 10.6%), entry of wild birds into poultry sheds (21.9 % ± 4.8%), visits of intermediaries and service providers (21.2% ± 7.1%), and sharing equipment with other farms (38.7 ± 7.2). The analysis of the survey results also revealed differences of opinion among the respondents as indicated by standard deviation. The risk factors showing the highest standard deviation in each category were (i) presence of farm close to main road [± 11.5]

(ii) access of feral and wild animals into farm premises [± 12.6] (iii) involvement of relatives of workers with poultry production and /or marketing chain [± 9.7] and (iv) use of feed contaminated before /or during delivery [± 17.3] .

Table 5.2 Mean relative importance of attributes ranked as first, second, and third within each risk category

Risk category (*n)	Attribute (potential risk factor)	**Mean ± SD***
Area (8)	Short buffer distance among the farms	23.9 ± 10.6
	Place near the farm where organic wastes (e. g. dropped feathers, droppings) from other poultry farms are disposed (≤ 1km)	20.3 ± 6.7
	Distance to live bird market (≤ 1km)	18.3 ± 10.7
Animals (9)	Entry of wild birds into poultry sheds	21.9 ±4 .8
	Access of feral and wild animals into the farm	20.7 ± 12.3
	Keeping backyard poultry or pet birds at farm	15.2 ± 2.2
People (12)	Visits of intermediaries and service providers	21.2 ± 7.1
	Contacts of owner or worker with other farmers	14.4 ± 6.8
	Visit of farm owner to potential cross contamination points	13.6 ± 4.6
Organic and inorganic vectors (7)	Sharing equipment	38.7 ± 7.2
	Admission of vehicles without cleaning and disinfection	33.9 ± 13.1
	Use of feed contaminated before /during delivery	14.4 ± 12.6

*n= Number of interviews included in analysis, **Relative importance was calculated based on the difference between risk estimates of L1 and L2 of each attribute for each expert, ***SD = Standard deviation As risk factors within each risk category were weighted with respect to each other and independent of those belonging to other categories, the relative importance of a risk factor falling into one category cannot not be compared with that of a risk factor belonging to any other risk category.

5.4 Discussion

The findings of the survey appeared plausible and all the respondents showed a high level of consistency in their answers. For some risk factors, however, we observed high standard deviation which may be due to the small sample size, uncertainties associated with the disease or tendency of the respondents to select middle or end choices of the Likert scale.

Between 2003 and 2004, HPAI H7N3 caused serious losses to the poultry industry in Pakistan. Exaggerated messages in the media created havoc and shunted the public to non-poultry protein sources. HPAI is therefore a sensitive issue and still a matter of great concern to the government, industry and the community. This was one of the reasons for the reluctance of respondents to participate in this survey. Another possible reason for the poor response rate appears to be lack of motivation. This might partially be overcome by providing incentives to the respondents or by collecting data during a workshop.

As in all expert elicitation methods, the selection of appropriate experts to participate is vital. Selection of inappropriate, incapable, or misrepresentative experts will compromise the process and therefore the opinion elicited (Webler et al., 1991). Previously, experts have been selected for participation in expert elicitation procedures based broadly on their experience in the field of interest and professional criteria such as education, publication record and membership of professional societies (Gallagher, 2005). Under the conditions prevailing in Pakistan, feasible criteria for selection of an expert panel may be experience in the field, willingness to participate in a survey and qualification. To get meaningful results, the knowledge of the veterinarians should be updated about the risk factors being considered. Inserting hyperlinks of the relevant publications in the questionnaire may be helpful in this regard.

The limitations of this survey are inherent to those of small pilot projects and include small sample size and limited generalizability. Another limitation of this survey was the fact that we divided the attributes into categories. As the risk factors within each risk category were weighted with respect to each other and independent of those belonging to other categories, the relative importance of a risk factor falling into one category could not be compared with that of risk factor belonging to any other risk category. In future surveys using ACA, all the risk factors should therefore be considered together.

In trade-off questions, combinations of levels from two or more attributes are presented side-by-side on the display of the computer. Ideally and technically, the respondents should consider the levels conjointly. The rank order of the risk factors may be distorted if the respondents subconsciously ignore some levels in decision making (B .McEvan, personal communication, June 6, 2009).

Due to its computer interface, web-based implementation, and questionnaire format, ACA appears to be an attractive alternative to paper-and-pencil techniques for elicitation of expert opinion, however further research is required to prove its feasibility and validity. This can be accomplished by repeating ACA questionnaire-based interviews twice on consistent respondents and by calculating Lin's concordance correlation coefficient (Lin, 1989; Cunningham et al., 1996). On the same respondents, results of ACA can be compared with those from the analytical hierarchy process (pair-comparison approach). In general, expert opinions may have a high degree of uncertainty and be subjected to reporting bias depending upon the political, economic and social implications of the disease under consideration. Experts cannot provide accurate information on the actual impact of a risk factor on the incidence of any disease partially due to spatio-temporal instability of the risk factors; however, an unbiased expert opinion elicited may improve policy making in the absence of data of optimum quantity and quality. Expert opinion-based risk modeling using accurate methods may provide a mechanism of sharing experiences among HPAI-endemic countries and those, which are at-risk or naïve for the disease, without a breach in data privacy.

6 General Discussion

Trans-boundary animal diseases (TADs) are livestock diseases that are important to many countries in economic, trade and/or food safety and sometimes in public health terms - as is the case with HPAI. A high level of importance is often attached to TADs because they have the potential to spread rapidly and reach epidemic proportions, but also because their control and eradication require cooperation between several countries (Obi et al., 2008). Like other highly contagious livestock diseases, HPAI affects poultry production via three main pathways: (i) through the direct impact of disease-related morbidity and mortality and the costs associated with ex-ante risk mitigation and/or ex-post coping measures that affect the incomes of producers and other stakeholders connected to poultry production and marketing, (ii) through government interventions aimed at disease control which include culling, marketing and movement restrictions, and investment in animal health infrastructure and disease preparedness, and (iii) through consumer and market reactions, both domestic and international, affecting demand for poultry and poultry products and their substitutes, and thus prices of products and production inputs (Otte et al., 2009).

HPAI (and LPAI)/AIVs) control in domestic poultry poses an unprecedented challenge for the veterinary profession because of the genetic versatility of these viruses, their invasion of large and geographically dispersed, high turnover domestic poultry populations, the possibility of asymptomatic persistence in domestic ducks and possibly other animal reservoirs (Otte et al., 2010). HPAI viruses may arise in terrestrial poultry from LPAI viruses which are prevalent in wild water fowl populations. Reports of HPAI infection in domestic poultry (mainly chickens and turkeys) have increased since the late 1990s. Current poultry production and marketing systems enhance the probability of AI virus selection for increased pathogenicity. H5N1 emerged in South China in 1996. Despite major efforts to control the virus, it is now firmly established in a number of countries in Asia and Africa and continues to evolve. Threats to human health are not restricted to H5N1 from poultry but can arise through the emergence of any novel influenza A virus from livestock with sufficient human-to-human transmissibility. Regional poultry production systems are extremely diverse, in terms of species, production methods, and marketing channels, but traditional smallholder production is ubiquitous. Market-oriented poultry producers are more important to the spread of infection than subsistence-oriented backyard poultry keepers. The poultry trade network operating through live bird markets is of key importance to the spread and maintenance of HPAI infection. These markets can themselves maintain infection chains and potentially have an important role in the molecular evolution of H5N1 virus (Otte et al., 2010).

Until now, significantly less emphasis has been placed on assessing the efficacy of risk reduction measures, including their effects on the livelihoods of smallholder farmers and their families. To improve the local and global capacity for evidence-based decision making on the control of HPAI and other diseases with epidemic potential, which inevitably has major social and economic impacts; studies have been carried out in Africa and Asia under the pro-poor HPAI risk reduction project (www.hpai-research.net/). For various countries, information was compiled in the form of background papers, on the current state of knowledge of poultry systems and their place in the larger economy of the study country, the current HPAI situation (and its evolution) , and institutional experiences with its control.

This thesis comprises of a series of studies which were carried out to provide insight into risk factors which may affect the epidemiology of AI in domestic poultry of Pakistan. Chapter 1 is an introduction into poultry industry, history, and epidemiology of previous detections of AI viruses, surveillance activities, and institutional responses. Poultry production is an important part of the agro industry and plays its role in food security of Pakistan. It comprises of several sectors that are interconnected and have stakes in each other. These include feed manufacturers, breeders, hatcheries, broiler and layer farms, besides companies dealing in poultry medicines etc. The first confirmed introduction of AI in Pakistan occurred in 1994. During 2003-2004, a HPAI H7N3 was diagnosed in the southern part of the country. Local and international news of human mortalities in Asia caused public panic, severe demand shock, and collapse of the prices. This caused tremendous economic losses to the poultry industry. As a part of its HPAI risk management, the Government prepared a contingency plan and established a surveillance system. Between 2006 and 2008, the country experienced several sporadic outbreaks of H5N1 including a cluster of human cases.

The comprehensiveness of chapter 1 is limited due to dearth of published and grey literature, national statistics, journal articles, and reports from other research efforts. From the available literature, conclusive answers to the following questions could not be found (i) how are the farms selected for active surveillance, (ii) what is the country-specific definition for sectors 1 to 3, (iii) Does the surveillance in general includes hatcheries and live bird markets, iv) what is the role of private veterinarians in surveillance, (v) what are the thresholds for clinical surveillance, (vi) What could be the possible factors which may mask the circulation of virus in domestic poultry, (vii) how does the information flow within the surveillance network?

There are no formal risk assessment studies on the introduction of AIVs into the country. As with many other countries, the possible routes may be wild migratory birds, legal and illegal transboundary trade of poultry and poultry products. The legal trade can involve import of the following (i) fancy, captive, game, and hobby birds from South Africa, (ii) cooked poultry products from South Africa and Malaysia, (iii) day-old grandparent stock chicks, day-old parent stock or breeder's chicks of layers and broilers and hatching eggs from France, Germany, Iran, and United Kingdom, and (iv) processed and cooked poultry products from China. The types of commodities, motivation, frequency, and volume of illegal trade through roads, air, and sea (if any) are unknown. It is also not clear whether the country has a risk of outbreaks due to poor quality vaccines, laboratory escape, and perhaps even bio-terrorism.

The rich Indus delta and the highlands are a great attraction for huge number of migratory wild birds coming from Siberia and central Asian states. A number of lakes, ponds, marshes, canals, and rivers make an ideal wetland habitat for the waterfowl and offer excellent harbour to a large variety of migratory population during each winter. During their journey, the birds make stopovers at lakes and water basins at Nowshera, Tanda Dam in Kohat, Swat, Chitral, Punjab, and Sindh. They spend the winter in warm lakes and wetlands along the Indus and Kabul rivers, or travel further south to India's swamps and reserves (especially in Rajasthan's Bharatpur wetland reserve) before returning in the spring to their northern breeding grounds (Anjum, 2004). Presently, there are more than 225 wetlands and 5 eco-regions in the country. There is not much information on the interface between wild birds and domestic poultry. Possibly infected waterfowl may come in direct contact with resident wild birds; backyard chickens or may contaminate the environment e. g. surface water. They may also be attracted by water and feed on poultry farms. Village chickens are more vulnerable as they may make effective contact with wild birds. Moreover, the sale of infected village chickens in live bird markets may introduce the virus into the commercial poultry circuit. Spill of infection from infected poultry to waterbirds may take place through resident wild birds, village chickens, meat, or offal of dead or slaughtered birds offered to wild birds, disposal of poultry manure and other effluents into the environment. The migratory season ranges from September to March and may be considered as a high risk period.

Wild bird surveillance programs have been initiated in several countries worldwide. These can provide epidemiological information about circulating viruses and identify changes in subtype prevalence in reservoir species. To identify geographic areas relatively more vulnerable to exposure of AI during the wild bird migratory period, a subset of AWC data was analyzed (chapter 3). Maps were created showing the distribution of monitoring sites and maximum count reported from various sites during 1987-2007. The number of sites was relatively high in Sindh. The data provided a crude approximation of the distribution of waterbirds during the migratory season. It was also possible create a list of H5N1 affected avian species reported in Pakistan. The data was found to contain a high proportion of missing values. Analysis was also carried out to locate clusters and outliers based on number of missing values per site. The AWC coordinates were geoprocessed with polygons of waterbodies and a raster map of predicted poultry density from FAO. Pixels representing estimated poultry per square kilometer were detected within 3 to 9 km range of the census sites or waterbodies in their proximity. Poultry rearing areas close to AWC sites or waterbodies as mapped in this study may be given priority to assess, communicate, and manage the risk of transmission of AIVs between poultry and wild birds.

Retrospective analysis of the H5N1 situation revealed that the number of outbreaks was significantly higher during the migratory season. This should not be taken as a cause-effect relation rather a hypothesis that needs further investigation. This study also showed the limitations of the available datasets and acknowledged their importance in context to AI. AWC sites and associated professionals can be a source of epidemiological and ornithological data. A combination of healthy, live, and hunter-killed wild birds (active surveillance, in particular for LPAI) and sick/dead wild birds (passive surveillance with a focus on HPAI) needs to be sampled. The sampling should be based on such factors as practical considerations, the species most likely to carry the virus, their relative abundance, migratory patterns, seasonal fluctuations in virus prevalence, and locations where these species have the greatest likelihood of interacting with poultry. There are no data on the epidemiological role of H5N1 and other AIVs in non-migratory wild birds. In addition, the susceptibility of these wild birds to the virus is unknown. The susceptibility and the role of indigenous resident wild-birds and local breeds of poultry in the epidemiology of AI also need attention.

Wild birds have long been known to play a role in the maintenance and transmission of LPAI viruses but were not considered an important means of spread of HPAI, other than having a potential role in local spread when wild birds are infected by poultry. However, events from 2003 onwards in Asia suggest that wild birds also play some role in the transmission of the H5N1 virus over relatively long distances, although the wild bird species responsible and mechanics of transference are still unclear. Studies carried out in Asia and Africa have revealed that trade in live poultry (legal and illegal) represents a much higher risk than wild birds in spreading the disease. Once the virus is introduced in a country the factors that may aid maintenance of disease may be (i) the structure of the poultry industry, consisting predominantly of backyard poultry and small scale market oriented commercial poultry production with minimum to moderate biosecurity, (ii) passive resistance to, or active efforts to circumvent state policies such as buying and selling chicks despite the government ban, (iii) existence of open live poultry markets characterized by interspecies mixing and poor sanitary conditions, (iv) deteriorating animal health delivery services, (v) under reporting, (vi) misdiagnosis with Newcastle disease, (vii) lack of integration e. g. purchasing farm inputs from different companies , and (viii) delay in depopulation for more than 48 hours (Obi et al., 2008; Sumiarto and Arifin 2008). As new information has become available, the understanding of the risk of disease introduction and spread posed by different actors in the poultry "business" has changed. Early in the HPAI pandemic, backyard producers were seen as the main "culprits." Blame later shifted to large producers, and then to small scale commercial farmers and traders. There is increasing realization that more resources should be allocated to control of HPAI in the small scale producers. Improving biosecurity in this sector will reduce the likelihood of flocks becoming infected and therefore, reduce the risk of large numbers of infected birds being dumped in live bird markets. If smallholder layer and broiler farmers are to improve their biosecurity measures and hence increase productivity and profitability, it is necessary to identify the appropriate biosecurity activities that will be worthwhile to implement. These activities should minimize the risk of disease entry and spread, cost-effective to implement, and financially rewarding for smallholders. Simply implementing a large number of biosecurity measures does not necessarily reduce disease risk. The biosecurity measures must match the existing but not necessarily all possible biosecurity risks. To do this, there is a need to understand what smallholders are currently doing, their economic circumstances, their understanding of risks and consequences, and their capacity to implement biosecurity (Patrick and Sudaryanto, 2010).

To describe how the disease may be transmitted between open-sided chicken farms, a cross-sectional survey was carried out in Kamalia, a sub-district of the Punjab. Between April and June 2009, an interview-based questionnaire was administered to a sample of 78 growers. The survey identified the following biosecurity risks for outbreak propagation: i) short buffer distances between farms, ii) disposal of carcasses and other organic wastes into the environment, iii) entry of feral birds into poultry sheds, iv) visits of poultry farmers to possible cross-contamination sites, v) absence of boundary walls, vi) incomplete biosecurity on high-risk visitors (i. e. those going inside the poultry houses), essential vehicles, and equipment used by vaccination crews, vii) visits of intermediaries and service providers and, viii) sharing of egg trays between layer farms at production. For most of the variables, there was no significant difference between the broiler and layer type of farms ($p \leq 0.05$). The risk of an extensive outbreak in Kamalia was concluded to be high due to its high poultry density, ubiquitous small-scale, market-oriented poultry production with medium to low biosecurity, and the affiliation of the farmers to multiple service providers. Improvement in biosecurity and targeted surveillance are therefore considered critical to limit the spread of infection should an outbreak occur.

The findings of this survey have implications for improving biosecurity on small scale commercial poultry farms. Biosecurity can be defined as the implementation of preventive measures to reduce the risk of introduction and spread of disease agents. Biosecurity includes bioexclusion (efforts to prevent diseases entering the farm) and biocontainment (prevention of disease spread on the farm). It is essentially a defensive health plan against poultry diseases that have the potential for reducing the magnitude of important factors associated with the transmission of these diseases e. g. the basic reproductive number (R_0), the period of infectiousness, and the probability of transmission (Obi et al., 2008). Although biosecurity is a private preventive investment constituting a necessary production input for each farmer, it has been recognized that poor biosecurity is a public bad because inadequate investment by a single farming agent increases exposure of other farmers within a susceptible region. Improved biosecurity in poultry production and trade is not only an important longer-term strategy to guard against the damaging effects of HPAI but also a complicated intervention that requires understanding of the entire market value chain (Obi et al., 2008). Adoption of cost-effective biosecurity requires an understanding of three issues. Firstly, the risks faced by a farmer; secondly, the effectiveness of control measures in minimizing these specific risks, and thirdly, the cost of implementing these control measures. Each farm is faced with a unique set of risks, and therefore requires a unique and individually tailored farm biosecurity plan. Several factors influence the adoption of farm biosecurity. Economic concern is the main factor, although other factors include the characteristics of farmers (farm experience, age, education, understanding of biosecurity, etc.), characteristics of farms (number of farms, size and capacity of shed, etc.), farm location, management and marketing systems, resource availability, whether other economic enterprises are undertaken by the family, and farmer attitudes to risk. In addition, the type of poultry operation (broiler or layer) influences the type of biosecurity adopted. Therefore, in order to provide recommendations for improving farm biosecurity more information is required on current biosecurity implementation at farm level, including the factors influencing the adoption of biosecurity measures (Patrick and Sudaryanto, 2010).

One of the risk factors which make Kamalia vulnerable to a large outbreak of AI is short buffer distance between the farms. HPAI epidemics in Europe, Canada and Southeast Asia have demonstrated the potential risks and major detrimental effects of HPAI in areas with a high density of poultry referred to as “densely populated poultry areas (DPPA)” (Marangon et al., 2004). These areas are highly susceptible to HPAI due to the fact that an outbreak on a single large farm can directly and indirectly infect or affect neighbouring farms. In many of these areas, there is considerable movement of vehicles and people from farm to farm (Capua and Alexander, 2004) leading to conditions that facilitate the spread of a virus once it has established. Control measures implemented by the veterinary authorities in DPPA can also contribute to the spread of virus to neighboring farms, for example through contaminated dust particles disturbed during the culling process or possibly through inadvertent carriage of virus by investigators checking farms for excess mortality or other evidence of infection (Power, 2005). Transmission by flies and vermin is also possible, given the fact that virus has been isolated from blow flies in Japan (Sawabe et al., 2006) and that HPAI viruses can multiply in a range of mammalian species, including mice without prior adaptation (Sims and Narrod, 2009). In DPPA, a "stamping out" policy involving the culling of poultry on infected farms, neighboring farms, contact premises, or in a zone of a certain diameter around an infected farm, can lead to the destruction of millions of poultry, as was seen in the Netherlands, where some 30 million heads of poultry were culled or died (Stegeman et al., 2004), and Canada (Bowes et al., 2004) following outbreaks of HPAI in 2003 and 2004. Airborne spread of virus over short distances has probably occurred, especially from heavily infected farms (Brugh and Johnson, 1986). Keeping in view the average flock size and structure of open-sided chicken farms, the extent of dispersion of small size dust particles (called PM_{10}) was calculated. At a velocity of 1 m/s, stable conditions, and higher emissions (broiler type of farms), concentrations of more than 0.01 $\mu g/m^3$ was simulated at a distance of 3 km. As previously noted, this investigation focused on the risk of exposure, rather than infection. Infection involves multiple factors, including viral adaptation to the host species, dose, and route of exposure (Leibler et al., 2010).

A case control study was designed to quantify the risk factors for secondary spread of HPAI but the study could not be fielded due to absence of cases and a possibility for selection and information bias. As the farms have affiliation with different companies and services providers, it was difficult to find suitable controls matched for these distance-independent exposures (confounders). In order to generate hypotheses, expert opinion was used. ACA is an individually tailored preferences elicitation technique that mimics actual decision- making processes by asking participants to make trade-offs between the various dimensions that underlie decision problems (Pieterse et al., 2010). In the past, the technique has been used to establish relative importance of the risk factors of bovine respiratory disease (Fels-Klerx et al., 2000), foot and mouth disease (Nissen, 2001), classical swine fever (Staerk et al., 1997), bovine herpes virus 1 infection, and John's disease (Milne et al., 2005). ACA interviews based on 21 attributes were completed by 13 local veterinarians with at least five years experience in poultry medicine (chapter 5). Each attribute contained two levels in natural order. The top-ranked risk factors identified in this survey were: short buffer distance between the farms, entry of wild birds into poultry sheds, visits of intermediaries and service providers, and sharing equipment with other farms. In a limited study, a set of six risk factors was further evaluated by 5 respondents. ACA and the paired comparison approach were used simultaneously. Despite a high level of consistency (more than 80%), there was a substantial mismatch in the rank order of the risk factors.

Recommendations

1) Disease awareness and surveillance during migratory season should be more focused on areas of waterbird concentration and poultry production. A buffer could be drawn around major inland waterbodies to map high risk areas. The Government has planned to register all commercial poultry farms in the country. For research, surveillance, risk analysis, and outbreak management, we recommend a geospatial database of the poultry holdings and live bird markets. A survey on the adoption of appropriate biosecurity measures, and a participatory disease surveillance programme in poultry rearing areas close to important wetlands are also recommended.

2) There is urgent need to design a biosecurity programme for small scale open-sided broiler and layer farms. In this context, special emphasis should be given to the disposal of carcasses of dead birds, manure, and other poultry effluents into the environment, covert sale of infected birds or their products into market (if any), high-risk visitors (vaccination crews, veterinarians, drivers, catching teams), shared materials (e. g. syringes, egg trays, equipment for postmortem), essential vehicles, and potential cross-contamination sites (live bird markets , poultry diagnostic laboratories, offices of the distributors). If smallholder layer and broiler farmers are to improve their biosecurity measures and hence increase productivity and profitability, it is necessary to identify the appropriate biosecurity activities that will be worthwhile to implement. These activities should minimize the risk of disease entry and spread .They should also be cost-effective to implement and financially rewarding for smallholders.

3) An epidemic of avian influenza has the potential to spread rapidly within, or through Kamalia. Therefore, structure and dynamics of the poultry industry should be documented in more details in order to determine how horizontal contacts could potentially affect the spread of infectious diseases. It may also be helpful to devise routine (level 1) and high-risk (level 2) biosecurity plans for all components of the poultry production and marketing chain. Future research may be targeted at issues such as delayed reporting by the farmers, failure to report, lapses in bio-containment, and within-country transmission.

4) Precautions should be adopted while culling in a high poultry density area to prevent risk of wind born transmission. A separate study is required to determine PM emissions rates from open sided chicken farms.

5) The validity and reliability of adaptive conjoint analysis as a tool for the elicitation of expert opinion in veterinary epidemiology needs further experimentation.

6) The main input for open-sided broiler and layer farms are day-old-chicks and feed. The hatcheries and feed mills are commercial and therefore assumed to practice high standards of production. For research, we recommend risk assessment studies on following worst-case scenarios (i) a vaccinated breeder flock become infected with H5N1 and does not maintain sentinel birds, (ii) infected offals from live bird market are used for preparation of crumbed feed.

7 Summary

Studies on potential risk factors for introduction and spread of avian influenza in domestic poultry of Pakistan

Since 1994, the domestic poultry in Pakistan has experienced several outbreaks due to avian influenza viruses (AIVs) of subtypes H7N3, H5N1, and H9N2. Many aspects of the epidemiology of the disease are unknown. Assessment of the risk factors for introduction, spread and persistence of AIVs is necessary so that informed decisions can be made by the government and the industry to manage outbreaks. In this thesis, available country-specific information on poultry production, avian influenza situation (and its evolution) and institutional responses was collated for epidemiological analyses and to assist in the design of control strategies. In addition, studies were carried out to provide insight into risk factors of the disease.

One possible route by which AIVs may be introduced into domestic poultry is through migratory wild birds. Pakistan is situated within the Central Asian flyway of migrating birds and contains more than 225 wetlands. The wetland areas provide wintering and staging grounds for a large number of migratory birds coming from Siberia and Central Asian states. The migratory season ranges from September until March. A retrospective case-series analysis of previous H5N1 outbreaks (2006-2008) was performed which revealed that 64% of outbreaks reported to the *Office International des Epizooties* (OIE) occurred during the migratory period. To answer the question, which areas should be given priority in surveillance and prevention of AIVs transmission during the migratory season, a subset of Asian waterbird census (AWC) data was reviewed and mapped. The data contained local names of 535 sites and annual mid-winter counts of waterbirds from 1987 to 2007. The majority of the sites were not counted regularly leading to gaps in sites-by-years data matrix, (here called missing values). The location of AWC sites provided a crude approximation of spatial distribution of waterbirds during the migratory period. It was also possible to map the maximum reported count per site and find out clusters of under-sampled sites (i. e. those with high number of missing values). With improved data on the distribution of migratory/waterbirds, the established geographic information system may help to

assess the risk of transmission of avian influenza viruses between migratory birds and domestic poultry. A list of wild bird species was generated that occur in Pakistan and were known to be infected with H5N1.

Another focus of this project was the investigation of the contact structure and possible transmission pathways among traditional open-sided chicken farms, a sub-sector of commercial poultry. Between April and June 2009, a cross-sectional survey was conducted in Kamalia, which is a part of the district Toba Tek Singh in central Punjab. Data were collected from 78 growers in interviews based on a standard questionnaire. Important findings of the survey regarding the transmission of AIVs were: short buffer distances among the farms, inappropriate methods for disposal of carcasses of dead birds, entry of bridge species into poultry sheds, incomplete biosecurity on high-risk visitors and essential vehicles, sharing of equipment (e. g. re-use of egg trays), and visits of farmers to potential cross-contamination points.

Considering flock size and structure of the farms and conventional meteorological assumptions (very stable boundary layers, very low and constant wind velocity as 1 m/s over 24 h and a wind direction straight towards the next adjacent settlement), the extent of the dispersion of small-sized particulate matter (PM_{10}) was simulated using a Lagrangian dispersion approach. For a velocity of 1 m/s, stable conditions and higher emissions (meat producing birds) concentrations of more than 0,01 $\mu g/m^3$ were simulated at a distance of 3 km. Compared to velocities of 3 m/s and indifferent conditions, the same concentration was detected up to a distance of 2.5 km.

Finally, a pilot study was conducted to elicit the opinion of poultry veterinarians regarding potential risk factors for HPAI outbreaks in Pakistan. For this purpose, the technique of adaptive conjoint analysis (ACA) was used which involves computer-mediated interactive interviewing optionally over the internet. A total of 21 risk factors “attributes” were divided into four categories namely area, pests, people, organic (and inorganic) items. The ACA interview was emailed to 33 local veterinarians in Pakistan. The response rate was 39%. Potential risk factors with the highest mean relative importance were: short buffer distance between the farms, entry of wild birds into poultry sheds, visits of intermediaries and service providers, and sharing high-risk equipment with other farms.

8 Zusammenfassung

Studien zu potentiellen Risikofaktoren für die Einschleppung und Verbreitung von aviärer Influenza beim Hausgeflügel in Pakistan

Seit 1994 kam es in Pakistan zu einer Reihe von Geflügelpestausbrüchen, die durch aviäre Influenzaviren der Subtypen H7N3, N5N1 und H9N2 verursacht waren. Viele Aspekte der Epidemiologie der Geflügelpest blieben dabei bislang unerforscht. Die Bewertung von Risikofaktoren für die Einschleppung, Verbreitung und dauerhaften Etablierung ist zumindest bezüglich der hochpathogenen aviären Influenza erforderlich, um auf dem Stand des Wissens beruhende Entscheidungen der Regierung und der Geflügelindustrie zum Umgang mit Seuchenausbrüchen zu ermöglichen. In der vorliegenden Arbeit wurden verfügbare Informationen zur Geflügelproduktion, zur aviären Influenza und der Entwicklung der Seuchenlage sowie der behördlichen Maßnahmen in Pakistan zum Zweck der epidemiologischen Analyse und zur Planung von Bekämpfungsstrategien zusammengestellt. Darüber hinaus wurden Untersuchungen durchgeführt, die Einsicht in Risikofaktoren für die Tierseuche vermitteln.

Ein Weg, über den aviäre Influenzaviren in Hausgeflügelbestände eingeschleppt werden können, stellen Zugvögel dar. Pakistan liegt im Bereich der zentralasiatischen und der ostafrikanisch-westasiatischen Zugroute und beherbergt mehr als 225 Feuchtgebiete. Diese Feuchtgebiete bieten einer großen Zahl von Zugvögeln, die aus Sibirien und zentralasiatischen Ländern stammen, von September bis März Gelegenheit zum Überwintern und Sammeln. Eine retrospektive Analyse der Fälle ergab, dass sich 64% der Primärausbrüche von hochpathogener aviärer Influenza des Subtyps H5N1, die der Weltorganisation für Tiergesundheit im Zeitraum 2006-2008 gemeldet worden waren, während der Zeit des Vogelzugs ereignet hatten. Die Ausbrüche befanden sich meist in Distrikten mit größeren Feuchtgebieten. Zur Beantwortung der Frage, welche Gebiete bei der Überwachung und der Verhinderung der Übertragung von aviärem Influenzavirus des Subtyps H5N1 auf Hausgeflügel während des Vogelzugs Vorrang gegeben werden sollte, wurden Daten des Asian Waterbird Census (AWC) aufbereitet und kartiert. Die AWC-Daten ermöglichte eine grobe Einschätzung der räumlichen Verteilung von Wasservögeln.

Das Maximum der pro Zählstandort registrierten Vögel konnte in einer Karte dargestellt und Cluster von fehlenden Angaben ermittelt werden. Mit besseren Daten zur Verbreitung von Zug- und Wildvögeln kann das etablierte Geografische Informationssystem behilflich sein, das Risiko der Übertragung von aviären Influenzaviren von Zugvögeln auf Hausgeflügel besser einzuschätzen. Darüber hinaus wurde eine Liste von Vogelarten erstellt, die Pakistan vorkommen und bei denen Infektionen mit aviärem Influenzavirus des Subtyps H5N1 gezeigt worden waren.

Ein weiterer Schwerpunkt des Projektes war die Untersuchung von Kontaktstrukturen und möglichen Übertragungswegen zwischen den traditionellen seitlich offenen Hühnerhaltungen, die einen erheblichen Teil der kommerziellen Geflügelproduktion ausmachen. Im Zeitraum von April bis Juni 2009 wurde eine Querschnittstudie in Kamalia, einem Teil des Distriktes Toba Tek Singh in Zentral-Punjab durchgeführt. Mit Hilfe eines Fragebogens wurden in 78 Betrieben Daten erhoben. Zu den für die Ausbreitung von aviären Influenzaviren wichtigen Ergebnisse der Studie gehörten: kurze Entfernungen zwischen den Betrieben, unangemessene Methoden der Beseitigung von Vogelkadavern, Zugang von Brücken-Vogelarten zu den Geflügelställen, unvollständige Biosicherheitsmaßnahmen in Bezug auf Besucher- und Fahrzeugverkehr mit hohem Risiko, gemeinsame Nutzung von Ausrüstung (z.B. Wiederverwendung von Eierkartons) sowie Besuche von Betriebsinhabern an Orten, wo Kreuzkontaminationen möglich waren.

Unter Berücksichtigung der Herdengröße, der Struktur der Betriebe und bei Annahme von in der Region üblichen meteorologischen Bedingungen (sehr stabile Grenzlagen, sehr geringe, konstante Windgeschwindigkeiten von 1 m/s für 24 Stunden, sowie einer Windrichtung direkt auf die nächstliegende Siedlung zu), wurde das Ausmaß der Dispersion von potentiell Virus-kontaminiertem Feinstaub (small-sized particulate matter; PM_{10}) mit Hilfe eines lagrangischen Dispersionsansatzes simuliert. Bei einer Windgeschwindigkeit von 1 m/s, stabilen Bedingungen und stärkeren Emissionen (Fleischproduktion) ergab die Simulation Konzentrationen von mehr als 0,01 $\mu g/m^3$ in 3 km Entfernung. Im Vergleich dazu wurde dieselbe Konzentration bei einer Windgeschwindigkeit von 3 m/s und ansonsten unveränderten Bedingungen bis zu einer Entfernung von 2.5 km gefunden.

Um Expertenwissen zu potentiellen Risikofaktoren für Ausbrüche von hochpathogener aviärer Influenza zu erheben, wurde schließlich bei Geflügeltierärzten eine Pilotstudie durchgeführt.

Dabei fand die Methode der der “Adaptive Conjoint Analysis” (ACA) Anwendung, einer Computer-vermittelten interaktiven Befragung, die über das Internet erfolgen kann. Insgesamt 21 potentielle Risikofaktoren (‘Attribute’) wurden vier Kategorien (Raum, Schädlingsbekämpfung, Menschen und Gegenstände) zugeordnet. Der ACA-Fragebogen wurde 33 in Pakistan ansässigen Tierärzten per E-mail zugesandt. 39% beantworteten die Fragen. Die potentiellen Risikofaktoren mit dem höchsten mittleren Gewicht waren: kurze Entfernungen zwischen den Betrieben, Zugang von Wildvögeln zu den Ställen, Besuche von Zwischenhändlern und Dienstleistern, und gemeinsame Nutzung von Gegenständen, die einem hohen Kontaminationsrisiko ausgesetzt sind, in mehreren Betrieben.

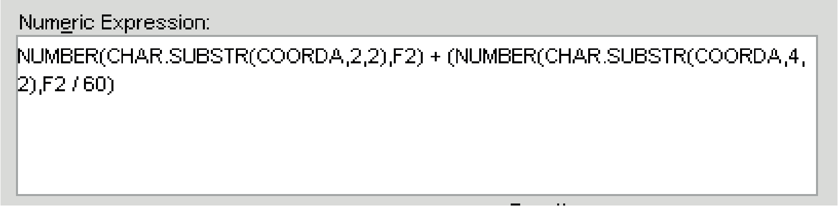
9 Appendices

9.1 Appendix A: Important operations performed in ArcGIS 10 and SPSS 19

Conversion of coordinates from Decimal Minutes (DM) to Decimal Degree (DD)

In the data provided by WI, the coordinates of each site were given as single string e. g. N2625E06740.

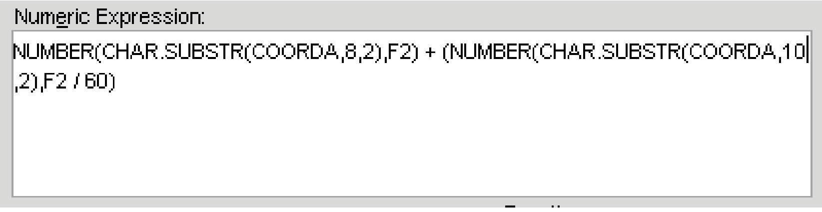
The following expression, “sub-stringed Y/North coordinate,” “changed it to numeric format” and then to a decimal value



Numeric Expression:

```
NUMBER(CHAR.SUBSTR(COORDA,2,2),F2) + (NUMBER(CHAR.SUBSTR(COORDA,4,
2),F2 / 60)
```

The following expression, “sub-stringed X/East coordinate”, changed it to “numeric format” and then to a decimal value

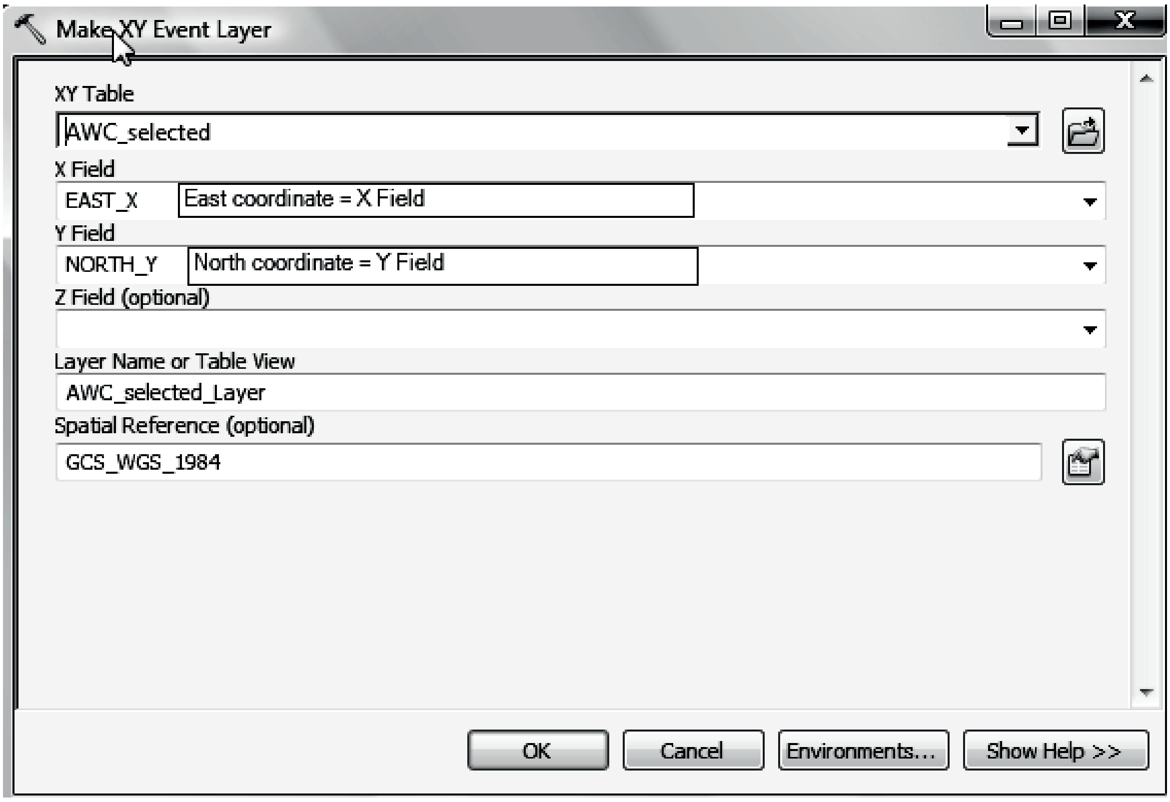


Numeric Expression:

```
NUMBER(CHAR.SUBSTR(COORDA,8,2),F2) + (NUMBER(CHAR.SUBSTR(COORDA,10
,2),F2 / 60)
```

Using XY event table create an XY event Layer and export that to a shape file

File > Add data > select file (. DBF) containing AWC coordinates and other relevant data, then Arc Toolbox > Data management tools > Layers and Table Views > Make XY event layer, then Select event layer > Data > Export data


Make XY Event Layer

XY Table: AWC_selected

X Field: EAST_X

East coordinate = X Field

Y Field: NORTH_Y

North coordinate = Y Field

Z Field (optional):

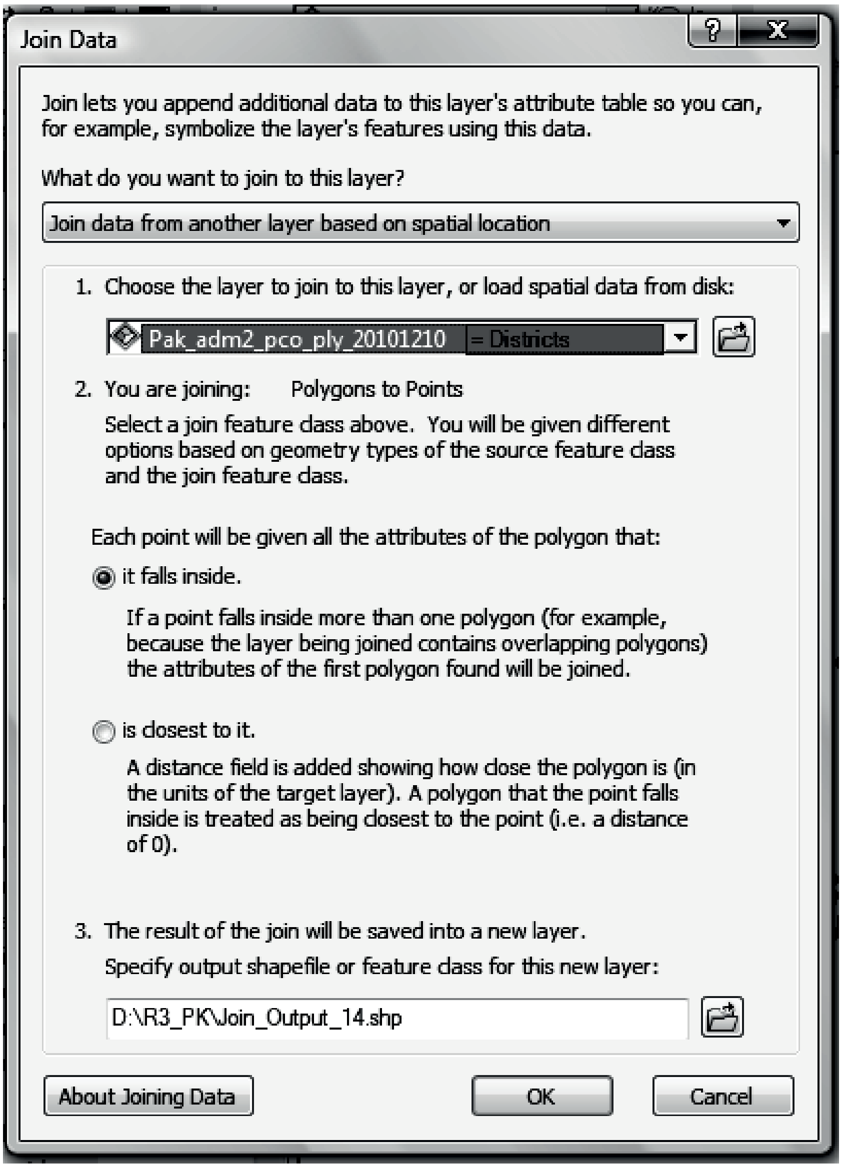
Layer Name or Table View: AWC_selected_Layer

Spatial Reference (optional): GCS_WGS_1984

Appendix A

Spatial join of AWC layer to districts or SRTM water bodies

Select layer > Join > Join data from another layer on spatial location > Choose layer of districts



Join lets you append additional data to this layer's attribute table so you can, for example, symbolize the layer's features using this data.

What do you want to join to this layer?

Join data from another layer based on spatial location

1. Choose the layer to join to this layer, or load spatial data from disk:

   Pak_adm2_pco_ply_20101210 = Districts

2. You are joining: Polygons to Points

   Select a join feature class above. You will be given different options based on geometry types of the source feature class and the join feature class.

   Each point will be given all the attributes of the polygon that:

   ● it falls inside.

   If a point falls inside more than one polygon (for example, because the layer being joined contains overlapping polygons) the attributes of the first polygon found will be joined.

   ○ is closest to it.

   A distance field is added showing how close the polygon is (in the units of the target layer). A polygon that the point falls inside is treated as being closest to the point (i.e. a distance of 0).

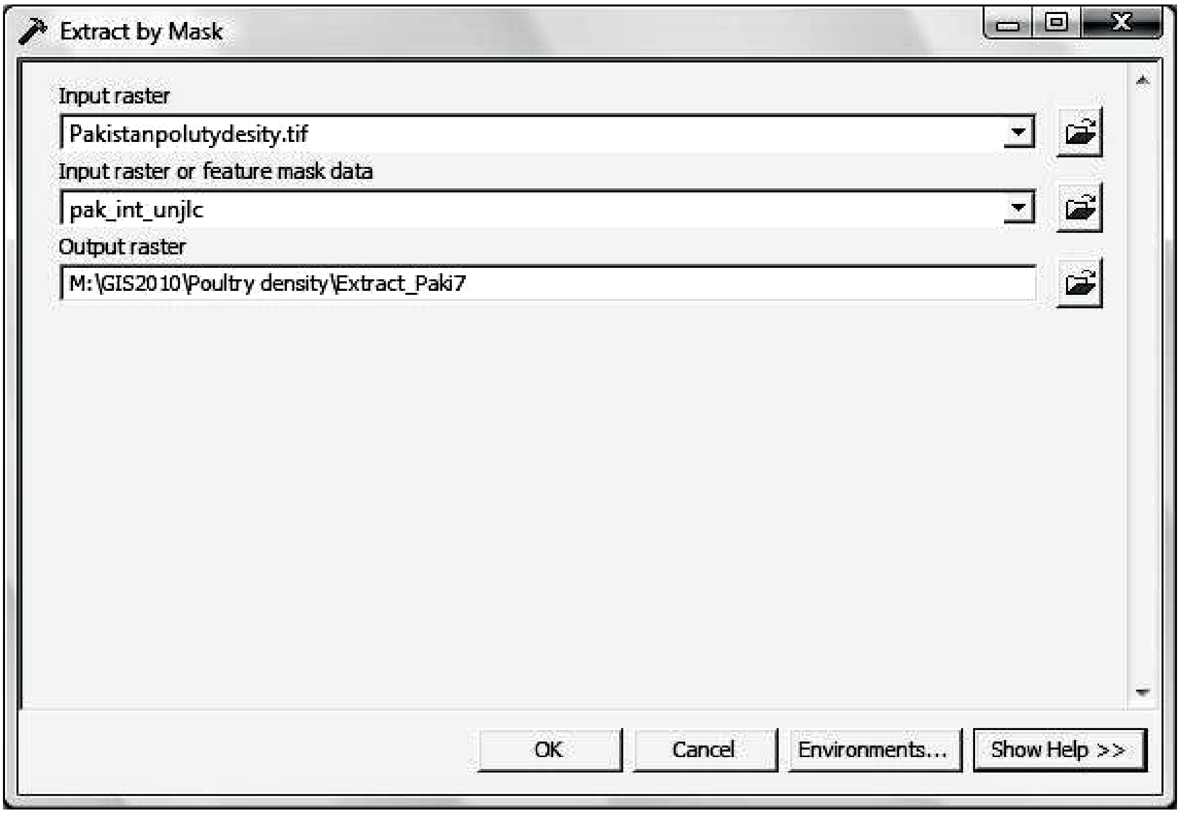
3. The result of the join will be saved into a new layer.

   Specify output shapefile or feature class for this new layer:

   D:\R3_PK\Join_Output_14.shp

Note: The same procedure was adopted for SRTM waterbodies

Extraction of cells of poultry density raster corresponding to administrative boundaries of Pakistan



Input raster: Pakistanpolutydesity.tif

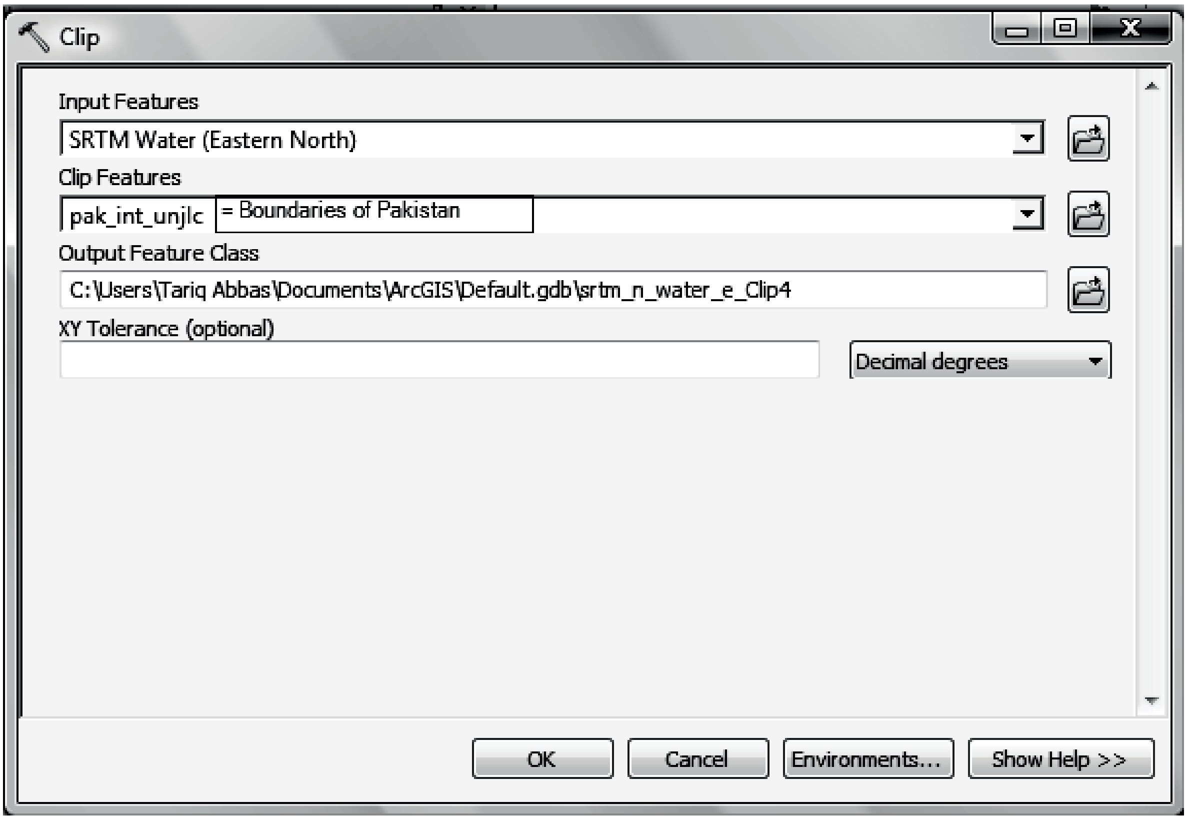
Input raster or feature mask data: pak_int_unjlc

Output raster: M:\GIS2010\Poultry density\Extract_Paki7

The poultry density map obtained from FAO GeoNetwork was for whole Asia. The data relevant to Pakistan was extracted by a mask of a polygon representing the borders of Pakistan (pak_int_unjil). To avoid misalignment, the extent of the output was defined equal to input raster in Environmental settings. The raster was floating point and had no Value Attribute Table (VAT). The following command (in raster calculator) was used to convert it into integer

Int [(Raster) + 0.5]

Clipping of SRTM waterbodies according to the administrative boundaries of Pakistan



Clip

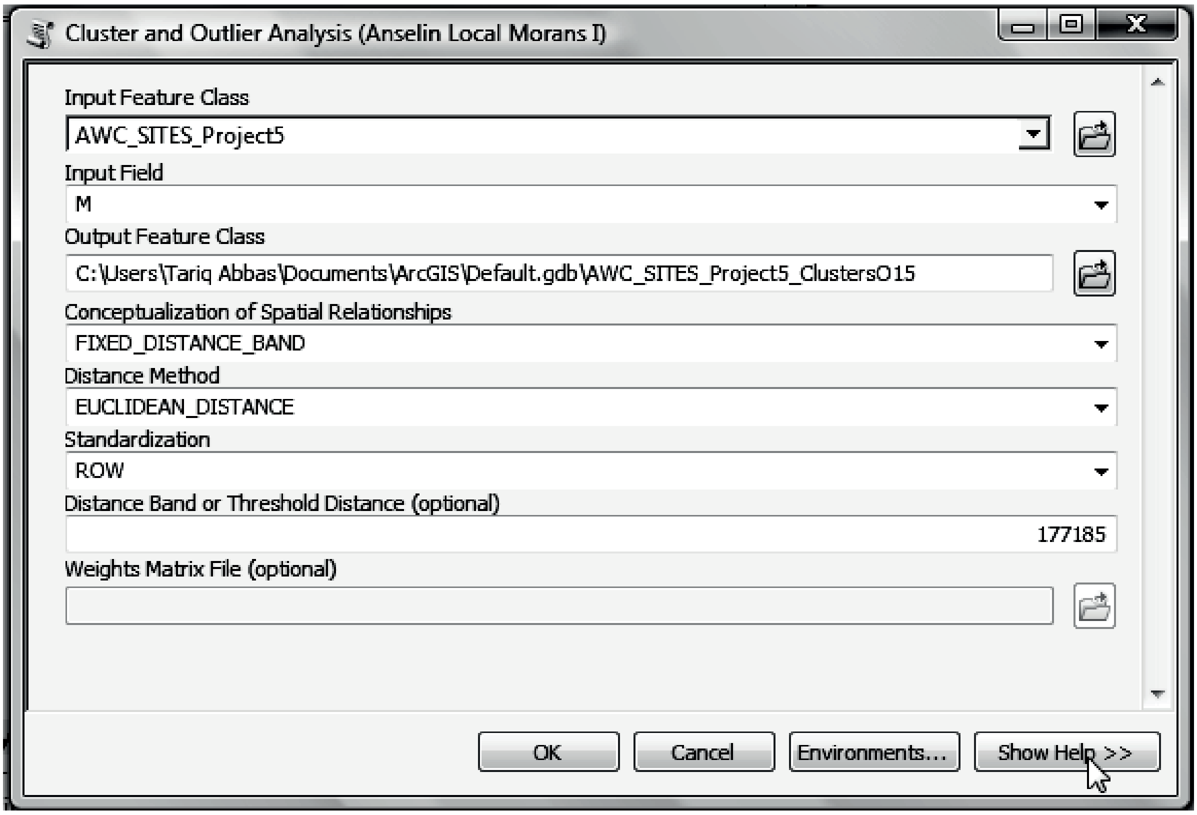
Input Features: SRTM Water (Eastern North)

Clip Features: pak_int_unjlc = Boundaries of Pakistan

Output Feature Class: C:\Users\Tariq Abbas\Documents\ArcGIS\Default.gdb\srtm_n_water_e_Clip4

XY Tolerance (optional): Decimal degrees

Cluster and outlier analysis (Anselin Local Morans I)



Cluster and Outlier Analysis (Anselin Local Morans I)

Input Feature Class: AWC_SITES_Project5

Input Field: M

Output Feature Class: C:\Users\Tariq Abbas\Documents\ArcGIS\Default.gdb\AWC_SITES_Project5_ClustersO15

Conceptualization of Spatial Relationships: FIXED_DISTANCE_BAND

Distance Method: EUCLIDEAN_DISTANCE

Standardization: ROW

Distance Band or Threshold Distance (optional): 177185

Weights Matrix File (optional):

The local Morans I statistic [17] is given as

$$\mathrm{I_i} = \frac{\mathrm{x_i} - \overline{\mathrm{X}}}{\mathrm{S_i^2}} \sum_{\mathrm{j=1,j\neq i}}^{\mathrm{n}} \mathrm{w_{i,j}}(\mathrm{x_i} - \overline{\mathrm{X}})$$

Where i is a feature (site) with attribute value = number of missing observations (1987-2007), j represent neighboring sites within threshold distance (search radius), $\bar{X}$ is the mean of the missing values, $w_{i,j}$ is the spatial weight between feature i and j, and: With n equating to the total number of features.

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[17] http://resources.esri.com/help/9.3/ArcGISDesktop/com/GP_ToolRef/spatial_statistics_tools/how_cluster_and_outlier_analysis_colon_anselin_local_moran_s_i_spatial_statistics_works.htm

9.2 Appendix B: Questionnaire

What type of farm is this? ☐ Broiler ☐ Layer (brooding/growing) ☐ Layer (at egg production)

Approximately, how many birds are being reared on this farm?

Which of the following choices describe production/husbandry system being practiced on this farm? ☐ All-in-all-out ☐ All-in-gradual out ☐ Non-specific

How much is the distance of the farm to the nearest commercial poultry holding? Provide approximate straight line distance along with a unit!

Which of the following methods is being used to dispose carcasses of dead birds?

☐ Bury on-site ☐ Burry off-site ☐ Through in nearby field ☐ Other

Do feral birds (e. g. sparrows) enter into poultry sheds? ☐ Yes ☐ No

[Hint: Check “Yes” if window gills or building of the shed is porous and /or door is permissive e. g. Open, damaged, poorly structured]

Do feral and wild animals have access to the farm premises? ☐ Yes ☐ No

[Hint: Check “Yes” if boundary wall is absent, damaged, and/or gate is permissive e. g. open, damaged, poorly structured]

Which of the following biosecurity measures are usually implemented on visitors who enter the sheds?

- Provision of clean footwear or scrubbing of shoes in use ☐ Yes ☐ No
- Provision of clean clothes ☐ Yes ☐ No
- Use of footbath or disinfectant spray [Hint: confirm through inspection] ☐ Yes ☐ No
- Washing of hands before handling the birds ☐ Yes ☐ No

Which of the following biosecurity measures are usually implemented on the vehicles which enter the farm premises or near the poultry sheds?

- Cleaning the wheels with a hose or wet brush ☐ yes ☐ No
- Spraying disinfectant on the wheels ☐ Yes ☐ No

Does the owner of the farm ensure disinfection of the equipment used for vaccination?

☐ Yes ☐ No

Which of the following biosecurity measures, the owner usually practice after visiting a poultry diagnostic laboratory, another poultry farm, office of the feed distributor or live bird market?

▪ Shower	☐ Yes ☐ No
▪ Scrubbing and disinfection of footwear in use or change into clean shoes	☐ Yes ☐ No
▪ Washing and disinfection of clothes in use or change into clean clothes	☐ Yes ☐ No

For broiler growers only

During the previous flock, how many times, the following people visited the farm?

Veterinarian from feed company (routine visits)	
Commercial vaccinators (for immunization)	
Feed delivery personnel	
Persons (owner/workers) from other poultry farms	

During previous flock, how many times, the owner visited the following places

Office of the feed distributor	
Other poultry farms	
Live bird market	

During the previous flock, did the owner transported diseased or dead birds to a poultry diagnostic laboratory or office of the feed distributor?

For layer growers with age more than 4 weeks

During previous four weeks, how many times, the following people visited the farm?

Veterinarian from feed company (routine visits!)	
Feed delivery personnel	
Persons (owner/workers) from other poultry farms	

Appendix B

During previous four weeks, how many times, the owner visited the following places?

Office of the feed distributor	
Other poultry farms	
Live bird market	

What is the frequency of visits of the following at this farm?

	Number of visits per flock
Commercial vaccinators	
Crew for beak trimming	
	Number of visits during laying period
Cake out personnel	
Waste haul personnel	

[Hint: Cake out = removal of manure from sheds while the farm is operational, waste haul = removal of manure from the property, personnel = staff from companies dealing in manure]

For layer farms at egg production

Do you re-use paper /cardboard egg trays? ☐ Yes ☐ No

How many times per month, egg transporters visit this farm in order to collect eggs?

During this flock, did the owner transported diseased or dead birds to a poultry diagnostic laboratory or office of the feed distributor?

9.3 Appendix C: OLS utility calculation in ACA Sawtooth software[18]

Consider a small ACA study with three attributes, each having 3 levels.

Attribute	Levels
Risk factor A	A1, A2, A3
Risk factor B	B1, B2, B3
*Risk factor C	C1, C2, C3

**Rank order of the levels known, C1: Best (=high risk), C3: Worst (=low risk)*

Assume an expert answers as follows

ACA rating questions

Question	Response (7-point scale)
A1	3
A2	2
A3	6
B1	7
B2	1
B3	5
As rank order of C is known, ACA rating questions were omitted.	

(Note: For the study, ACA rating questions were not asked in interview as hierarchy of levels was known)

ACA importance questions

Attribute (“Best “ versus “Worst “level)	Importance rating (7-point scale)
Risk factor A	6
Risk factor B	2
Risk factor C	3

ACA pair questions

Left concept	Right concept	Answer (9-point scale)
A3, C3	A1, C2	2
B3, C2	B2, C1	5
B3, A3	B1, A2	3
A1, B2	A2, B1	8
A1, C1	A2, C3	1
C1, B3	C3, B1	2

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[18] This document has been adopted from the material provided by Sawtooth Software, Inc, Sequim, USA

Appendix C

Calibration concepts [“worst (low risk) “, “Best (high risk)”, then “in-between”]

Concept	Combination of levels	Answer (0-100)
1	A2, C3, B2	0
2	A3, C1, B1	90
3	A2, C3, B1	50

Initial estimates

The first step in the computation of prior utilities is reversing of the rank order. For example, ranks of 1, 2, and 3 would be converted to values 3, 2, and 1. The rank reversal converts preference into desirability.

The average desirability for each attribute is subtracted to center its values at zero. For example, desirability values 3, 2, and 1 would be converted to 1, 0, and -1.

The values for each attribute are scaled to have a range of unity. For example, values of 1, 0, and -1 would be converted to 0.5, 0, and -0.5.

The importance ratings for each attribute are used as multipliers for the unit-range desirability values. Multiplier = x*4/n, where x is the respondent’s importance rating and n is the number of scale points used.

4-point scale		**7-point scale**	
Importance	**Multiplier**	**Importance**	**Multiplier**
1	1*4/4=1	1	1*4/7=0.571
2	2*4/4=2	2	2*4/7=1.143
3	3*4/4=3	3	3*4/7=1.714
4	4*4/4=4	4	4*4/7=2.286
		…..	…….
		7	7*4/7=4.000

(Note: It is not necessary to scale the range for prior utilities within each attribute to have a maximum of 4, because we normalize the “sums of differences” across attributes in further steps below. It is due to historical reasons that a maximum utility range of 4 was used for priors)

For this example

Level	Raw desirability	Centered desirability range =1	Importance multiplier	Final priors
A1	3	-0.167	6*4/7= 3.4286	-0.571
A2	2	-0.417	-	-1.429
A3	6	0.583	-	2.000
B1	7	0.444	2*4/7= 1.1429	0.508
B2	1	-0.556	-	-0.635
B3	5	0.111	-	0.127
C1	3	0.500	3*4/7= 1.7143	0.857
C2	2	0.000	-	0.000
C3	1	-0.500	-	-0.857

Pair utilities

An independent variable matrix is constructed with as many columns as levels taken forward to the pairs questions. If a level is displayed within the left concept, it is coded as -1; levels displayed within the right-hand concept are coded as +1. All other values in the independent variable matrix are set to 0.

A column is created for the dependent variable as follows: the respondents' answers are zero-centered, where the most extreme value for the left concept is given 4 and the most extreme value on the right +4. Interior ratings are fit proportionally within that range.

Each pairs question contributes a row to both the independent variable matrix and dependent variable column vector. Additionally an n x n identity matrix is appended to the independent variable matrix, where n is the total number of levels taken forward to the pairs questions. Additional n values of 0 are also appended to the dependent variable matrix. The resulting independent variable matrix and dependent variable column vector each have t + n rows, where t is the number of paired questions and n is the total number of levels taken forward to the pairs questions. OLS utility estimates of the n attribute levels are computed by regressing the dependent variable column vector on the matrix of independent variables (no intercept computed).

Appendix C

	Left concept	**Right concept**	**Answer (9-Point scale)**
Pair 1	A3, C3	A1, C2	2
Pair 2	B3, C2	B2, C1	5
Pair 3	B3, A3	B1, A2	3
Pair 4	A1, B2	A2, B1	8
Pair 5	A1, C1	A2, C3	1
Pair 6	C1, B3	C3, B1	2

	A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**	**Dep.Var.**
Pair 1	1	0	-1	0	0	0	0	1	-1	-3
Pair 2	0	0	0	0	1	-1	1	-1	0	0
Pair 3	0	1	-1	1	0	-1	0	0	0	-2
Pair 4	-1	1	0	1	-1	0	0	0	0	3
Pair 5	-1	1	0	0	0	0	-1	0	1	-4
Pair 6	0	0	0	1	0	-1	-1	0	1	-3
Identity matrix	1	0	0	0	0	0	0	0	0	0
	0	1	0	0	0	0	0	0	0	0
	0	0	1	0	0	0	0	0	0	0
	0	0	0	1	0	0	0	0	0	0
	0	0	0	0	1	0	0	0	0	0
	0	0	0	0	0	1	0	0	0	0
	0	0	0	0	0	0	1	0	0	0
	0	0	0	0	0	0	0	1	0	0
	0	0	0	0	0	0	0	0	1	0

OLS “ridge” regression (9 independent variables, 15 “cases”) is run (without estimating an intercept).

“Pairs” Betas (utilities):

A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
-0.610	-0.592	1.202	0.596	-1.361	0.765	1.634	-0.828	-0.806

These utilities are then “normalized,” so they have the same “sums of differences” (maximum minus minimum utilities across attributes).

Sum of differences for the “Pairs” utilities is:

(1.202--0.610) + (0.765--1.361) + (1.634--0.828) = 6.400

Sum of differences for “Priors” utilities is 6.286

Multiply all Priors utilities by 6.400/6.286, putting them on the same “scale” as the Pairs utilities:

“Adjusted” Priors Utilities:

A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
-0.582	-1.455	2.037	0.517	-0.647	0.129	0.873	0.000	-0.873

Combining the Priors and Pairs Utilities:

The prior utilities for levels also included in the pairs questions are multiplied by n/ (n + t), where n is the total number of levels used in the Pairs section, and t is the number of pairs questions answered by the respondent. Any element in the priors that was not included in the Pairs section is not modified. The pairs utilities are multiplied by t / (n + t).

Priors “contribution” = 9 / (9 + 6) = 0.60

Pairs “contribution” = 6/ (9 + 6) = 0.40

The “Adjusted” Priors and “Pairs” utilities (after multiplication by the “contribution” weights specified above) are added together. These are the final utilities, prior to calibration.

A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
-0.593	-1.110	1.703	0.549	-0.932	0.384	1.177	-0.331	-0.846

Example:

A1 = (-0.582) (0.60) + (-0.610) (0.40) = -0.593

Appendix C

Calibration (using calibration concepts)

As a final step the utilities are calibrated. The procedure is as follows :

p=the predicted preference of applying a concept

x1=the concept’s utility based on the final “uncalibrated” utilities

b1 = the coefficient used to weight the utilities

α = an intercept parameter

Calibration concepts:

Concept	**Combination of levels**	**Answer (0-100)**
1	A2, C3, B2	0
2	A3, C1, B1	90
3	A2, C3, B1	50

Responses are trimmed to the range of 5 to 95. Then, a logit transform is applied, $\ln[x/(100-x)] \sim \alpha + b1x1$ here x is the trimmed response.

For each concept shown, compute the total utility based on the final utilities prior to calibration.

10	**Independent variable**	**Dependent variable**
	Total concept utility	**Logit transformed response**
Concept 1	-2.888	$\ln[5/(100-5)] = -2.994$
Concept 2	3.429	2.197
Concept 3	-1.407	0.000

Another OLS regression is estimated using the single independent variable above to predict the logit transformed response, computing an intercept this time.

Beta =0.725

Constant= -0.040

R-Squared= 0.862

If the regression coefficient (R-squared) is less than 0.00001, it is assumed that the estimation is faulty and a conservative positive value (0.00001) is used. The R-squared (measure of fit) is set to 0 in such cases.

To calibrate the utilities, each part worth is multiplied by the slope (beta). The intercept is divided by the number of attributes (3 in our example), and that quotient is added to the part worth for every attribute level.

Final Utilities:

A1	A2	A3	B1	B2	B3	C1	C2	C3
-0.443	-0.818	1.221	0.384	-0.689	0.265	0.840	-0.254	-0.627

Example: A1=-0.593*0.725+ (-0.04/3)=-0.4

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List of publications

Abbas T, Wilking H, Detlef HB, Conraths FJ (2011). Contact structure and potential risk factors for avian influenza transmission among open-sided chicken farms in Kamalia, an important poultry rearing area of Pakistan. Berl Munch Tierarztl Wochenschr (in press)

Abbas T, Wilking H, Staubach C, Conraths FJ. Framework for spatial analysis to identify hotzones for surveillance and prevention of avian influenza in domestic poultry of Pakistan. Robert Koch Institute, Berlin, Germany 22-24 November 2009.

Abbas T, Wilking H, Staubach C, Conraths FJ. Priority areas for surveillance and prevention of High Pathogenic Avian Influenza subtype H5N1 during waterbirds migration season in Pakistan. ESRI, Colorado, USA, 16-18 October 2010.

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T. Abbas, M. Ziller, H. Wilking, and F.J. Conraths (in process). Pilot study on the use of adaptive conjoint analysis to assess risk factors for high pathogenic avian influenza outbreaks on commercial poultry holdings of Pakistan

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Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

Berlin, den 10. Juni 2011

Tariq Abbas