EPIDEMIOLOGIC AND ECONOMIC ANALYSIS OF AVIAN INFLUENZA IN NEPAL

A Thesis

by

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ABSTRACT

Many countries, including Nepal, have been affected with highly pathogenic avian influenza (HPAI) outbreaks. There have been human mortalities in some countries and large numbers of poultry either died or were culled due to HPAI. The overall objective of this thesis was to improve our understanding of the epidemiology and economics of avian influenza (AI), and particularly HPAI, in Nepal.

We determined the seroprevalence of and risk factors for AI virus antibodies presence in ducks in Kathmandu, Nepal. The estimated true prevalence of AI viruses (AIV) antibodies was 27.2% [95% Confidence Interval (CI): 24.6- 29.5]. Age of the ducks was identified as the only risk factor for AIV seropositivity. Ducks older than one year were more likely to be seropositive compared to ducks less than six months of age [Odds Ratio= 2.17 (95% CI: 1.07- 4.39)]. This study provided baseline information about seroprevalence of AIVs in Kathmandu that will benefit further research to differentiate the subtypes of AIVs circulating in Kathmandu.

We also evaluated alternatives to the current control program (CCP) for HPAI in Nepal. The considered alternatives were: (i) absence of control measures (ACM) and (ii) vaccinating 60% of the domestic poultry flock twice per year. Cost-benefit analysis approach was used to evaluate the economic feasibility of the programs. In terms of the benefit-cost ratio, our findings indicated that there is a return of 1.96 dollars for every dollar spent in the CCP compared to ACM. The net present value of the CCP versus

ACM was US$ 989,918. The vaccination program yielded a return of 2.41 dollars for every dollar spent when compared to the CCP. The net present value of vaccination versus implementing the CCP was US$ 13,745,454. These results support a continued investment into the CCP rather than ceasing to implement government regulated control measures and suggest that vaccination may be an even better control alternative.

In summary, our studies have highlighted the value of epidemiologic and economic analysis in research of AI. Our results are expected to lead to an improved understanding and awareness of AI in Nepal and to formulation of better control strategies.

DEDICATION

I would like to dedicate this thesis to my parents; Mr. Chhabi Bahadur Karki and Mrs. Bishnu Maya Karki, for their continuous support and motivation in my studies for many years. I would also like to dedicate this work to my wife, Neeta Parajulee Karki and daughter Suvani Karki, who have been very supportive during my work and studies.

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NOMENCLATURE

ACM	Absence of Control Measures
AI	Avian Influenza
AICP	Avian influenza control project
AIV	Avian Influenza virus
BC	Backyard chicken
B-C	Benefit-cost
BD	Backyard duck
BP	Broiler parent
CBA	Cost-benefit analysis
CB	Commercial broilers
CCS	Current control scenario
CL	Commercial layers
CVL	Central Veterinary Laboratory
DIVA	Differentiate infected from vaccinated animals
DOCs	Day old chicks
ELISA	Enzyme-linked immune sorbent assay
FAO	Food and Agricultural Organization of the United Nations
FM	Frequency modulation
FV	Future value

HA	Haemagglutinin
HPAI	Highly Pathogenic Avian Influenza
HPAIV	Highly Pathogenic Avian Influenza Virus
HRD	High risk district
IVPI	Intravenous pathogenicity index
LP	Layers parent
LPAI	Low Pathogenic Avian Influenza
LPAIV	Low Pathogenic Avian Influenza Virus
LRD	Low risk district
MoAD	Ministry of Agricultural Development
MRD	Medium risk district
NA	Neuraminidase
NPV	Net present value
NRs	Nepali rupees
OIE	World Animal Health Organization
PCR	Polymerase chain reaction
PV	Present value
PVB	Present value of benefits
PVC	Present value of costs
WHO	World Health Organization
Yr	Year

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

1.1 Introduction

Avian influenza (AI) is an infectious disease primarily of birds caused by influenza A viruses. On their surface, AI viruses (AIV) have two types of glycoproteins: haemagglutinin (HA) and neuraminidase (NA). Based on these glycoproteins AIVs are divided into subtypes. Overall, 16 HA (H1 - H16) and nine NA (N1 - N9) subtypes have been reported. Recently, in Guatemala, a new subtype of HA (H17) has been discovered in little yellow shouldered bats (Tong et al., 2012). Depending upon its ability to cause disease, AI is classified into highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). All HPAI are caused by H5 and H7subtypes. However, not all H5 and H7 subtypes are highly pathogenic (Alexander and Brown, 2009). That being said, the World Organization for Animal Health (OIE) (2013) has defined notifiable AI as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AIV with an intravenous pathogenicity index (IVPI) greater than 1.2 (or based on an alternative measure of at least 75% mortality). All the H5 and H7 subtypes are considered notifiable because of the risk of low pathogenic H5 or H7 subtypes mutating into highly pathogenic ones. All AIVs, with HPAI H5N1 subtype in particular, are of concern because they have caused disease in humans, in addition to occurring in wild and domestic birds (Yee et al., 2009).

HPAI grabbed global attention in 1997 when, in Hong Kong, 18 humans became sick and 6 died from infection with the H5N1 virus. Around that time, there was also an outbreak of HPAI at one of the geese farms located in Guangdong Province, China (Xu et al., 1999). Subsequently, between 2001 and 2003, multiple outbreaks were detected in Hong Kong in wild and domestic birds (Sturm-Ramirez et al., 2004). Relatively quickly, H5N1 outbreaks became widespread in several countries in Southeast Asia (between 2003 and 2004) as well as in Europe and Africa (between 2005 and 2006) (Otte et al., 2008). As of 2013, more than 60 countries have reported HPAI H5N1 outbreaks (OIE, 2013). According to the World Health Organization (WHO), between 2003 and 2013 (April), 374 of 628 people with laboratory confirmed HPAI H5N1 infection have died worldwide (WHO, 2013). Mortality in poultry due to HPAI outbreaks is in the millions. In addition, in an effort to control the disease at the animal level, millions of poultry have been culled and the poultry trade disrupted. All of this has a serious impact on the national economies of the affected countries, with the magnitude of impact likely to differ across the countries.

Nepal was free from HPAI H5N1 until 2009, although the adjoining India and China were affected with the disease several years prior. The first HPAI outbreak was detected in January 2009 in Jhapa, a district bordering India and close to Bangladesh.

Since the detection of this first outbreak, there have been a number of additional outbreaks in Nepal. More than 35 outbreaks have been officially reported by the Nepali government to the OIE. As of January 2013, the total poultry loss in Nepal, either due to HPAI related mortality or culling activities to control its further spread, has reached nearly 120,000 animals (OIE, 2013).

Between 2007 and 2011, the government of Nepal has implemented AI surveillance activities and an awareness program through the Avian Influenza Control Project (AICP) funded by the World Bank. Since 2011, the Nepali government has been carrying out the AICP using its own resources. The focus of the control policy implemented by the Nepali government has been on the outbreak related culling of poultry in conjunction with cleaning and disinfection. After the government has declared an area affected by the outbreak, there is a ban on poultry production for a period of 45 days. Surveillance activities are also intensified near the outbreak areas and other high risks areas, such as districts with high poultry density. However, amidst these efforts, the number of HPAI outbreaks has been increasing in Nepal. Major commercial poultry areas, namely Chitwan and Kathmandu, have already been affected by the outbreaks. As poultry density is very high in these areas, culling of poultry has the potential to cause economic devastation in these regions.

The potential for substantial economic losses has triggered a discussion on alternative control strategies, such as vaccination of the national poultry flock. Likewise, the continued occurrence of outbreaks, despite the ongoing AICP, questions whether the resources spent on the AICP are well spent. However, before making any changes in the control strategy, there needs to be a careful evaluation of the economic feasibility of different options. There are several economic techniques for the evaluation of disease control programs to help in decision making, such as cost-benefit analysis (CBA), network analysis, mathematical programming and simulation (Bennett, 1992). When long-term control programs are desired at the national level, CBA is the method of choice (Dijkhuizen et al., 1995). CBA is mostly used by governments to choose the most desirable control policy, based on the comparison between the impact of an intervention and its operating cost (Tiongco, 2008).

Kathmandu is the capital city of Nepal. It is also the most important center for poultry trade in Nepal. Poultry produced in several other districts of Nepal are transported to Kathmandu for consumption. In addition, Kathmandu district in itself is an important poultry production district of the country. A large number of duck farms exist in Kathmandu district. On these farms, ducks are mainly raised in a scavenging system, where ducks are allowed to graze freely in the daytime and are kept in their shed during the nighttime. These ducks have access to ponds, rivers and other water bodies, where they have the opportunity to mingle with wild birds and backyard chickens. Due to these production practices, Kathmandu is considered to be one of the highest risk districts for HPAI outbreaks in Nepal. While Nepal has experienced outbreaks of HPAI since January 2009, no clinical outbreaks were detected in Kathmandu until January 2012. Nevertheless, it has been suspected that the virus had been circulating in Kathmandu even before the first detected outbreak.

It is known that infections of H5N1 in chickens and ducks exhibit different clinical presentations. Whereas in chickens the infection is characterized by clinical symptoms and high mortality, in ducks the infection is usually asymptomatic, leading to underestimation of the disease prevalence (Chantong and Kaneene, 2011). Therefore, infected ducks may “silently” help maintain and transmit the infection to other susceptible hosts (Henning et al., 2011). Because of its potential silent nature, H5N1 infection in ducks has been considered a threat to the national poultry flock and public health (Sturm-Ramirez et al., 2005). Thus, monitoring and surveillance activities focusing on ducks are very important for the control of AI. Serology is commonly used to detect AI in birds in surveillance programs (Brown et al., 2010). Finding ducks seropositive to AIV in the serum samples collected before January 2012 would corroborate the suspicion that AIVs (though not necessarily HPAI viruses) were circulating among ducks in Kathmandu even before the first detected outbreak.

This thesis focuses on the epidemiology and economics of AI in Nepal. In particular, our interest was in the seroprevalence of and risk factors for AI in Kathmandu, Nepal, and the costs and benefits of AI control in Nepal. The following sections summarize a review of literature pertinent to these questions.

1.2 Literature review

1.2.1 Epidemiology of AI

AI epidemiology is very complex due to the numerous host species involved, many existing and emerging subtypes of AIVs and the role of environmental factors in the persistence of AIVs. Outbreaks of HPAI, particularly H5N1, have caused mortalities of a huge number of birds. In addition, mortality due to the HPAI H5N1 infection in human population has made this disease of high public health concern. Therefore, timely control of the disease at the animal (poultry) level is necessary. It is essential to understand the epidemiology of AI for its effective control. When studying the epidemiology of AI, we need to approach it from the perspectives of the hosts, pathogen, environment and their interactions, together with the temporal and spatial patterns of its distribution.

Birds are the main hosts for AIV. As of now, AIVs have been isolated from at least 105 species of birds belonging to 26 families (Olsen et al., 2006). Among the wild birds, AIVs have mostly been isolated from Anseriformes (e.g. ducks, swans and geese) and Charadriiformes (e.g., gulls, waders and terns). Waterfowls are considered important reservoirs for AI, mainly LPAI, because they shed the virus through feces into water contributing to the fecal-oral spread of the disease. Among the domestic species, chickens and turkeys have been the major species involved in HPAI outbreaks

(Alexander, 2000). Domestic ducks can be infected but may not show signs of the disease (Songserm et al., 2006). Exceptionally, tigers and leopards were infected in Thailand in 2003 after the consumption of infected chicken carcasses (Songserm et al., 2006). HPAI H5N1 outbreaks in Asia, and later in Europe and Africa, have raised the concern that the wild birds are playing an important role in the maintenance of influenza viruses (Olsen et al., 2006). Among AIV subtypes identified thus far, most have been low pathogenic and they have been isolated from wild birds in surveillance studies with an overall prevalence of LPAI in ducks and geese of about 11% and about 2% in other wild bird species (Alexander, 2007).

AIVs are influenza A virus under the *Orthomyxoviridae* family. AIVs are RNA, segmented and negative stranded (Capua and Alexander, 2004). Though there are many influenza A viruses, HPAI H5N1 virus is of particular concern due to the economic losses it causes to the poultry industry and its zoonotic importance. Phylogenetic analysis of HPAI H5N1 has shown that the clade 2.2 was dominant both in Asia and Europe (Cattoli et al., 2009). Clades are distinct groups within a lineage that share a common ancestor or node on a phylogenetic tree (Lu et al., 2007). The clades of H5N1 have shown continuous evolution posing global threat to the poultry industry and humans (Guan et al., 2009).

Environment plays a crucial role in the transmission of AIVs. AIVs can survive in the environment outside the hosts for a considerable period of time. The length of persistence depends upon several factors such as temperature, salinity and pH. It has been reported that pH of 7.4- 8.2, temperature below 17°C and salinity (0- 20,000 parts per million) are favorable environmental conditions for AIV persistence (Brown et al., 2009). Nazir et al. (2011) reported that AIVs persisted for 5-11 days, 13-18 days, 43-54 days and 66-394 days at 30°C, 20°C, 10°C and 0°C, respectively, in the lake sediment. HPAI H5N1 viruses from domestic poultry are less persistent to the above environmental factors than wild type AIVs (Brown et al., 2009). In a study conducted in India, HPAI H5N1 virus survived for 18 hours, 24 hours, 5 days and 8 weeks at 42°C, 37°C, 24°C and 4°C, respectively (Kurmi et al., 2013). In detached feathers from infected domestic ducks, AIVs (H5N1) persisted for 160 days at 4°C and 15 days at 20°C (Yamamoto et al., 2010).

For better understanding of AI epidemiology it is necessary to evaluate the temporal and spatial patterns of its spread. HPAI outbreaks were very limited before they were observed in China in 1996 and in Hong Kong in 1997 (Suarez, 2010). After outbreaks of HPAI H5N1 in Hong Kong in 1997, this disease was not reported for almost six years from anywhere in the world. Then, in December of 2003, the Republic of Korea reported the outbreaks in poultry (OIE, 2013). After that, every year, HPAI H5N1 outbreaks have been reported to OIE by different countries. In 2004, outbreaks were mainly concentrated in the Asian nations, specifically in Southeast Asian nations including Thailand, Vietnam, Indonesia and Cambodia (OIE, 2013). There might be several reasons for the rapid spread of the disease in these countries, such as traditional backyard poultry husbandry system, large free grazing duck population in the paddy fields, weak bio-security system in the commercial poultry, large number of live bird markets and poorly monitored extensive poultry movements prevailing in these countries. The disease then spread further to other Asian regions, including Middle East and some European countries, including eastern part of Russia and United Kingdom in 2005 (WHO, 2013). In 2006, a record number of 48 countries reported HPAI H5N1 in poultry, with 36 countries reporting the disease for the first time. Among these countries were now also African nations, including Nigeria and Egypt (WHO, 2013). The higher number of countries reporting the disease reflected the fast spread of the diseases but might have also been due to an increased awareness and alerts created through OIE, FAO, WHO and media that resulted in enhanced surveillance activities and testing of the birds.

In summary, AI has rapidly spread from Asia to European and African countries and finally became endemic in Asia (Lupiani and Reddy, 2009). This has triggered the need for research to better understand its epidemiology.

1.2.2 Public health importance

AI became of a global concern when the first human cases of AI were detected in Hong Kong in 1997. After a series of additional human cases over the following few years, there was a discussion about the possibility of the next pandemic. In that regard, WHO defined six phases of pandemic treat to show how the influenza virus moves from the initial human cases to a pandemic state (WHO, 2013). Phase one is a situation in which influenza viruses are circulating in animals but there is no infection in humans, whereas in phase two, viruses are known to cause infections in humans and the infection is thus considered a pandemic threat. Phase three is a situation in which there might be a chance of limited human-to-human transmission of AI virus in case of a close contact between infected and non-infected humans but which is not sufficient to sustain outbreaks at the community level. In phase four, there is a verified human-to-human-transmission or re-assortment of human-animal influenza virus capable to cause community level outbreaks while in phase five there is a human-to-human spread of virus into at least two countries in one WHO region (there are six WHO regions: Africa, Americas, Southeast Asia, Europe, Eastern Mediterranean and Western Pacific). Phase six is a pandemic phase where community level outbreaks are seen in at least one other country in a different WHO region in addition to countries within the originally affected WHO region. As per an official WHO position, the world is currently at the pandemic alert phase three for H5N1 (Pappaioanou, 2009). Human casualties from H5N1 have occurred in 15 countries, mostly Indonesia, Egypt, Vietnam and China. The case fatality rate of nearly 60% observed for H5N1 HPAI has made this disease of a high public health concern (Suarez, 2010). Lack of prior immunity in humans against this subtype might be the reason for such a high case fatality rate. Another strain of AI, H7N9, has recently caused infections in humans in China. This has highlighted the further importance of AIVs as a zoonotic agent. As of May 9, 2013, 32 people (24.4%) out of 131 laboratory confirmed H7H9 cases have died (WHO, 2013). Though it is not clear whether the current H7N9 infections in humans in China were initiated from an animal contact (poultry and swine), more than two-thirds (77%) of the cases had a history of contact with live animals, including chickens and pigs, either in the live poultry market or on farms (Li et al., 2013).

There is also a concern about the potential mixing of several influenza viruses and creation of a novel influenza virus capable of human-to-human transmission. In this regard, mixing of HPAI H5N1 virus, that has already caused disease in humans, and the H1N1 virus that caused pandemic in 2009 or any other influenza virus resulting in a co-infection and possible re-assortment of the virus to create a new highly pathogenic and easily transmittable strain is of concern (Amendola et al., 2011). Concurrent circulation of HPAI H5N1 and H7N9 virus in China may also provide chance for re-assortment of the virus. H9N2 virus should also be included in the human pandemic strain list and more research should be conducted to better understand it as it has been done for H5N1 (Lupiani and Reddy, 2009).

As of now, the risk of H5N1 virus transmission among humans is little to moderate. However, close contact with poultry (infected sick or dead birds) and poor bio-security in poultry farms increases the risk for human infections (Rabinowitz et al., 2010; Van Kerkhove et al., 2011). The individuals at risk are recommended to follow precautions such as proper hand washing and reporting influenza like illness to the health authorities (Kelly et al., 2008). In summary, the number of deaths up to now is not that high. However, the high case fatality rate seen and the risk of virus re-assortment into a strain capable of human-to-human transmission, leading to a pandemic, have made this disease of high public health importance.

1.2.3 Avian influenza in ducks

While ducks (wild and domestic) are relatively frequently infected with LPAI viruses they can also get infected with HPAI viruses (Stallknecht et al., 1990). As ducks can become infected and co-infected with different AIVs, this provides a chance for re-assortment of the virus (Chua, 2009). Moreover, ducks are important in the epidemiology of AI because they vary in the extent of the expressed symptoms of HPAI. For example, in South Korea, breeder ducks showed reduced feed consumption and egg production without increase in deaths but in commercial ducks respiratory signs and moderate increase in mortality were observed (Kwon et al., 2005). Grazing ducks in paddy fields in Thailand did not show any disease symptoms though they were infected (Songserm et al., 2006). The expression of AI symptoms in ducks is contingent upon several factors, such as age of the hosts, strain of the virus and environmental conditions (e.g., weather extremes) causing physiological stress (Kwon et al., 2005). Regarding age, in a study conducted by Pantin-Jackwood et al. (2007), the mortality was higher in ducks of two weeks compared to those five weeks of age.

There is a variation in the period the ducks shed the virus once they are infected. Shedding of the virus has been reported to last one to two weeks in adults (Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005) and up to a month in juveniles (Hinshaw et al., 1980). The level of virus shedding peaks around the day three of shedding (Sturm-Ramirez et al., 2005). The long shedding period allows sufficient time for disease transmission from one country to another during long distance travel and migration. This has been substantiated by the H5N1 detection in apparently healthy ducks in South Korea brought from China for slaughter in 2001 (Tumpey et al., 2002).

Analysis of spatial data on HPAI outbreaks in Southeast Asia has shown that scavenging ducks are contributing to HPAI outbreaks in domestic poultry in that region (Pfeiffer et al., 2007). In Thailand, there was a positive correlation between grazing of ducks in the rice fields and their infection with H5N1 virus (Gilbert et al., 2006). When chickens get infected from ducks, signs of clinical disease and high mortality are observed (Chen et al., 2004). Thus, ducks are a probable source of disease transmission to chickens and even humans (Henning et al., 2010). In summary, ample evidence suggests that ducks are playing an important role in the spread of HPAI as they do not show symptoms of disease despite being infected. Serological testing of ducks can indicate their exposure to AIVs. Thus, serological studies of ducks are very important to know their exposure level to AIVs.

1.2.4 Risk factors for AI in poultry

Several risk factors for the spread of AI in poultry have been identified. The most important one is the global movement of poultry and their products through trade (Steensels et al., 2006; Alexander, 2007; Van den Berg, 2009; Yee et al., 2009). In addition to formal trade, illegal and informal imports of infected poultry that mainly occur between neighboring countries are contributing to the spread of AI (Beato et al., 2009). Besides trade, the poultry husbandry system is an important risk factor. Specifically, the traditional backyard poultry raising and free ranging duck farming systems, especially in the developing countries of Asia and Africa, are contributing to the spread of AIV as bio-security measures are often weak in these types of husbandry systems (Chantong and Kaneene, 2011). Similarly, in Thailand, seasonal flooding of paddy fields contributes to AIV dissemination (Gilbert et al., 2006).

The introduction of AI infection to domestic poultry often occurs through the contact with wild birds. AI infection status in wild birds is thus an important risk factor for AI infection in domestic birds. In wild birds, different physiologic stresses, such as molting and environmental stress due to cold weather, increase their susceptibility to AI (Feare, 2010). Climate change is considered another risk factor because it can influence the AIV ecology by changing the virus survival outside the host, migration patterns of wild birds and the infection transmission cycle (Gilbert et al., 2008).

In summary, several risk factors have been identified for the spread of AI in poultry. The most important ones are the global movement of the poultry and poultry products through legal and illegal trade and the husbandry practices involving traditional backyard poultry and free grazing duck farming in developing countries.

1.2.5 Costs-benefit analysis

The CBA technique was first used in the 19th century to analyze the cost and benefits of a bridge construction project in France (Ramsay et al., 1999). Though CBA has been widely used to make decisions on the economic worth of national or regional level projects, it can be equally applicable to make decisions at the farm level (Marsh, 1999). In CBA, all the relevant costs (C) and benefits (B) are identified and then quantified by giving them monetary values, after which they are compared to make decisions (Bennett, 1992). In disease control programs, benefits mainly include the prevented losses that would have occurred in the absence of a disease control program.

There are both advantages and disadvantages of using CBA approach. The advantage is that costs and benefits are assigned with monetary values. That aids decision-making because one can see how much money will be saved or earned in return for the money spent. Furthermore, CBA can compare the competing programs. As a down side of CBA, it may be tedious to give monetary values to every relevant cost and benefit (Bennett, 1992). Sometimes, the market values are unavailable or are distorted. Sometimes, the return in the absolute amount might be more important for the decision making than just the ratio. Similarly, CBA compares the advantage in an aggregate and doesn't consider which particular groups in a society are getting more benefits (Ramsay et al., 1999). The outcome from a CBA indicates economic profitability of the change being assessed. If carried out at a national or regional level it does not necessarily indicate who might bear the costs of the change and who might benefit from that change. The CBA also gives no impression of the social acceptability or the financial feasibility of the change.

While performing CBA, one needs to keep in mind that the same amounts of money today and in the future have different values. In other words, the money we have today is more valuable than the same amount of money in the future. Discounting takes account of the time value of money by converting future values to a present value (Dijkhuizen et al., 1995). The formula used in the calculation of Present Value (PV) from a future value (FV) is: $PV= FV / (1+r)^{n}$, where r = periodic interest (discount) rate and n = number of periods (Marsh, 1999).

The B-C ratio is calculated by dividing the benefits and costs in their present value. It is worth to invest when B-C ratio is greater than one (Dijkhuizen et al., 1995).

For example, if the B-C ratio is 5, this means that for each dollar invested, $5 will be saved or returned, indicating that the investment is worth pursuing. On the other hand, if the B-C ratio is 0.5, this means that for each dollar invested; only half a dollar will be saved (or earned) in return indicating that it is not worth to invest. In addition to the B-C ratio, the Net Present Value (NPV) can be used to aid decisions about an investment. The NPV is the difference between the benefits and costs in terms of their present values. A positive NPV indicates that the investment has greater return for that investment than its opportunity cost (Dijkhuizen et al., 1995).

In conclusion, CBA is a method of economic analysis where all the relevant costs and benefits of different programs are identified and then compared to choose the one worth pursuing. There are both advantages and disadvantages of the CBA approach, as we discussed above, nevertheless, this is the most commonly used approach to evaluate the disease control programs at a national or regional level.

1.2.6 AI vaccination

Vaccination has been a very important tool in the control of many infectious diseases, including AI. Vaccination against AI boosts an individual host's immunity to an infection and thus the population of susceptible hosts decreases (Capua and Marangon, 2003). In case of virus introduction, it reduces the load of circulating viruses (Hinrichs et al., 2006). Vaccination has been considered as an option in the control of

HPAI when the outbreaks are massive and culling of poultry becomes uneconomic and impractical. However, there are debates over the use of vaccination for controlling AI. The main concern is the possibility of silent spread of the disease as vaccinated birds may still shed the virus (Ellis et al., 2004). It is also difficult to distinguish vaccinated-infected from vaccinated-non-infected animals (Capua and Marangon, 2003). On these grounds, several poultry importing countries have imposed trade bans from countries having vaccination policy for AI control. Recently, a new technology has been developed, called DIVA (differentiating infected from vaccinated animals), that can differentiate infected from vaccinated birds (Suarez, 2012). With the development of DIVA, poultry trade between countries can resume even if vaccination is used. However, vaccination program should always be conducted together with strict bio-security and surveillance activities (Koch et al., 2009) because of the possibility of vaccinated birds shedding the virus and silently spreading the disease.

Several countries have used vaccination against AI. Countries that have used vaccination to control HPAI H5N1 are Pakistan, Vietnam, Egypt, France, Russia and the Netherlands. Mexico, Italy, USA, El Salvador and Guatemala have used vaccination against LPAI H5 and H7 (Swayne et al., 2011). Pakistan successfully controlled the spread of HPAI H5N1 through mass vaccination and bio-security measures (Naeem, 2003). Success of vaccination depends on the proportion of the population that is vaccinated. For the effective control of the infection, it has been considered that at least

80% of the total poultry population needs to be vaccinated (Tiensin et al., 2007). However, in practice, that is difficult to achieve because of the fast turnover of poultry and large number of backyard poultry particularly in developing countries. In a nutshell, vaccination is one of the several options for the control of AI particularly when outbreaks are massive and stamping out is not feasible. However, there are several constraints of AI vaccination, as we discussed earlier, which makes its use debatable.

1.3 Overall objectives and outline of this thesis

The overall objective of this thesis was to improve understanding of the epidemiology of AI and the economic worth of its control in Nepal. This overall objective has been addressed through two independent chapters. In Chapter II, we have estimated the seroprevalence of ducks carrying antibodies against AIV in the major duck raising areas of Kathmandu, Nepal and assessed the effect of age, sex and size of the farm on the presence of AIV antibodies in domestic ducks. In Chapter III, we evaluated the costs and benefits of AI control in Nepal. This assessment was important as Nepali government has been interested in the economic worth of alternatives to the current control program for HPAI implemented since 2007. Finally, in Chapter IV, conclusions and summary of the methods and results of the two studies have been presented with recommendations for potential future research.

CHAPTER II

CROSS-SECTIONAL SEROSURVEY OF AVIAN INFLUENZA ANTIBODY CARRIAGE IN DUCKS OF KATHMANDU, NEPAL

2.1 Introduction

Avian influenza (AI) is caused by influenza A viruses. While AI is mainly a disease of birds, humans and other mammals can also become infected (OIE, 2013). AI viruses (AIV) are classified based on their surface glycoproteins. The diverse AIV are not equally pathogenic. Highly pathogenic avian influenza (HPAI) is caused by viruses of the H5 and H7 subtypes; however, not all viruses of the H5 and H7 subtypes are highly pathogenic (Alexander and Brown, 2009). There have been outbreaks of HPAI H5N1 in poultry in several countries and humans have been infected in some of those countries. As of April 26, 2013, out of 628 laboratory confirmed human cases of HPAI H5N1 since 2003, 374 have died (WHO, 2013). Due to this reason and huge economic loss it causes to the poultry industry, AI, particularly HPAI, has been of major concern worldwide, including in Nepal. Nepal was free from any HPAI until January of 2009, when the first outbreak of HPAI H5N1 was detected in backyard chickens in the eastern district of Nepal. This region of Nepal borders West Bengal, India and is in close proximity to Bangladesh where HPAI outbreaks have been reported. Since this initial outbreak, Nepal has faced several additional outbreaks of HPAI, predominately in the winter months.

Ducks play an important role in the ecology of AIV. In the HPAI H5N1outbreaks that occurred in the Southeast Asian countries, grazing ducks were found to have played a key role in the transmission of the infection (Tiensin et al., 2005). This is likely due to the fact that ducks can harbor the virus, yet they remain asymptomatic, thus, helping in the silent spread of the disease (Songserm et al., 2006). When domestic and wild ducks share the same wetlands, they can transfer the infection to each other and help in the maintenance and spread of AIV (Kim et al., 2009). Serological tests are commonly used to detect AIV infections (Brown et al., 2010). Due to the role ducks play in the epidemiology of AI, it is of interest to determine the seroprevalence of antibodies to AIV in ducks.

Nepal is a Himalayan country that lies between India and China. The country is divided into 5 developmental regions, 14 zones and 75 districts. Each district consists of village development committees and municipalities. Livestock sector is important contributor to the gross domestic product (around 10%) in Nepal and duck farming is a small component of it. Ducks are mostly raised as backyard birds under scavenging system where they are allowed to graze and have access to nearby ponds and streams during the daytime and are kept in enclosures during the nighttime.

Based on the national surveillance plan for HPAI in Nepal, Kathmandu district is classified as a high risk district for the disease by the Nepali Government. Kathmandu's high risk classification is based on the high density of commercial poultry in the district, the high volume of poultry being moved into Kathmandu from other districts, the large number of free ranging ducks, the large number of natural and man-made ponds and lakes (where large numbers of migratory birds come every winter), the presence of live bird markets, and poor bio-security in commercial poultry farms. No outbreaks of HPAI were detected in Kathmandu until January 2012. However, it was suspected that the virus circulated in Kathmandu before that, but the country's surveillance system did not detect it because only a small number of samples were tested. Motivated by this suspicion, in 2011 we initiated a serosurvey of domestic mallard ducks (*Anas platyrhyncos domesticus*) in Kathmandu with the following objectives: (1) to estimate the prevalence of seroconversion to AIV in domestic ducks and (2) to assess the effect of age, sex and size of the farm on the carriage of AIV antibodies in domestic ducks. Additionally, we were interested in the number of duck farms, particularly those with seropositive ducks, that also keep pigs because pigs could serve as mixing vessels for re-assortment of influenza viruses (Yasuda et al., 1991).

2.2 Methods

2.2.1 Study design

We conducted a cross-sectional study from April through July of 2011 in the major duck-raising areas of Kathmandu district. The target population consisted of domestic ducks from the major duck raising areas of Kathmandu district where the risk of HPAI was considered to be high because of the large duck population, frequent mixing of chickens and ducks and weak bio-security measures on the farms. The source population consisted of ducks from 9 of those areas (Figure 1) in the Kathmandu district. These 9 major duck raising areas were identified by the district livestock service office of Kathmandu based on a high duck population size. To select the study farms, within each area, we selected the first farm randomly. A farm that was located 3-4 farms away from the first farm in a random direction was selected for sample collection and this process was repeated for selection of additional farms. Within each farm we collected blood samples from a certain number of ducks (described in the next section). A target number of farms to be enrolled were not predetermined. Rather, the farms continued to be enrolled and the ducks sampled until the estimated duck sample size was reached.

2.2.2 Sample size estimation and sampling

Duck sample size was calculated using WinEpiscope® 2.0 Software (University of Edinburgh, 2007, United Kingdom). Assuming that seroprevalence would be at least

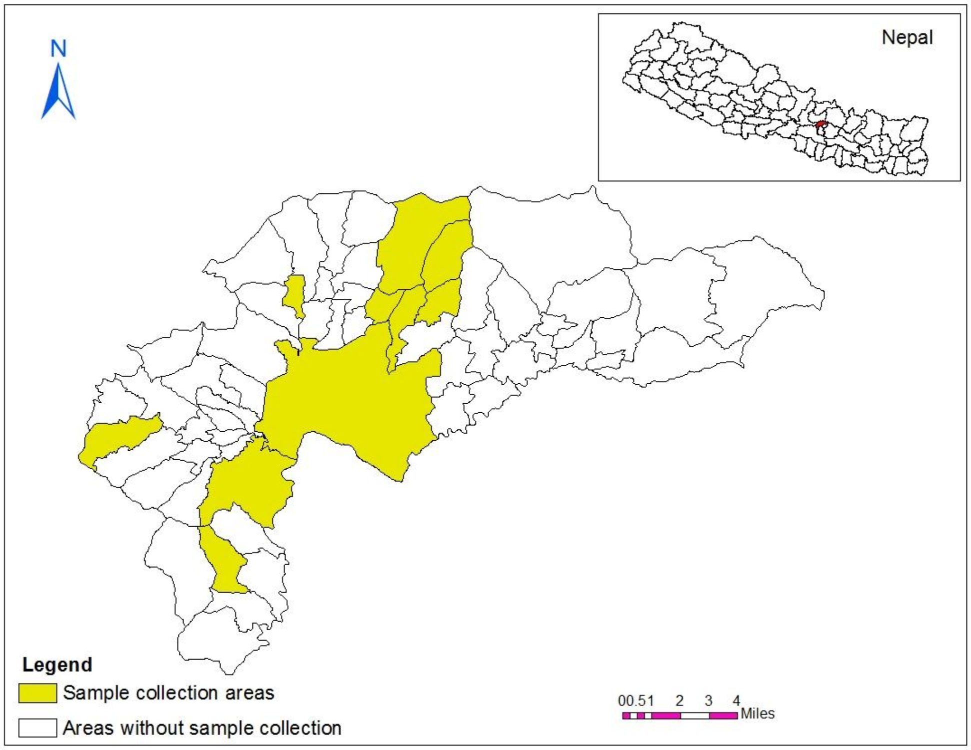

Figure 1. Map of Kathmandu district showing sample collection areas (village development committees) in yellow.

1% if AI infection is present in the area, with a 95% confidence interval (CI) and the population size of 25,000 ducks in the nine enrolled areas, the estimated sample size was 297 ducks. We collected 310 samples to accommodate for possible losses during serum separation. These samples were collected from 62 farms of different sizes in terms of the number of ducks they kept, which was in the range from 1 to 1,050 ducks per farm. To select individual ducks within a farm, we asked farmers to enclose all of the ducks in the enclosure where they were normally kept during feeding. The number of ducks sampled per farm ranged from 1 to 17 depending upon the farm size and farmer's cooperation; the mean proportion of birds sampled per farm was 18% (median= 7%, range= 1-100%). Likewise, the distribution of sex among the sampled ducks roughly matched the sex distribution in the flock. Age, farm size and the presence of swine on the farm were recorded based on the information provided by farmers. Sex was recorded based on the farmers and investigator's (SK) personal observation on the basis of feather colors and the sound produced. Ducklings, less than 4 weeks old, were excluded as it was difficult to collect serum samples from that age group. Samples of five ml of blood were collected from a wing vein from individual birds.

Serum was separated by keeping the syringe containing the blood on a 45^o slant for about 2 hours at room temperature. The separated serum samples were collected in a serum vial and transported to the Central Veterinary Laboratory, Kathmandu, in a cool box containing ice packs at 4ºC and stored at -20ºC for 2 weeks until testing. The samples were tested for the presence of antibodies to AIV using IDEXX Influenza A Ab test kit (IDEXX Laboratories, USA) with results being expressed as sample to negative control (S/N) ratio. To classify the ducks as positive or negative, the manufacturer recommended cut–off value was used. Specifically, $S/N \geq 0.5$ was considered as negative and $S/N < 0.5$ as positive. According to the manufacturer, the sensitivity and specificity of this test are 95.4% and 99.7% respectively (IDEXX, 2013).

2.2.3 Data management and analysis

Unless otherwise stated, the analyses were performed at the individual duck level. The result of the ELISA test (positive/negative) for the individual ducks represented the outcome variable. The considered explanatory variables were: duck's age (categorized as less than 6 months old, 6 – 12 months old and more than 1 year old) and sex, as well as the farm size. We categorized age into 3 categories based on the practice of maintaining the ducks in the flock. Below six months of age, they are not yet considered ready for market. From 6 to 12 months of age, they are ready to be marketed and may be sold at any time. Ducks older than 1 year of age are generally maintained in the flock for laying and breeding purposes and kept for a longer time period, generally 3-4 years. Regarding farm size, accounting for the husbandry practices of ducks in Kathmandu, the farms were categorized as small (less than 50 ducks), medium (50 to 500 ducks) and large (more than 500 ducks). The apparent duck seroprevalence, with 95% CI, was calculated by dividing the number of positive samples by the total number of samples tested. The true prevalence, with 95% CI, was then estimated by using the formula: True prevalence = (Apparent prevalence+Specificity-1)/(Sensitivity+Specificity-1) (Dohoo et al., 2003). Farms were considered positive when at least one sample from that farm tested positive.

For statistical analyses, we used SAS 9.3 software (SAS Institute Inc., North Carolina, USA). The Pearson's Chi-square test was used to evaluate the bivariate association between the outcome and the individual explanatory variables. Variables with a P-value < 0.2 were considered in the multivariable analysis. The liberal significance cut-off of 20% was used to assure that potentially influential variables (including potential confounders) were evaluated in the multivariable analysis while keeping in mind the risk of making a Type I error at the bivariate analysis level. Multivariable analysis was performed using the generalized estimating equation (GEE) approach (the PROC GENMOD command in SAS) to account for the clustering effect at the farm level. To evaluate the appropriate correlation matrices, the QIC criterion was used (Pan, 2001). We ran the model with different correlation matrices and used a Toeplitz correlation matrix in the final model based on its lowest Quasi-likelihood under the independence model criterion (QIC) value. To select the final multivariable model, a backward variable selection approach was used. Two-way interactions between individual explanatory variables were also assessed. P-values less than 0.05 were considered significant. The final GEE model was selected by excluding non-significant variables. Odds ratio (OR) was used as a measures of association. OR is "the odds of the disease in the exposed group divided by the disease odds in the non-exposed group" (Dohoo et al., 2003). When OR=1, it means that there was no difference between the odds of seropositivity in the exposed and non-exposed groups. The statistical significance of OR can evaluated by examining the estimated CI; for example if the estimated 95% CI for a OR does not include 1 we are 95% confident that the estimated

OR is truly different from 1 meaning that the odds of seropositivity among the exposed differs from the odds of seropositivity among non-exposed ducks.

2.3 Results

A total of 310 ducks on 62 enrolled farms were sampled. Among them, 97 were males and 213 were females. The mean and median age of sampled ducks were 49.3 weeks (95% CI: 41.9-56.6 weeks) and 20 weeks, respectively. Using the Shapiro-Wilk test we determined that the distribution of age was not normal (p <0.0001). Thus, rather than using age as a continuous variable we classified the sampled ducks into three age categories as explained in the Data Management and Analysis section. The mean and median number of ducks on enrolled farms were 250 ducks (95% CI: 217- 283) and 113 ducks, respectively. In total, 31 enrolled farms had swine (200 samples were collected from farms having swine) and 31 farms did not have swine (110 samples were from farms not having swine). Considering that the number of ducks sampled per farm was proportional to the farm size, this means that the farms that had pigs also tended to keep more ducks.

Among 310 tested ducks, 81 were seropositive. At least one duck tested positive on 26 out of 62 enrolled farms. The mean and median numbers of positive ducks per positive farms were 3.1 and 3.0, respectively. The apparent seroprevalence, at the individual duck level was 26.1% (95% CI: 23.6-28.6%) whereas it was 41.9% (95% CI:

29.5- 55.2) at the farm level. The true duck seroprevalence, estimated by accounting for sensitivity and specificity of the ELISA test used, was 27.2% (95% CI: 24.5- 29.7). At the farm level, 51.6% (95% CI: 33.1- 69.8%) of farms with swine had seropositive ducks while 32.3% (95% CI: 16.7- 51.4) of farms without swine had seropositive ducks. However, the difference in the proportion of seropositive ducks between farms with and without pigs was not statistically significant (OR 2.2: 95% CI: 0.8- 6.3).

In the bivariate analysis (Table 1), at the individual duck level, AI seroprevalence was significantly associated with age, sex and farm size at the 20% significance level. The apparent seroprevalence was 22.7% (95% CI: 16.5- 29.9), 11.7% (95% CI: 4.8- 22.6), and 42.5% (95% CI: 32.0- 53.6) in ducks less than six months of age, between six months and one year of age, and older than one year of age, respectively. The seroprevalences in male and female ducks were 19.8% (95% CI: 12.2- 29.4) and 32.3% (95% CI: 25.8–39.4), respectively. The seroprevalence was 27.4% (95% CI: 21.1- 34.4%) when swine were present and 30.0% (95% CI: 21.2- 40.0) when swine were absent from the farm. The seroprevalences were 19.8% (95% CI: 12.0- 29.8), 29.9% (95% CI: 22.5- 38.0), and 37.5% (95% CI: 24.9- 51.5) on small, medium and large farms, respectively.

In the multivariable analysis using GEE, after controlling for clustering of ducks within farms, the age effect remained significant (p= 0.01) albeit that was not the case for the sex and farm size variables. The model containing only the age was selected as the final model. Based on the final model (Table 2), the odds of being seropositive was 2.17 (95% CI: 1.07- 4.39) times higher in ducks older than one year compared to ducks aged less than six months of age.

Table 1. The results of bivariate analysis of association between avian influenza seroprevalence in ducks and the individual explanatory variables

Variable and category	Ducks negative No. (%)	Ducks positive No. (%)	OR* (95% CI*)	P value
Age				
<6 months	126 (0.55)	37 (0.46)	1	<0.0001
6 mths-1 year	53 (0.23)	7 (0.08)	0.44 (0.18- 1.01)	
>1 year	50 (0.22)	37 (0.46)	2.52 (1.43- 4.41)	
Sex				
Male	79 (0.34)	18 (0.22)	1	0.04
Female	150 (0.66)	63 (0.78)	1.84 (1.02- 3.38)	
Farm size				
Small farms	74 (0.32)	17 (0.21)	1	0.12
Medium farms	112 (0.49)	43 (0.53)	2.12 (1.02- 4.43)	
Large farms	43 (0.19)	21 (0.26)	1.27 (0.67- 2.38)	

*OR: Odds ratio; CI: Confidence interval

Table 2. The final multivariable GEE model of avian influenza antibodies in ducks

Variable and category	OR[*] (95% CI[*])	P value
Age		
<6 months	1	0.01
6 mths-1 year	0.50 (0.18- 1.36)	
>1 year	2.17 (1.07- 4.39)	

*OR: Odds ratio; CI: Confidence interval

2.4 Discussion

This study describes a cross-sectional study of AI seroprevalence in ducks of Kathmandu district, Nepal, which was the first study of the kind conducted in Kathmandu. The main finding of the study was seropositivity against AIVs in a quarter of the ducks tested in the major duck raising areas of Kathmandu. Ducks older than 1 year of age were 2.17 times more likely to be positive compared to ducks less than 6 months of age. Implications and limitations of these findings are discussed below in the context of the published literature.

In late spring/early summer of 2011, the seroprevalence of ducks carrying AI antibodies in the major duck raising areas of Kathmandu district, which is classified by the Nepali government as a high risk area for HPAI, was 27.2% (95% CI: 24.5–29.7%).

This finding supported the suspicion that AIVs were circulating in Kathmandu before the first outbreak in poultry reported in January 2012.

Studies on the seroprevalence of AI antibodies in domestic ducks have been conducted in other parts of the world. In cross-sectional studies, high seroprevalences have been reported in domestic ducks in Iran (80.9%) (Hadipour et al., 2011), Saudi Arabia (35.9%) (Alkhalaf, 2010), New Zealand (30.0%) (Zheng et al., 2010) and West Bengal, India (40.6%) (Pawar et al., 2012). The study in New Zealand was conducted in high risk areas, which is similar to our study. The higher seroprevalence observed in Iran might be due to the reason that samples were primarily obtained from ducks near the wintering grounds of migratory birds where there is a possibility of mixing with wild migratory birds. In contrast, antibodies to AIV were not found in domestic ducks in Grenada, West Indies (Sabarinath et al., 2011). This might be due to the low sample size (n=16) tested. A lower seroprevalence was reported in domestic ducks in Mali (1–18.3%) (Molia et al., 2011).

There have been no reports of HPAI from Kathmandu before January 2012. However, the high seroprevalence to AIV among ducks in Kathmandu before that date, as determined by the present study, suggests that ducks have been exposed to AIVs before the detected HPAI outbreaks. Ducks raised in Kathmandu are transported to various locations within the district and even to other districts, for consumption in hotels and restaurants. If these ducks are infected, particularly if infected with HPAI virus, their movement could cause considerable damage to the poultry industry in Kathmandu and other parts of Nepal. This supports the importance of continued monitoring of commercial ducks for AI infection in Nepal. Indeed, in Thailand, distribution of free-grazing ducks and outbreaks of H5N1 in domestic chicken were correlated (Gilbert et al., 2006). Similarly, there is evidence that the virus could spread over long distances through trade (Tumpey et al., 2002). The detection of H5N1 virus in ducks in South Korea that were imported from China indicates that ducks that look healthy for slaughter could also carry the infection (Tumpey et al., 2002).

In our study birds older than one year showed a higher seroprevalence than those younger than 6 months. The reason for such higher seroprevalence in older ducks might be due to their longer length of exposure, and the related increased chance for seroconversion, compared to young birds. Antibodies become detectable one week post infection, peak at about 2 weeks (Jourdain et al., 2010) and may remain in the serum for months (Wilson et al., 2013). Most of the association studies of seroprevalence with host related risk factors, such as age and sex, have been done in wild birds. Higher seroprevalences were reported in adult waterfowls (adults Vs sub-adult (hatch year birds of 2-6 months)) in different wild bird species in Alaska (Wilson et al., 2013) and in Northwestern Europe (56% in adults, which experienced two winters, Vs in 8% juveniles) (Hoye et al., 2011). If we look at the result of infection prevalence studies, the prevalence of AIVs are high in juveniles. For example, in a study of wild birds in Texas, USA, juveniles showed a higher prevalence of AIVs than adults (Ferro et al., 2010). This may be due to juveniles being immunologically naive compared to adults. When young birds become infected with AIVs, they will seroconvert and as antibodies persits in the blood for months, antibodies will still be detected when these birds become adults. In addition, they can get infected at older age and seroconvert to become AIV antibody positive. Thus, it is not surprising to see high seroprevalence in the older ducks, either wild or domestic. However, it was surprising to find the lower seroprevalence, though not statistically significantly lower, in the ducks aged 6 months to 1 year category compared to ducks aged less than 6 months of age. This may be simply due to a low statistical power related to a low number of samples tested in this category compared to younger and older ducks. Tolf et al. (2013) have reported higher levels of AIV antibodies in the first autumn of ducks' life, a marked drop during the following summer, followed by a rise again in the second autumn. Since ducks in different age categories in our study hatched at different seasons, they were exposed to AIV for the first time at different seasons, after which they may have followed their cohort's pattern of seroconversion and even drop in the antibody levels. Therefore, it is also possible that the detected lower seroprevelance among ducks 6 -12 months of age is real and it may reflect the natural variation in the immune response to AIV exposures during a duck's life. The problem is that the natural variation in the immune response affects our ability to detect flocks which had experienced AI infection. Therefore, it is important to elucidate further the effect of age on the probability of seroconversion in future studies.

In our study, female ducks showed a higher seroprevalence than males (OR 1.6: 95% CI: 0.8–3.2), however that was not statistically significant in the final model. Though not significant, females having higher rates of seroprevalence in our study might be due to them being maintained in the flock for longer duration of time than males. No sex-related differences in wild birds were reported in the seroprevalence of AIV in Italy (De Marco et al., 2010). However, they were statistically significant in a study done in different wild bird species in Alaska with females having higher seroprevalence than males (OR 1.2 (1.1- 1.4)) (Wilson et al., 2013). In another study among wild birds in Alaska, males (1.3%) had a higher overall AI prevalence than females (0.6%) (Ip et al., 2008). These differences might be due to the differences among species, season, immunological status of the birds during sampling and sampling variations.

Pigs are considered to be an important player in the ecology of influenza virus. A pig can get infected with influenza A virus from birds as well as humans, which makes it a potential mixing vessel. This can be facilitated in an environment where birds, pigs and humans remain in close proximity to each other (Brown, 2000). In our study, duck seroprevalence did not differ significantly between farms with and without swine present. While this may seem reassuring, it is important to remember that 50% of duck farms enrolled in this study kept both ducks and pigs in the close proximity thus providing an opportunity for virus exchange.

In our study, seroprevalence was higher in ducks from larger compared to smaller farms. In the larger farms, generally the number of contacts between the individual ducks is higher than on smaller farms. If there is an infected duck on a farm, the susceptible ducks would soon become infected after having adequate contacts with the infected duck. As chance for exposure between the susceptible and infected ducks is generally higher on the larger farms compared to the smaller ones, the observed increasing seroprevalence on the farms of increasing sizes was expected. However, the difference in seroprevlence among farm size categories was not statistically significant. This lack of statistically significant difference may be due to an insufficient power of the study to detect the difference or similar husbandry practices and bio-security regardless of the size of a farm. Mostly, ducks are allowed to go to nearby ponds or streams for grazing during the daytime. During this time, ducks from different farm sizes comingle and may transmit the infection to each other.

The major limitation of this study was its limited geographic coverage and the fact that it was conducted at a single point in time. Another limitation is related to the focus of our study on high risk sites of major duck raising areas of Kathmandu. In this regard, our seroprevalance estimation may represent the upper limit and other areas in

Kathmandu might have lower seroprevalence than what we found in this study. Therefore, care is needed in extrapolation of results to all the ducks of Kathmandu.

Nevertheless, as this was the first study to estimate the seroprevalence of AI in ducks of the major duck raising areas in Kathmandu, Nepal, this study provided a valuable baseline information about the AIV seroprevalence in ducks in the region. The findings indicate that AIV circulate widely in Kathmandu (at least in the major duck raising areas) with older ducks having higher levels of seroprevalence which may be explained by their longer length of exposure. After this study was conducted in 2011, the Kathmandu poultry industry experienced several outbreaks of HPAI. Therefore, in future studies, we recommend conducting tests, such as hemagglutination inhibition, to differentiate the AIV subtypes present in Nepal with a particular interest in the presence of HPAI viruses.

CHAPTER III

COST-BENEFIT ANALYSIS OF AVIAN INFLUENZA CONTROL IN NEPAL

3.1 Introduction

Avian influenza (AI) is a highly contagious disease caused by type A influenza viruses. These viruses infect several species of food-producing birds (chickens, turkeys, quails, guinea fowl, ducks, etc.), as well as pet and wild birds (OIE, 2013). Highly pathogenic avian influenza (HPAI) H5N1 infection has been reported in domestic poultry, wildlife, and human populations (Yee et al., 2009).

HPAI became of global concern when six people died out of 18 laboratory confirmed cases in Hong Kong due to HPAI H5N1in 1997 (Sturm-Ramirez et al., 2004). However, it is believed now that the virus emerged in 1996 on a goose farm in Guangdong Province, China (Xu et al., 1999). After 2003, there were outbreaks of H5N1 in East and Southeast Asia, which gradually spread into Europe and Africa (Otte et al., 2008).

Due to its zoonotic potential and ability to cause high mortality in poultry, HPAI has received much attention around the world. Of 628 laboratory confirmed human cases of HPAI globally between 2003 and 2010, 374 have died (WHO, 2013) and millions of birds have either died or been killed in an effort to control the disease. This has caused significant economic losses and has provoked discussions about animal welfare. Civic

Consulting and Agra CEAS Consulting (2007) are two consulting agencies based in Germany and the United Kingdom, respectively, which submitted a report to the World Organization for Animal Health (OIE) were they identified direct and indirect losses due to HPAI. Direct losses included production losses through culling and mortality, costs associated with control measures and other direct production losses, such as staying out of business for a period of time. Indirect losses included ripple effects (such as price and demand shocks), trade impact, spill-over effects (such as effects on tourism and service sectors), and effects to the wider society, such as loss of workforce due to human sickness and mortality.

Nepal faced its first outbreak of HPAI in January 2009 in the eastern part of the country, 600 km from the capital of Kathmandu. Since then, multiple outbreaks have been reported and thousands of poultry have been destroyed in an effort to control the disease. By April, 2013, Nepal had reported a total of 54 outbreaks of HPAI to the OIE (OIE, 2013). As a consequence of the outbreaks, either because of culling to control the infection or because of the infection-induced mortality, as of mid-April, 2013 Nepal had reportedly lost nearly 150,000 birds of various domestic species.

The Nepali government has been implementing a prevention and control program for HPAI since 2007 in an effort to contain the disease as early as possible and minimize possible poultry losses due to disease outbreaks. From 2007 to 2010, avian influenza control project (AICP) was supported by the World Bank whereas after that Nepali government has been carrying out the control program using its own resources. Major control policies implemented are the surveillance of poultry farms and stamping out of poultry flocks in the outbreak area up to 3 km from the index case, followed by disinfection of the farms. In addition, there is a ban on poultry rearing in the declared outbreak area for 45 days after the outbreak has been declared over. Surveillance is carried out in a 7 km zone outside the outbreak area for 90 days after the outbreak has been declared over. Despite these control efforts, the number of outbreaks of HPAI in Nepal has been increasing. Moreover, outbreaks have been reported in the Chitwan and Kathmandu districts, which are the hubs for commercial poultry production in Nepal. This increase in HPAI outbreaks has questioned whether the effectiveness of the current HPAI control program warrants the societal resources spent on it and led to the discussion of alternative control strategies, such as vaccination of the national poultry flock.

A decision to change strategies can be facilitated by a comparative economic analysis of the current control strategy and possible alternatives. A number of economic techniques, such as mathematical programming, network analysis, decision analysis, simulation, and cost-benefit analysis have been applied to livestock disease-control decisions (Bennett, 1992). Cost-benefit analysis (CBA) is typically the method of choice when assessing a change in strategy over a long-term period (Dijkhuizen et al., 1995). CBA evaluates the impact of an intervention versus the cost of such intervention and is typically used by governments to evaluate the desirability of a given intervention (Tiongco, 2008). The objective of this study was to perform a CBA in order to evaluate the economic worth of two control alternatives to the HPAI control program currently implemented by the Nepali government. The two evaluated control alternatives were: (i) ceasing the current control program (i.e., absence of control measures) and (ii) vaccinating 60% of the domestic poultry flock twice a year.

3.2 Methods

We performed a CBA to evaluate the current control program (CCP) in comparison with the alternatives (of implementing no control measures or vaccinating 60% of domestic flock twice a year) that have been considered for control of HPAI in Nepal. We used the time frame of 3 years for evaluation of control measures to demonstrate the cumulative effect of the considered control options. We used this time frame based on the experience of Nigeria where they have performed the CBA of HPAI control over a 3 years-time frame (Fasina et al., 2007). The following assumptions were made:

- Unless stated otherwise, control options were evaluated assuming 19 outbreaks of HPAI occurring annually under the CCP, which was the number of outbreaks that had occurred in 2012.

- Ceasing to implement control measures or absence of control measures (ACM) would result in a 100% increase of the number of birds dying and being culled due to HPAI annually (i.e., the number of affected birds will double every year during our evaluation period). This assumption reflected the complete lack of information about the expected increase of the number of affected birds under the hypothetical absence of control measures and was tested in the sensitivity analysis.
- The market loss due to HPAI outbreaks occurring under the CCP and ACM was assumed to be 10% while we assumed no market loss under the vaccination option.
- The vaccination program would prevent 80% of the losses, in terms of death and culled birds, which would otherwise occur under the CCP (baseline).
- A 5% discount rate was used on all costs and benefits to correct the estimated costs and benefits for time value of money.

For each of the control options, we estimated the involved costs and benefits and evaluated them as described in the followings sections. Next, the estimated costs and benefits were discounted. The discount rate represents the real interest rate which is the rate of interest that would be earned in excess of the inflation rate. We used the formula by Marsh (1999) to derive the present value (PV) of a future value (FV): $PV= FV / (1+r)^n$, where, n= number of periods and r = discount rate per period. We calculated the present value of costs (PVC) and the present value of benefits (PVB) using this approach. Finally, the control programs were evaluated based on the ratio of the estimated and discounted benefits and cost, i.e., benefit-cost ratio (B-C). Furthermore, we estimated the net present value (NPV) for the evaluated control programs as the difference between the PVB and the PVC. Unless otherwise stated, all the monetary values in this study are expressed in US dollars. As of May 20, 2013, the exchange rate was 1 US$ = 87.5 Nepali currency (rupees) (Central Bank of Nepal).

3.2.1 Description of the current control program (CCP)

CCP is the program currently implemented by the Nepali government for the control of HPAI in Nepal. This includes surveillance, stamping out operation followed by compensation and training and information dissemination activities. These activities are guided by a Bird Flu Control Order (BFCO) of Nepali government. The BFCO is a legal document by the Nepali Government that officially outlines the country's AI prevention and control practices.

3.2.1.1 Identification of relevant costs under the current control program (CCP)

Costs incurred during the CCP are related to: (1) Cost of surveillance including (a) Cost of farm visits (b) Cost of sampling (c) Cost of testing samples (laboratory costs), (2) Cost of stamping out operations and compensation, (3) Cost of training, communication and information dissemination. Each of these costs is elaborated below.

3.2.1.1.1 Cost of surveillance

Surveillance activities include visiting farms to monitor for the presence of disease, sample collection and testing of those samples. The cost of surveillance (which includes the costs associated with farm visits by the Animal Health Officials, sample collection, and sample testing) were calculated as follows.

3.2.1.1.1.1 Costs of farm visits in the absence of an outbreak

As part of Nepal's national surveillance plan for HPAI, the 75 districts within the country have been divided into three categories: high risk, medium risk and low risk districts based on the criteria that include the poultry population size, domestic duck population size, presence of lakes and water bodies, presence of migratory birds, and poultry movements. Based on the criteria, Nepal has 20 high risk districts (HRD), 21 medium risk districts (MRD) and 34 low risk districts (LRD). For the purpose of active surveillance, 8 and 4 risk sites have been identified in each HRD and MRD, respectively. Only passive surveillance, which is based on the monthly reporting of livestock diseases by the district livestock services offices to the veterinary epidemiology center, is conducted in the LRDs. An Animal Health Official visits each HRD and MRD risk site once a week to inspect farms and look for unusual poultry mortalities. During these visits, samples are collected if deemed necessary. Animal Health Officials receive US$ 4.5 per week to cover the cost of gas for their motorcycles and costs associated with using their cell phones. As there are 248 total active surveillance sites in the HRDs and MRDs, this leads to the total estimated cost of US$ 58,002 per annum (Table 3).

Table 3. Annual cost of farm visits in the absence of HPAI outbreaks

	Numbers	Total sites	Costs per week	Costs per year
HRD*	20	20*8= 160	$4.5*160= $720	$720*52= $37,440
MRD*	21	21*4= 88	$4.5* 88= $396	$396*52= $20,592
Total Costs			$1,116	$58,032

*HRD: High risk districts; MRD: Medium risk districts

3.2.1.1.1.2 Costs of farm visits during outbreaks

During an outbreak, active surveillance is conducted within a 7 km radius outside of the stamping out zone. Generally, 2 teams, each comprised of 3 Animal Health Officials, are deployed for this purpose. The same teams monitor the stamping out zone for 6 weeks, as per the provision of the BFCO, after the completion of the stamping out operation. They monitor for violation of the re-stocking ban before completion of the 6-

week stamping out period and for breakage of seals that are kept in the gates of affected commercial farms after the completion of stamping out and cleaning. Each member of the team receives US$ 4.5 per week as a compensation for fuel and the cost of using their cell phones for a total of 6 weeks. Assuming that 19 outbreaks would continue to occur annually under the CCP (the baseline), the total cost of farm visits during outbreaks was estimated at US$ 3,078 per annum (Table 4).

Table 4. Annual cost of farm visits during outbreaks

Cost / outbreak	$4.5 *6*6= $162
Total cost for 19 outbreaks	$162*19= $3,078

3.2.1.1.3 Costs of farm visits after outbreaks

After the completion of the stamping out operation, post-outbreak surveillance activities are conducted in a 7 km area outside the stamping out zone. For this purpose, generally 2 teams, each comprising of 3 Animal Health Officials, are deployed. Each member of the team receives US$ 4.5 per week for fuel and the cost of using their cell phones for a total of 6 weeks, as per the provision of BFCO. Considering 19 outbreaks per year, the total cost of postoperative surveillance is US$ 3,078 per annum (Table 5).

Table 5. Annual cost of farm visits as part of post-outbreak surveillance

Cost / outbreak	\$4.5 *6*6= \$162
Total cost for 19 outbreaks	\$162*19= \$3,078

3.2.1.1.4 Cost of sample collection

As per the national surveillance plan for AI in Nepal, a total of 12,780 samples (tracheal and cloacal swabs, serum samples, dead birds, and fresh feces) (Appendix 1) are collected across the country annually. Based on the tentative market costs in Nepal, US\$ 0.57 should be sufficient to purchase a sampling kit set, comprising of 1 syringe and needle, 1 pair of gloves, cotton swabs, disinfectant (70% alcohol), small plastic bag and serum vials. The total estimated cost for collection of samples was thus 12,780*\$0.57 = US\$ 7,285.

3.2.1.1.5 Cost of testing samples (laboratory costs)

In 2011, a total of 6,596 samples were tested with the type A AI antigen rapid test kit (Bionote, Republic of Korea), 524 serum samples were tested with the type A AI antibody ELISA test kit (Idexx Laboratories, USA), and 191 samples were tested with the reverse transcription polymerase chain reaction (RT-PCR) (Bio-Rad Laboratories, USA) at the Central Veterinary Laboratory and in regional veterinary laboratories in

Nepal (Annual Bulletin of CVL, 2010/11). We assumed the same numbers were tested in 2012 as data for 2012 was not available. The lower number of samples tested than collected might be due to pooling of samples from the same farm or household for testing. In addition, some samples may have been rejected by laboratory due to quality issues. When a sample is positive for H5 by RT-PCR, it is sent to the OIE reference laboratory at Weybridge, United Kingdom, for final confirmation of HPAI for the first case in a year. Subsequent samples are tested only in Nepal with the RT-PCR and an outbreak is declared when there is a positive result. The total annual cost of laboratory testing was calculated as the sum of the costs associated with the cost of type A antigen rapid test kits, type A antibody ELISA tests and RT-PCR tests as shown in Table 6. The estimated total cost was US$ 46,817.

Table 6. Annual cost of laboratory tests

Tests	Number tested	Cost / test	Total costs
Type A AI rapid tests	6,596	$6.7	6,596*$6.7= $44,193
RT-PCR	191	$7.7	191*$7.7= $1,471
Type A Ab ELISA tests	524	$ 2.2	524*$2.2= $1,153
Total costs of all tests			$46,817

3.2.1.2 Cost of stamping out operation and compensation

The average cost of stamping out operation and compensation based on the experience of previous HPAI control in Nepal, as mentioned in the Implementation Completion and Results Report, is US$ 10 per bird (World Bank, 2013). During the 19 outbreaks that had occurred in 2012, 18,110 birds were destroyed in an effort to control the disease. Thus, the total cost of stamping out and compensation was estimated as 18,110 birds culled* US$ 10 = US$ 181,100.

3.2.1.3 Cost of training, communication and information dissemination

Public awareness campaigns are conducted at the central level through related directorates under the Department of Livestock Services. In the field, respective District Livestock Services Offices are responsible for public awareness. Generally, information is circulated through mass media such as the national newspaper, national television channels, local radio stations, local newspapers, pamphlets and posters. Based on experience in 2012, there will be four, two and one training sessions provided annually to the farmers in each HRD, MRD and LRD, respectively. Per unit costs are based on personal communication with an officer who had worked in AICP (Dr. Nabin Ghimire, personal communication). The breakdown of the costs is shown in Table 7. The total costs associated with training, communication and information dissemination is estimated at US$ 77,207/year.

Table 7. Annual costs of training, communication and information dissemination

Activities		No. of districts	No. of training sessions	Per unit cost	Total costs
1.	Training				
	a. High risk	20	20*4= 80	$229	$229*80= $18,320
	b. Medium risk	21	21*2= 42	$229	$229*42= $9,618
	c. Low risk	34	34*1= 34	$229	$229*34= $7,786
	d. Regional		5	$1,143	$1,143*5= $5,715
	e. Central		1	$1,143	$1,143*1= $1,143
2.	Broadcasting				
	a. High risk	20		$229	$229*20= $4,580
	b. Medium risk	21		$114	$114*21= $2,394
	c. Low risk	34		$57	$57*34= $1,938
	d. Regional				$2,857
	e. Central				$11,428
3.	Pamphlets, posters printing				$11,428
			Total costs		$77,207

The summary of the annual cost calculated for HPAI control under the CCP, assuming the number of birds being affected by HPAI per year being the same as in 2012 (i.e., 19 outbreaks) is presented in Table 8:

Table 8. Summary of the annual costs of the current control program

Activities	Year 1
Surveillance	$118,290
Stamping out operation and compensation	$181,100
Training, communication and information	$77,207
Total	$376,597

3.2.2 Identification of relevant losses under the current control program

Losses incurred under the CCP were grouped as (1) Losses due to HPAI caused mortality among poultry, (2) Losses due to culling of poultry beyond the losses covered by government compensation, (3) Losses due to production, movement and a trade ban period imposed by the government and (4) Losses due to market reaction. Each of these losses is elaborated below.

3.2.2.1 Losses due to HPAI caused mortality

In 2012, a total mortality of 41,100 poultry in Nepal was reported to OIE. Out of this total, 34,872 were commercial layers, 2,850 were commercial broilers, 1,410 were backyard poultry and 1,968 were broiler parents. As farmers receive compensation only for poultry killed by the government as part of the control program, farmers' losses were calculated based on the prevailing farm gate price of the respective category of poultry. The farm gate price used in this study was obtained from the World Bank's Implementation Completion and Results Report of HPAI control project in Nepal. The total annual direct loss due to HPAI related mortality based on the farm gate price was US$ 298,922. The breakdown is shown in Table 9.

Table 9. Annual direct loss to farmers due to HPAI related mortality

Type of poultry	Cases	Farm Gate Price	Total
CL	34,872	$7.4	$258,053
CB	2,850	$3.0	$8,550
BC	1,410	$3.8	$5,358
BP	1,968	$13.7	$26,961
Total	41,100		$298,922

[*] CL: Commercial layers; CB: Commercial broilers; BC: Backyard chicken; BP: Broiler parent

3.2.2.2 Losses due to culling of poultry

Whenever an index case is identified and officially declared, the Nepali government imposes stamping out operations as per the BFCO. Based on the epidemiological situation, a team under the Livestock Department decides how wide (commonly 0 to 3 km) the culling area will be. As farmers received some compensation for poultry killed (which was accounted for under the costs of the program), farmers' losses were calculated by subtracting the compensation they received from the prevailing farm gate price of the respective category of poultry. The total annual direct loss due to the culling of poultry was estimated at US$ 101,826. The breakdown is shown in Table 10.

Table 10. Annual direct losses to farmers due to the culling of poultry

Type of poultry	No. Culled	Compensation	Farm Gate Price	Per unit loss	Total Loss
CL	1,6748	$1.5	$7.4	$5.9	$98,813
CB	150	$1.5	$3.0	$1.5	$225
BC	1,212	$1.5	$3.8	$2.3	$2,788
Total	18,110				$101,826

*CL: Commercial layers; CB: Commercial broilers; BC: Backyard chicken

3.2.2.3 Losses due to a production ban period imposed by the government

The Nepali government imposes a production ban period of 45 days according to the provision of the BFCO in the outbreak zone. This causes additional losses to the farmers, elaborated below, as they will be out of business at least for a period of 45 days.

3.2.2.3.1 Losses to backyard farmers

Although the ban period is 45 days, it generally takes 6 months for backyard farmers to resume their poultry business (Dr. N.P.S Karki, personal communication). This is because of the difficulty of obtaining replacement flocks. It is estimated that during this 6 month period, each affected household loses 22 marketable chickens and on average 35 households are affected in each outbreak (Dr. N.P.S Karki, personal communication), with US$ 3.8 per kg and an average weight per chicken of 2 kg, the farmers' loss is estimated to be US$ 5,896 per outbreak. With 19 outbreaks per year, the total annual loss is estimated at US$ 112,024. In addition, traders lose US$ 22,405 (20% of farm gate price). Therefore, the total loss in the backyard poultry system due to the ban in rearing was estimated at US$ 134,429.

3.2.2.3.2 Losses to broiler farmers

It has been observed that broiler farmers lose 2 cycles of broiler production due to HPAI (Dr. N.P.S Karki, personal communication). The average margin in each broiler is US$ 0.4. In 2012, 3,000 broilers were culled or died from HPAI H5N1. If farmers do not raise broilers for 2 cycles, they will lose an estimated 2*3,000*$0.4= US$ 2,400. In addition, traders lose US$ 480 (20% of farm gate price) and meat processors lose US$ 600 (25% of farm gate price) (Dr. N.P.S Karki, personal communication). Therefore, the total loss to the broiler industry due to the ban period was estimated at US$ 3,480.

3.2.2.3.3 Losses to layer farmers

The average profit per egg for farmers is US$ 0.009. As 51,620 layers died or were destroyed in 2012, we assumed an average daily egg loss of 37,166 (assuming an average laying capacity of 72%). Over the course of 45 days, it is estimated that farmers lose 37,166*45 days*$0.009= US$15,291 from eggs. After the ban period is over, if farmers restock immediately, they need to wait another 5 months for layers to produce eggs. From this, farmers will additionally lose income for 150 days*$0.009*37,166= US$ 50,971. The cumulative loss will thus be US$ 66,262. Traders lose US$ 13,252 (20% of farm gate price) and egg retailers lose US$ 6,626 (10% of farm gate price). Therefore, the total loss by the layer industry, due to the ban period, is estimated at US$ 86,140 annually.

3.2.2.3.4 Losses due to market reaction

It is very difficult to accurately estimate the losses associated with the market reaction due to HPAI. The most important losses are those due to the reduction in the consumption of poultry and poultry products, and losses due to the reduction in prices of poultry and poultry products. The sale of day old chicks (DOCs) will decrease as many farmers remain out of business and new farmers do not want to start poultry farming around the time of outbreaks. These types of effects are not uniform across the country. For the purpose of this study, we assumed that HPAI outbreaks and concurrent poultry mortalities falsely believed to be due to HPAI will affect 10% of the total volume of national commercial poultry production annually. With this volume (10%) of affected production, we assumed that consumption will remain fairly similar while there will be 20% reduction in poultry meat price and 10% reduction in egg price (Dr. Rajesh Bhatta, personal communication). Backyard poultry are mostly fed leftovers and, therefore, backyard producer are able to wait and sell their products when prices stabilize. We assumed that there will be no indirect effects for backyard poultry farmers other than those directly affected by the outbreaks.

According to the data published in the MyRepublica national daily newspaper, published in Kathmandu quoting Dr. T.C Bhattarai, leading poultry entrepreneur in Nepal, on October 7, 2012 (www.myrepublica.com), the estimated total broiler meat production was 132.17 million kg and total egg production was 1.11 billion eggs in

2012. Likewise, 78.87 million broiler DOCs and 10 million layer DOCs were produced. More than 646,000 tons of poultry feed were produced. We used these data to calculate the losses to the commercial sector, as official government data were not available for 2012.

3.2.2.3.4.1 Loss due to the reduction in the price of poultry

The loss due to the reduction in the price of poultry and poultry products was estimated at US$ 6,037,317. A breakdown is shown in Table 11.

Table 11. Loss due to the reduction in the price of poultry and its products

Items	Total Production	10% of production	Price / Unit	% of price reduction	Price loss / unit	Total loss
Broiler meat	132,170,000	13,217,000	$1.9	20	$0.38	$5,022,460
Eggs	1,110,000,000	111,000,000	$0.09	10	$0.009	$1,014,857
Total						$6,037,317

3.2.2.3.4.2 Loss due to reduction in the price of DOCs

In Nepal, 78.87 million of broiler DOCs and 10 million of layer DOCs were produced in 2012 (www.myrepublica.com). The price of a broiler DOCs ranges from US$ 0.63 - 0.86 depending upon the supply and demand situation whereas the price of a layer DOC varies from US$ 0.74 - 0.97. For the purpose of this study, we assumed that 10% of the total DOCs produced will suffer price reduction due to HPAI (Dr. Rajesh Bhatta, personal communication). We assumed US$ 0.74 as the average price for a broiler DOC while US$ 0.86 for a layer DOCs (Dr. Rajesh Bhatta, personal communication). During outbreaks, generally DOCs prices come down by about US$ 0.23 per DOC (Dr. Rajesh Bhatta, personal communication). This will cause a loss of 7,887,000*$0.23= US$ 1,814,010 to the broiler DOC producer while it will cause a loss of 1,000,000*$0.23= US$ 230,000 to the layer DOC producers. The total loss to the DOC producers will be US$ 2,044,010.

3.2.3 Description of the absence of control measures (ACM)

3.2.3.1 Costs under the absence of control measures

The absence of control measures (ACM) means that the government would take no action towards the control of AI. Under this option, there would be no active surveillance and subsequent sample testing, stamping out operations, training seminars, or communication and information dissemination activities focused on AI. Farmers will not receive any compensation for losses. There will be no ban period for the poultry in the outbreak zone. Therefore, the cost of the ACM would be zero.

3.2.3.2 Losses under the absence of control measures

It is impossible to predict with certainty how many additional outbreaks and the associated losses would occur if the CCP is withdrawn. For the purpose of this study, under the ACM, we assumed that without the current control efforts, the number of birds that would die due to HPAI would double each subsequent year during our evaluation period. Under these assumptions, the following losses have been identified for the ACM: (1) Losses due to HPAI caused mortality and (2) Losses due to market reaction.

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3.2.3.2.1 Losses due to HPAI caused mortality

Based on the assumed consequences of ceasing any control strategies, we estimate the number of birds dying from HPAI would double each subsequent year. Farmers’ loss was calculated based on the prevailing farm gate price of the respective category of poultry. The total direct loss due to HPAI related mortality based on farm gate price was US$ 597,845 in the first year, $1,195,690 in the second year and $ 2,391,319 in the third year (Table 12).

Table 12. Loss due to HPAI related mortality under absence of control measures

	CL[*]	CB[*]	BC[*]	BP[*]	Total
Farm gate price	$7.4	$3.0	$3.8	$13.7	
Cases 1st yr	69,744	5,700	2,820	3,936	
Losses 1st yr	$516,106	$17,100	$10,716	$53,923	$597,845
Cases 2nd yr	139,488	11,400	5,640	7,872	
Losses 2nd yr	$1,032,211	$34,200	$21,432	$107,846	$1,195,690
Cases 3rd yr	278,976	22,800	11,280	15,744	
Losses 3rd yr	$2,064,422	$68,400	$42,864	$215,693	$2,391,379
Total loss					$4,184,914

[*] CL: Commercial layers; CB: Commercial broilers; BC: Backyard chicken; BP: Broiler parent

3.2.3.2.1 Losses due to market reaction

For the purpose of this study, we assumed the losses due to market reaction would be identical to the losses under the CCP, i.e., the estimated total annual loss was US$ 8,081,920. Assuming the same market loss under ACM as under the CCP may be considered as an underestimation of the loss because a larger number of outbreaks may provoke a stronger market reaction. On the other hand, an invalid assumed higher market loss could lead to an overestimated benefit of the current program.

3.2.4 Description of the vaccination program

Vaccination is one of the options employed to control HPAI. Inactivated AI vaccines have helped prevent morbidity, mortality, egg production loss, and control the spread of disease and reduce economic losses (Halvorson, 2002).

At least 80% of the susceptible poultry population in a flock needs to be vaccinated to control the infection (Tiensin et al., 2007). However, it is very hard to achieve this level of vaccination coverage in a country like Nepal, where there are large numbers of backyard birds. Thus, it would seem reasonable to target vaccination of 60% of the national flock two times per year using an H5 vaccine, e.g. A/Goose/Guangdong/1996 (Harbin Veterinary Research Institute, Harbin, Heilongjiang province, China). Under this program, it is reasonable to expect that a few outbreaks (possibly smaller scale) would still occur. Thus, it would seem reasonable to expect that approximately 20% of the birds lost on under the CCP would die under the vaccination program.

3.2.4.1 Identification of relevant costs (inputs) under the vaccination option

Costs incurred during the vaccination option are: (1) Cost of vaccine, (2) Cost of administering vaccine, (3) Costs of surveillance (farm visits sample collection and laboratory testing), (4) Cost of stamping out operation and compensation (5) Cost of training, communication and information dissemination.

3.2.4.1.1 Costs of vaccine

The average cost per dose of AI H5 vaccine is about US$ 0.04 as per prevailing market price. The total cost of the vaccine purchase would depend upon the poultry population to be vaccinated. Based on the current population size and the growth rate of 7% for broilers, 5% for layers (Dr. N.P.S. Karki, personal communication) and 3% for backyard chickens while duck population were decreasing by 2% annually (MoAD, 2011), the population size for the coming years and the number of birds to be vaccinated were predicted (Table 13).

Table 13. Poultry population in Nepal

Type	Yr 1	60% of Yr 1	Yr 2	60% of Yr 2	Yr 3	60% of Yr 3
CB*	50,000,000	30,000,000	53,500,000	3,2100,000	57,245,000	34,347,000
CL*	6,000,000	3,600,000	6,300,000	3,780,000	6,615,000	3,969,000
LP*	120,000	72,000	126,000	75,600	132,300	79,380
BP*	1,130,000	678,000	1,209,100	725,460	1,293,737	776,242
BC*	11,592,168	6,955,301	11,939,933	7,163,960	12,298,131	7,378,879
BD*	379,753	227,852	372,158	223,295	364,715	218,829

* Data source for commercial: www.myrepublica.com
Data source for backyard: Statistical information on Nepalese agriculture, MoAD, 2011
*CL: Commercial layers; CB: Commercial broilers; LP: Layers parent; BP: Broiler parent; BC: Backyard chickens; BD: Backyard ducks

The cost of vaccine was calculated as: Cost of vaccine = cost of one dose * number of vaccinations per year*60% of the population. The total cost of vaccine purchased for 3 years would be US$ 10,589,663. Breakdown of this cost is shown in Table 14.

Table 14. Cost of vaccine

Type	Yr 1	Yr 2	Yr 3	Total
CB*	$2,400,000	$2,568,000	$2,747,760	$7,715,760
CL*	$288,000	$302,400	$317,520	$907,920
LP*	$5,760	$6,048	$6,350	$18,158
BP*	$54,240	$58,037	$62,099	$174,376
BC*	$556,424	$573,117	$590,310	$1,719,851
BD*	$18,228	$17,863	$17,506	$53,598
Total	$3,322,652	$3,525,465	$3,741,546	$10,589,663

*CL: Commercial layers; CB: Commercial broilers; LP: Layers parent; BP: Broiler parent; BC: Backyard chickens; BD: Backyard ducks

3.2.4.1.2 Costs of vaccine administration

AI vaccines are administered subcutaneously (Steitz et al., 2010). This makes vaccination tedious and costly. The average prevailing price for vaccinating an individual bird in Nepal is US$ 0.002 (Dr. Rajesh Bhatta, personal communication). The cost of vaccine administration was calculated as: Cost of vaccine administration per bird* number of vaccinations per year*60% of the population. The total cost of vaccine administration for 3 years was US$ 605,124. Breakdown of this cost is shown in Table 15.

Table 15. Costs of vaccine administration

Type	Yr 1	Yr 2	Yr 3	Total
CB*	$137,143	$146,743	$157,015	$440,901
CL*	$16,547	$17,280	$18,144	$51,881
LP*	$329	$346	$363	$1,038
BP*	$3,099	$3,316	$3,549	$9,964
BC*	$31,796	$32,749	$33,732	$98,277
BD*	$1,042	$1,021	$1,000	$3,063
Total	$189,866	$201,455	$213,803	$605,124

*CL: Commercial layers; CB: Commercial broilers; LP: Layers parent; BP: Broiler parent; BC: Backyard chickens; BD: Backyard ducks

3.2.4.1.3 Cost of surveillance during vaccination program

We assumed that the cost of surveillance under vaccination program will be identical to the cost of surveillance incurred under the CCP. The cost, under this heading, is estimated to be US$ 118,290 annually.

3.2.4.1.4 Cost of stamping out operation and compensation

We assumed that under the vaccination program, there would be a few outbreaks where 20% of the birds affected under the CCP (baseline) would die from HPAI. Considering US$ 10 as the cost of stamping out operation and compensation per bird, the cost of stamping out and compensation would be 3,622 (20% birds of the baseline) * US$ 10= US$ 36,220 annually.

3.2.4.1.5 Cost of training, communication and information dissemination

We assumed that the cost of training, communication and information dissemination under the vaccination program would be identical to the cost incurred in the CCP. The total cost, under this heading, was estimated to be US$ 77,207 annually.

3.2.4.2 Identification of relevant losses under the vaccination option

Losses under the vaccination option are: (1) losses due to HPAI related mortality, and (2) losses due to culling of poultry.

3.2.4.2.1 Losses due to HPAI related mortality

Under our assumption that 20% of the birds would die from HPAI compared to the baseline (CCP), the total estimated direct loss due to HPAI related mortality based on the farm gate price in 3 years was US$ 59,787 annually (Table 16).

Table 16. Annual loss to farmers due to HPAI caused mortality in vaccination program

	CL*	CB*	BC*	BP*	Total
Farm gate price	$7.4	$3.0	$3.8	$13.7	
Cases	6974	570	282	3,94	
Losses	$51,608	$1,710	$1,071	$5,398	$59,787

*CL: Commercial layers; CB: Commercial broilers; LP: Layers parent; BP: Broiler parent; BC: Backyard chickens

3.2.4.2.2 Losses due to culling of poultry

Under our assumption that 20% of the birds would be culled due to HPAI compared to the baseline, the total direct loss due to HPAI related culling based on per unit loss (farm gate price after deducting compensation) in 3 years was US$ 20,365 annually (Table 17).

Table 17. Annual loss to farmers due to culling of poultry under vaccination program

	CL*	CB*	BC*	Total
Per unit loss	$5.9	$1.5	$2.3	
Cases	3,350	30	242	
Losses	$19,762	$65	$558	$20,365

*CL: Commercial layers; CB: Commercial broilers; BC: Backyard chickens

3.2.4.3 Identification of benefits under the vaccination program

We assumed that the vaccination program would help resume the domestic poultry market to a pre-outbreak level and it would prevent part of the costs associated with stamping out operation and compensation. As Nepal is primarily an importer of poultry and export is negligible, there will be no effect on the international poultry trade.

The benefits of the vaccination program would be the losses prevented that would have otherwise occurred under the CCP. Annual losses prevented (benefits) was US$ 8,707,116.

3.2.5 Sensitivity analysis

We performed a sensitivity analysis to evaluate the robustness of our program under different discount rates (3%, 10% and 15%), different numbers of birds dying under the ACM (200% increase, 50% increase and 50% decrease compared to the CCP baseline), different market reactions (5% and 15%) and different number of birds dying under the vaccination program (10%, 20% and 50% compared to the CCP baseline). We also calculated the break-even points for the market loss and the number of birds dying.

3.3 Results

3.3.1 "Current control program" vs "absence of control measures"

Economic evaluation showed that CCP is better than ACM option. The B-C was 1.96 and the net present value (NPV) was US$ 989,918 (Table 18).

Table 18. Summary of the cost and benefits of "current control program (CCP)" vs "absence of control measures (ACM)"

	Yr 1	Yr 2	Yr 3	Total
Loss in ACM	$8,679,172	$9,277,017	$10,472,706	$28,428,895
Loss in CCP	$8,707,117	$8,707,117	$8,707,117	$26,121,351
Benefit	-$27,945	$569,900	$1,765,590	$2,307,545
PVB at 5% discount rate	-$26,614	$516,916	$1,525,183	$2,015,485
Costs of CCP	$376,597	$376,597	$376,597	$1,129,791
PVC at 5% discount rate	$358,664	$341,584	$325,319	$1,025,567
NPV (Net present value)	-$385,278	$175,332	$1,199,864	$989,918
B-C ratio				1.96

3.3.2 “Vaccination” vs “current control program”

The economic evaluation showed that vaccination is better than CCP. The B-C was 2.41 and the NPV was US$ 13,745,454 (Table 19).

Table 19. Summary of the cost and benefits of “vaccination” vs “current control program (CCP)”

	Yr 1	Yr 2	Yr 3	Total
Loss in CCP	$8,707,117	$8,707,117	$8,707,117	$26,121,351
Loss in “vaccination”	$80,150	$80,150	$80,150	$240,450
Benefit	$8,626,967	$8,626,967	$8,626,967	$25,880,901
PVB at 5% discount rate	$8,216,159	$7,824,913	$7,452,298	$23,493,370
Added Costs of “vaccination”	$3,367,638	$3,582,040	$3,810,469	$10,760,147
PVC at 5% discount rate	$3,207,274	$3,249,016	$3,291,626	$9,747,916
NPV (Net present value)	$5,008,885	$4,575,897	$4,160,672	$13,745,454
B-C ratio				2.41

3.3.3 Sensitivity analysis

The sensitivity analysis was conducted to test the effect of the assumed discount rate, market reaction, the number of predicted birds affected under the ACM and vaccination program on the results of CBA.

3.3.3.1 Discount rate

In addition to the 5% discount rate assumed for the main analysis, we evaluated the control options using the discount rates of 3%, 10% and 15%. The NPVs and B-C ratios under different discount rates are presented in Table 20. Under the considered discount rates, the calculated NPVs were positive and B-C ratios were still higher than 1 which indicates that it is better to implement either of the two control programs than doing nothing.

Table 20. Net present values and benefit-cost ratios under different discount rates

		NPV	B-C
	CCP vs ACM		
Discount rates	3%	$1,060,573	1.995
	5%	$989,918	1.965
	10%	$835,560	1.892
	15%	$707,675	1.823
	Vaccination vs CCP		
Discount rates	3%	$14,269,250	2.408
	5%	$13,745,454	2.410
	10%	$12,568,275	2.414
	15%	$11,554,944	2.419

3.3.3.2 Market reaction

Sensitivity analysis was performed to evaluate the magnitude of a different market loss than that assumed in the main analysis. Specifically, we evaluated the effect of changing market loss in the CCP and ACM from 10% (main analysis) to 15% and 5% in ACM, while for all analysis the market loss under the vaccination program was 0%. The NPVs and B-C ratios under different market loss scenarios are presented in Table 21. Additionally, we calculated a break-even point (a situation of no gain or loss), where the NPV becomes zero, for both the ACM and vaccination options. Break-even analysis showed that there would be no gain or loss from application of the vaccination program if the market loss under CCP would be only 3.75% (US$ 3,033,879) (note that a fixed 0% market loss was assumed for the vaccination program). Since market loss could be different under the CCP and ACM programs, different combinations of market losses for the two programs could present the break-even points. For example, a break-even point was identified when the market loss was 9.55% (US$ 7,717,821) under ACM and 10% under CCP. Another break-even point was identified when the market loss was 0% under the CCP albeit it was 4.49% (US$ 363,506) under ACM.

Table 21. Net present value and benefit-cost ratio under different market loss

		NPV	B-C
	CCP vs ACM		
Market loss	15%	$11,993,648	12.694
	10%	$989,918	1.965
	5%	-$10,013,812	-8.760
	CCP vs Vaccination		
	15%	$24,749,184	3.538
	10%	$13,745,454	2.410
	5%	$2,741,723	1.281

3.3.3.3 Number of birds affected

In the main analysis, we assumed that there would be a 100% increase in the number of birds affected each year under the ACM option. For sensitivity analysis, we evaluated scenarios assuming that there could be a 200% or 50% increase or a 50% decrease in the number of birds affected by HPAI under the ACM option. The results are shown in Table 22. The break-even point analysis showed that it is justified to continue with the CCP only if the number of birds dying due to HPAI increases by more than 76% every year during the evaluation period. The CCP would not be justified if the number of birds dying due to HPAI would increase by less than 76% every year and obviously if it would decrease (which is unlikely).

Table 22. Net present value and benefit-cost ratio under different assumptions for the change in the number of birds affected under absence of all measures

	NPV	B-C
CCP vs ACM		
100% increase in birds dying	$989,918	1.965
200% increase in birds dying	$7,539,153	8.350
50% increase in birds dying	-$818,473	0.201
50% decrease in birds dying	-$2,484,640	-1.422

3.3.3.4 Outbreaks under the vaccination program

In the main analysis, we assumed that under vaccination, a few smaller outbreaks would continue to occur. These outbreaks would incur a loss of 20% of the birds affected under the CCP. To test this assumption, we evaluated the different scenarios where 0%, 10%, and 50% of birds lost under the CCP baseline would die despite vaccination. Results showed that when the number of birds affected under the vaccination option is at 0%, 10%, and 50% of the baseline, B-C ratio was 2.457, 2.340

and 2.433 while NPV was US$ 14,062,357, US$ 13,903,906, and US$ 13,270,099, respectively. In these cases, it is worth to invest in the vaccination even if there are outbreaks. However this conclusion would change if the market loss under the CCP was different from the assumed 10%. The break-even point would occur when the market loss was 3.75% in CCP compared to no market loss in vaccination.

3.4 Discussion

We performed CBA to evaluate whether the investment into the current control program (CCP) to control HPAI currently being operated in Nepal, is justified compared to alternatives of absence of control measures (ACM) and the vaccination options. In terms of the B-C, our findings indicated that there is a return of 1.96 dollars for every dollar spent by the CCP compared to ACM. The net present value of the CCP versus absence of control measures was US$ 989,918. The vaccination program yields a return of 2.41 dollars for every dollar spent when compared to CCP. The net present value of vaccination versus the CCP was US$ 13,745,454.

Fasina et al. (2007) have reported a return of $52 for every dollar invested in vaccination program compared to doing nothing in Nigeria. Such a high estimated B-C compared to ours might be due to the differences in the baseline outbreak losses between Nigeria and Nepal. Outbreak loss was very high in Nigeria compared to Nepal. This resulted in the prediction of prevention of a huge loss after vaccination compared to Nepal.

Nepal’s export market of poultry and poultry products is negligible. However, it is near to being self sufficient to meet the domestic demands for poultry meat and eggs; albeit it has to import parent stocks and vaccines. Under these conditions, safeguarding domestic poultry industry is a priority for Nepal. In case that the number of outbreaks continues to increase, it would be highly recommended for Nepal to adopt the vaccination control program. Naeem (2003) has reported that mass vaccination program and bio-security helped overcome HPAI in Pakistan. However, though the vaccine protects from the clinical disease, there is a possibility that asymptomatic virus circulation may continue and result in the spread of infection (Ellis et al., 2004). Our B-C and NPV for vaccination program compared to CCP might be an overestimation as we had assumed there would be no market loss under the vaccination program. If there would be a market loss under vaccination, then the B-C ratio and the NPV would be lower.

The performed CBA has important limitations. We did not consider the public health implications of HPAI in Nepal as there have been no recorded HPAI related human illnesses in Nepal. Therefore, we implicitly assumed that there will be no human health losses in the future and under the evaluated alternative control scenarios. If human health losses would indeed occur, our estimate of the benefits would be underestimated.

We did not consider other indirect benefits of HPAI control programs in Nepal, such as an increased availability of animal proteins to the backyard poultry farmers. Backyard farmers mostly rely on the eggs and chicken meat produced in their own house for protein source. When birds die due to HPAI and they remain out of poultry rearing during production and production ban period, their protein supply would decrease but it is difficult to quantify. Nevertheless, not accounting for this indirect benefit may have underestimated the total benefits of the evaluated CCP and vaccination programs.

In conclusion, implementation of one of the control programs, either the CCP or the proposed vaccination program, should be used rather than ceasing to implement HPAI control measures in Nepal. Vaccination would be better than the CCP; however, the concerns related to AI vaccination regarding the possibility of asymptomatic virus circulation need to be further evaluated before its implementation.

CHAPTER IV

CONCLUSIONS

Avian influenza (AI), a viral disease caused by influenza A virus, is mainly a disease of birds. Avian influenza viruses (AIVs) can be divided into highly pathogenic (HPAI) or low pathogenic (LPAI) based on their ability to cause disease. Among these two types, HPAI are of concern mainly for two reasons. First, they infect humans (Yee et al., 2009) and second, they cause huge monetary losses to poultry farmers, industry and government through poultry mortalities and the control programs implemented to control the infection spread. The overall economic loss due to HPAI is negligible compared to their gross domestic product (GDP). However, the economic loss to the smallholder farmers, whose major source of income comes from selling poultry, is particularly important because of low resilience of smallholders to recover from the economic loss they suffer. HPAI became of global concern when human infections were noticed in Hong Kong in 1997 where18 people were laboratory confirmed with HPAI H5N1 infections and six people died among them (FAO, 2013). Before the confirmed human infections in Hong Kong in 1997, there was an outbreak of HPAI H5N1 in a geese farm in Guangdong province, China (Xu et al., 1999). HPAI H5N1 then spread to Southeast Asian countries and gradually to Europe and Africa and finally became endemic in Asia (Lupiani and Reddy, 2009). In Nepal, HPAI H5N1 infection was detected for the first time in January 2009 in domestic chickens. Since this first outbreak, more than 54 outbreaks have been officially reported by the Nepali government to the World Animal Health Organization (OIE) as of April 2013 (OIE, 2013). The objective of this thesis was to improve the understanding of the epidemiology of AI and the economic worth of its control in Nepal. The objectives were addressed through two independent studies. The main conclusions of these studies are summarized below.

It is necessary to improve understanding of the epidemiology of AI to better control it. In this regard, understanding the epidemiology of AI in ducks is important as ducks may “silently” harbor the infection and transmit it to other susceptible hosts, such as chickens (Henning et al., 2011). A large number of duck farms exist in Kathmandu district of Nepal. On these farms, ducks are mainly raised in a scavenging system. In that system, ducks have access to ponds, rivers and other water bodies in the daytime where they have the opportunity to mingle with wild birds and backyard chickens. Due to such production practices, Kathmandu has been considered as a high risk district for HPAI outbreaks in Nepal since 2007. However, no clinical outbreaks were detected in Kathmandu until January 2012. Nevertheless, it was suspected that the AIVs had been circulating in Kathmandu even before the first detected outbreak. To assess that suggestion, we conducted a cross-sectional study to determine the presence of AI in Kathmandu ducks and estimate the seroprevalence of AIV antibodies in domestic ducks of Kathmandu using serum samples collected in 2011 (Chapter II). The estimated prevalence of AIV antibodies was 27.2% [95% Confidence Interval (CI): 24.6- 29.5]. This indicates that AIVs were circulating in domestic ducks in Kathmandu even before the detected outbreak of HPAI in poultry. However, the subtypes of AIVs circulating among ducks in Kathmandu in the summer of 2011 remain unknown. Having discovered that AI is present among ducks in Kathmandu, the next question was to identify the risk factors for the carriage of antibodies. As potential risk factors, we considered age and sex of ducks and farm size. In bivariate analysis at 20% confidence level, all the three risk factors were significantly associated with the antibodies carriage in ducks. However, the final multivariable model that controlled for clustering of ducks within farms, identified age as the only significant risk factor. Based on this model, ducks older than one year were more likely to be seropositive compared to ducks less than six months of age [Odds Ratio (OR) = 2.17 (95% CI: 1.07- 4.39)]. However, there were no statistically significant differences between the ducks ages less than six months of age and six months to one year of age [OR= 0.50 (95% CI: 0.18- 1.36)]. Finally, we wanted to know what proportion of farms raising pigs also has seropositive ducks. This is relevant considering that a pig could become infected with AI virus (Yasuda et al., 1991) providing an opportunity for the mixing of influenza viruses from ducks and pigs and possible emergence of new influenza viruses. Among all enrolled farms, 50% raised pigs and of the farms that raised pigs 51.6% (95% CI: 33.1- 69.8%) had seropositive ducks. We did not test the pigs for AIVs in those farms; however the presence of seropositive ducks on farms having pigs indicates the necessity of monitoring and testing of pigs on those farms.

Nepali government has been implementing a control program for HPAI since 2007. Despite the control program, outbreaks have continued to occur. This raised suggestions for implementation of alternative programs. However, careful evaluation of the economic feasibility of the alternatives is necessary before deciding on any new control strategy. Cost-benefit analysis (CBA) is often a method of choice when long-term control programs are desired at the national level (Dijkhuizen et al., 1995). This is the approach we have taken to evaluate the costs and benefits of HPAI control in Nepal (Chapter III). First, we compared the current control program (CCP) for HPAI in Nepal and the alternative of absence of control measures (ACM). Our analysis indicated a cost-benefit ratio of 1.96, which indicates that there is a return of 1.96 dollars for every dollar spent on the CCP compared to ACM. The cost-benefit ratio as a measure is to show how much return we obtain from each dollar we invest. This approach allows quick assessment of the worth of an investment but has limitations, such as it doesn’t take account of the investment scale while decision making; rather it just looks at the ratio. Therefore we also used another measure, the net present value (NPV) which gives the actual difference between the benefits and costs. The net present value of the CCP versus ACM was US$ 989,918. That means that Nepal should continue with the ongoing control program. That being said, despite the ongoing control efforts, outbreaks of HPAI still continue to occur in Nepal. Therefore, vaccination has been considered as a potential alternative control strategy. Hence, we compared the CCP for control of HPAI in Nepal to a potential new strategy that would involve vaccination of 60% of the domestic poultry flock twice a year. The vaccination program yielded a B-C ratio indicating a return of 2.41 dollars for every dollar spent when compared to the CCP. The NPV of vaccination versus CCP was US$ 13,745,454. These results mean that vaccination program is more cost effective compared to the CCP. Because of missing information and because future can never be predicted with certainty, our analysis was based on several assumptions, the most important ones being about the appropriate discount rate, the future annual number of birds affected, the extent of market loss, and the effectiveness of vaccination to control the infection. Sensitivity analysis of these uncertainties indicated a reasonable robustness of the results to the assumptions made. However, we recommend additional studies in Nepal before choosing the most appropriate option for HPAI control because there might be other options for HPAI control such as strengthening bio-security measures on the farms, vaccinating only commercial poultry or poultry in high risk districts only. Since we did not conduct analysis for these options in the current study, it is recommended that they be analyzed in the future.

In summary, this thesis improved understanding of the overall epidemiology and economics of AI, particularly HPAI, in Nepal. The conducted cross-sectional serosurvey was the first study of the kind conducted in Kathmandu, Nepal to estimate the seroprevalence of AIV antibodies presence in ducks of Kathmandu. This study serves as a baseline for the AIV antibodies presence in ducks in the major duck raising areas of Kathmandu and identified the high-risk group that can be targeted in surveillance activities. We recommend that future studies should be conducted to differentiate the subtypes of AIV present among domestic ducks in Kathmandu, with particular interest in the presence of HPAI virus. In the CBA, the returns in benefits for the costs of the evaluated control programs supported a continued investment into the CCP as opposed to ceasing control measures and suggested that vaccination may be an even better control alternative. The CBA study was also the first study of the kind conducted to estimate the costs and benefits of alternatives to the CCP for HPAI control in Nepal. However, for further CBA of HPAI control in Nepal, it is necessary to find the baseline information, such as about the true market loss due to HPAI outbreaks. Amidst several limitations, we believe that our studies contributed to an improved overall understanding of the epidemiology of AI and the economic worth of its control in Nepal.

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APPENDIX A

Total samples collected as a part of national surveillance plan for HPAI per year

Appendix A. Total samples collected as a part of the national surveillance plan per year

Types	Number of samples
Commercial poultry	5,700
Backyard poultry	5,900
Wild freshly dead birds from wild life areas / national parks	100
Wild water birds (fresh feces)	560
Domestic backyard ducks in wild water birds buffer zones	560
Total	12,780