

INVESTIGATION OF SEASONAL PREVALENCE OF LOW PATHOGENIC AVIAN INFLUENZA (LPAI) IN A HETEROGENEOUS WILD WATERFOWL POPULATION IN PRETORIA

by

Thandeka P. Phiri

Student Number: 206065825

A Dissertation submitted in fulfilment of the requirements for the

degree Magister Technologiae (M.Tech.) Biotechnology

Department of Biotechnology Faculty

of Applied and Computer Sciences

Vaal University of Technology

Vanderbijlpark, South Africa

Supervisor: Dr Naser Aliye Feto

Co-supervisor: Prof Celia Abolnik

June 2018

Private Bag X021 ~ Vanderbijlpark ~1900 Andries Potgieter Boulevard ~ South Africa Tel: +27 16 950 9000 ~ www.vut.ac.za

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidate for any degree.

Signed: Date: 08 June 2018

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people who have encouraged me to further my studies and better myself in my work and career.

First and foremost, I would like to acknowledge my creator my GOD for without him nothing is possible for keeping me safe and guarded throughout my whole life, I give all praise to him. I would also like to give a big thanks to my mother (Nomvula) she has been my pillar of strength helping in raising my daughter and for the love and support that she has always given. I sure hope to make her very proud of me. To my daughter for she is my all for she is the reason I keep going forward with my studies, I sure hope to open up doors for her and show her that anything is possible when you put your mind to it.

To my supervisor Dr Naser Aliye Feto, thank you sir for your continuous assistance towards my completion for my project, for your advice and patience it has been a great pleasure to meet and work with you. I would like to give my gratitude again to my co- supervisor Prof Celia Abolnik, for believing in me and giving me a chance to better myself in both my work and studies. I have learnt a lot working with you, your determination, knowledge and strengths are things that I admire the most about you. I thank you for your guidance and support.

I would also like to give thanks to VUT for granting me the opportunity to further my postgraduate studies and for granting me bursary that has enabled me to finish off my studies smoothly and carrying through my project.

Lastly, I would like to thank Technology Innovative Agency (TIA) for providing finance to the project, and giving me an opportunity to present the project to fellow scientist in the industry

ABSTRACT

Influenza-A virus is a single stranded negative sense RNA virus that is a member of a Orthomyxoviridae group. The virus is diverse and consists of 16 haemagglutinin (H) and 9 neuraminidase (N) glycoproteins subtypes that form a serotype. Avian influenza virus (AIV) has been detected in more than 100 bird species from 26 different families, although Anseriformes and Charadriiformes are considered the natural hosts of the virus. A 12-month study was conducted at the African Pride Irene Country Club lodge in Pretoria where the prevalence of AIV was monitored in a community of wild birds. The African Pride Irene Country Club lodge houses a population of wild bird species such as Egyptian geese (Alopochen aegytptiaca), Yellow-billed duck (Anas undulata), Red knobbed coot (Fulica cristata), African sacred ibis (Threskiornis aethiopicus) and Hadeda ibis (Bostrychia hagedash). A total of 3674 faecal samples were collected over a period of 12 months and screened for AIV group using the Matrix gene (M-gene) real time reverse-transcriptase PCR (rRT-PCR). Positive samples were submitted for virus isolation in embryonated chicken eggs. In addition, the RNAs were screened using H5 and H7 subtype specific rRT-PCR and a conventional universal PCR assay that targets the HA gene was also used. Polymerase Chain Reaction (PCR) products were sequenced using Sanger sequencing and the viral isolates were subjected to Next Generation sequencing (NGS). Twenty percent of the samples tested positive for the AIV group and four virus subtypes were identified. One virus isolate was identified through NGS as H3N6; two through conventional PCR and Sanger sequencing as H9Nx and H6Nx. Of the twenty percent samples that tested positive for AIV 98% were identified as H7Nx by subtype specific through rRT-PCR. The highest frequency of AIV positive samples was detected between the months of January and February 2017 (20%), with smaller peaks detected in February and March 2016 (0.3%). Lower peaks were also detected between the months July and November 2016 (0.1%), respectively. A high prevalence of AIV was detected in the late summer months with a frequency of 65% positive, although a low prevalence was also detected in the autumn (0.6%) winter (0.6%) and spring (0.08%). Thus, the study provides a valuable insight into the seasonal prevalence of AIV in a heterogeneous wild duck population in Gauteng Province.

Table of Contents

CHAPTER ONE (INTRODUCTION)

1.1. Introduction	1
1.2. Research significance/ Motivation	4
1.3. Aim	7
1.4. Objectives	8

CHAPTER TWO (LITRATURE REVIEW)

2.2. Literature	8
-----------------	---

CHAPTER THREE (METHODOLOGY)

3.1. Methodology	18
3.1.1. Sample collection	18
3.1.2. RNA Extraction	18
3.1.3. Real Time reverse transcription PCR	19
3.1.4. Conventional PCR	20
3.1.5. Virus Isolation	20
3.1.6. Genetic Analysis	21

CHAPTER FOUR (RESULTS)

4.1. Results	22
4.1.1. Sample collection	24
4.1.2. rRT-PCR	24
4.1.3. Virus Isolation	25
4.1.4. Conventional PCR results	26
4.1.5. Genetic Analysis results	27

CHAPTER FIVE (DISCUSSION)

5.1. Discussion	29
5.2. Conclusion	32
5.3. References	

LIST OF FIGURES IN LITERATURE REVIEW

Figure 1a : Schematic diagram of Influenza A virus	10
Figure 2a : Image of Anseriformes species	11
Figure 3a : Image of Charadriiformes species	11

LIST OF FIGURES IN RESULTS

Figure 4.1 : Total number of samples collected from February 2016 to February 2017	22
Figure4.2: Graph representation of overall positive samples on a monthly basis	.23
Figure 4.3 : Seasonal prevalence of positive samples identified and isolated	23
Figure 4:4: rRT-PCR amplification plot with positive samples reflecting on the graph	24
Figure 4.5: Graph representation of positive samples detected monthly	25
Figure 4.6: Gel image of PCR results	26

LIST OF TABLES

APPENDIX	41
Table 4.4: Monthly viruses isolated, and overall viruses identified	28
Table 4.3: BLAST analysis results	.27
Table 4.2: DNA quantification using the Nano-droop device	26
Table 4.1: Total number of positive samples detected and isolated for February 2016-2017	.25

CHAPTER ONE

1.1. INTRODUCTION

Influenza A is a highly diverse virus that consists of different subtypes that have been isolated from waterbirds and other wild birds. Wild waterfowl are the natural reservoir of the low pathogenic Avian Influenza virus (LPAIV). However, in some countries these birds may also carry highly pathogenic (HP) strains such as H5N1, H5N8 and H5N6. The highly pathogenic form of the virus has caused major outbreaks throughout different parts of the world (Causey and Edwards, 2008). The epidemiology of the viruses is linked with the host's behavioural patterns, habitat, type of species and its dietary foraging factors. The role of wild birds in the introduction and transmission of the virus is largely dependent on a range of ecological factors including the distribution and density of susceptible animal host (Munster and Fouschier, 2009).

Wild birds and waterfowl species have a broad genetic heterogeneity as compared with domesticated bird species. These birds support a wide genetic diversity of influenza A virus. Waterfowl species such as the Anseriformes (ducks and geese) and Charadriiformes (shorebirds, such as waders) are considered the main natural hosts for influenza viruses. These birds have relatively long life cycle thus they live under variable densities with multiple exposure to pathogens and have a well-developed immunity (Caron et al., 2009). Thus, waterfowl species have adapted to carry and transmit a wide range of Influenza A viral strains in nature without showing clinical signs of infection. (Caron et al., 2009).

Some of the Influenza A viral strain that are carried by wild waterfowls have caused major pandemics in poultry, mammals and humans (Rustadler et al., 2007). The virus can be transmitted directly or indirectly to potential hosts such as domesticated birds, pigs, mammals and humans (Munster and Fouschier. 2009), thus posing a major threat for the emergence of new pandemics particularly in humans (Fouchier et al., 2004).

Severe disease epidemics can occur when there is a drift with significant antigenic change in the HA gene (Tracey et al., 2004). In addition, if humans are concurrently infected with both human and avian strain influenza virus there is an increased risk of a new subtype emerging, which could potentially result in direct transmission between humans and a possibility of a pandemic (Tracey et al., 2004). Live bird markets play a role in the transmission and evolution of influenza A virus (Vandergrift et al., 2010). Waterfowl are widely distributed globally except in semi-arid regions, these birds occupy seasonal habitats and their movements may range from short local flights to intercontinental migration. The transmission of the virus and its geographical spread is dependent on the ecology of the migrating bird host.

The first investigation that was undertaken for influenza virus was in feral birds, where it was revealed that enormous pools of influenza virus was present in wild birds particularly in waterfowl in the family of Anseriformes (Robert et al., 1992). Over the years numerous other studies have highlighted this fact and established key species and seasonal trends for the northern hemisphere. For example, Stallknecht and Shane (1988) conducted various studies on bird species

(*Anseriformes, Passeriformes and Charadriiformes*) for Influenza A virus, the outcome of the study highlighted that *Anseriformes* had the highest number of infections (15.2%) as compared to *Passeriformes* (2.9%) and *Charadriiformes* (2.2%) with the lowest infection rate (Capua et al., 2007).

A study conducted in Canada between 1976-1989 revealed that juveniles had the highest percentage of influenza infection, during which the birds congregate before migration period. These birds migrate from Canada to the south where they shed the virus (Robert et al., 1992). According to Gurberti et al (2007) hatchlings form a virgin population in which infectious viruses can spread during the mating period in late summer. The seasonal prevalence of the LPAI demonstrated a pattern in which juvenile birds were the main host of virus. Wild waterfowl migrate towards the wintering grounds in the sub-Saharan region in Africa prior to the mating season (Guberti et al., 2007). During the migrating period, these birds congregate around lakes where they share a common habitat with domestic ducks, thus providing an opportunity for virus re-assortment. However, in the winter season there is a sharp decline in viral subtype and isolates (Zhu et al., 2014).

Migrating birds play a major role in the long-distance dispersal of the virus. The interaction of the migratory birds with domestic fowl at the congregation site enables viral transmission of AI and facilitate genetic diversity through re-assortment. Subsequently periodic introduction of AIV from wild birds to domestic poultry causes outbreaks among domestic birds (Xu et al., 2017). The prevalence of Anseriformes species in North America is associated to be in the late summer and early fall, at this stage AI infection rates can exceed 30% in the juveniles. During the winter

season virus isolation in ducks is higher in America than in Asian countries. The predominant subtypes in the birds differ from year to year and migration prevalence of AI in waterfowl rapidly decreases in winter seasons and often drops lower to 1-2% (Robert et al., 1992; Swayne, 2008). In Spain during the autumn season thousands of migrating birds stop to rest and feed in the wetlands area before undertaking their journey to Africa and Europe (Perez-Ramirez et al., 2010).

1.2 RESEARCH SIGNIFICANCE / MOTIVATION

Avian Influenza Virus has a global distribution and has been isolated on almost all continents. A large body of evidence on the circulation of low pathogenic AIV of various subtypes in aquatic birds exists for North America, Northern Europe, and Asia (Perez-Ramirez et al., 2010). Non pathogenic AIV circulates in the community of wild water birds and can be transmitted to land birds such as quails, turkey, poultry, domestic ducks and geese. Highly pathogenic AI viruses have been restricted to the H5 and H7 subtype, however not all strains of these subtypes convert to high pathogenicity. The HPAI subtype H5N1 poses serious threat to animal health, wildlife conservation, human health as well as economic welfare. The HP virus causes a mortality rate of up to 100% in poultry and domesticated ducks and geese (Happold et al., 2008).

Wild waterfowls infected with AIV shed high doses of virus through fecal and oral secretions into the environment. These viruses persist in cold water or the frozen environment throughout winter. Therefore, birds that are returning during the spring migration will encounter the virus in thawing ponds or ground thus they are re-

infected (Rustadler et al., 2007). Waterfowl are known to maintain low pathogenic avian influenza virus (LPAI) strains. These birds play a role in the spread of the virus which could potentially become HPAI in a susceptible host. Therefore, these wild birds play a vital role as indicators or sentinels for AIV strains that can potentially infect poultry and humans.

Prevalence of Avian Influenza Virus (AIV) is affected by the host species, age, season and environmental factors. At any given location the prevalence of different viral strains may fluctuate from year to year. However, AI is more frequently detected in birds migrating south from breeding grounds in the Northern hemisphere in the late summer than birds migrating north from overwintering areas in the spring (Rustadler et al., 2007).

There are few studies conducted on a multi-year variability for AIV prevalence in a single sampling location, although in other continents such as Europe and North America, the prevalence of LPAIV in wild birds have been well documented. One of the largest single gaps in the geographical coverage of the virus sampling was in the Southern African region. The sub-Saharan region offers an interesting challenge for AIV research due different behavior patterns of wild birds in the southern hemisphere. Thus as the environment in Southern Africa is relatively dry with a variation in precipitation magnitude and pattern (Cumming et al., 2008).

The first surveillance conducted was in 1961 after an outbreak of AIV in the Southern African region (Swayne,2008), thereafter 49 years later another

surveillance was conducted of which the study comprised of two hundred and sixtytwo waterfowl from 14 species. These birds were captured in Oudtshoorn during the late autumn and winter of 1998 and tested for AIV. Another study pursued in 2004, which was conducted at Barbespaan wetlands South of Gauteng (Abolnik,2010). Three subtypes of AIV were isolated which were H3N8,H4N8 and H5N1 (LPNAI), these subtypes were isolated from endemic *Anatid* species. Subsequently in another study conducted subtype H1N8 was isolated from Egyptian goose (Abolnik, 2010).

Another surveillance which was conducted in the Zimbabwean lakes west of Harare (Chivero and Manyame lakes) for a period of twenty months, amongst a community of waterfowl revealed that multiple strains of AIV were detected in the bird's ecosystem (Cumming et al.,2011). According to the surveillance African waterfowl communities have potential to harbor multiple viral strains for an extended period thus they play a broad scale in the epidemiology of AIV. Another virus that was detected from the African region was from Niger of which H5N2 was isolated from a healthy waterfowl (Caron et al., 2011).

Numerous influenza subtypes have been detected in South Africa during recent years such as H7N1 which was isolated from Ostriches (*Struthio camelus*) and H5N9, H5N2, H9N5 and H6N8 which were isolated during the outbreak. These outbreaks were isolated in Southern Cape ostrich farming and were associated with high mortality (Pfizer et al., 2000). The LPAI H5N2 strain was detected and isolated from the Egyptian goose which was in the western Cape Province (Abolnik et al., 2009).

According to phylogenetic analysis that was conducted by Abolnik (2009) the H5 genes isolated from the Egyptian goose virus was closely related to the HPAI H5N2 isolated from the ostrich outbreak strain isolated in the Eastern Cape province (Abolnik et al., 2009). This demonstrated the link between AI which is carried by wild duck and those infecting ostriches. Southern African ducks and geese are short to medium distance migrants and thus, unlikely to be directly involved in the initial introduction of LPAI from the Eurasian into South Africa (Abolnik et al., 2009).

The present study was conducted to evaluate AIV in wild waterfowls residing at the African Pride Irene Country Club lodge in Pretoria, Gauteng Province. The study site was selected because a large number of free-living bird species connect and interact there, in addition to overnight roosting, some species such as the *Sacred ibises* and *Hadeda ibises* also nest there. In addition to presence of a large diversity of watebirds sampling fresh droppings from the environment was convenient.

1.3 AIM

The aim of the study was to investigate the prevalence of LPAI in the heterogeneous community of free-living waterfowl at the African Pride Irene Country Club lodge in Pretoria, Gauteng Province over a 12-month period.

1.4 OBJECTIVES

The objectives were the following:

 To collect fecal samples from the heterogeneous waterfowl species over a 12month period

- To screen the samples using AIV M gene matrix RT-PCR
- To submit AIV-positive samples for virus isolation
- To perform subtype specific H5 and H7 rRT-PCR assays on positive samples
- To identify non-H5/H7 subtypes by conventional RT-PCR and Sanger sequencing

 To identify if possible, the months or seasons in which AIV prevalence is highest in the population

CHAPTER TWO

2.1. LITERATURE

Influenza virus is a member of the Orthomyxoviridae group, which contains a segmented, single-stranded negative sense RNA genome. Influenza virus are classified in three different serotypes namely *Influenzavirus* A, B and C. Type A is commonly referred to as bird or avian flu, type B viurs is mostly restricted to birds however it is considered to play an important role in cause of human illnesses and type C is not known to cause any illness in humans. Both type B and C influenza virus have never been isolated from avian species (Suarez and Cherry, 2000; Muzaffar et al.,2006; Alexander, 2007;). According to Dejong and Hien (2006) all avian influenza viruses are classified as type A virus, of which are based on their antigenic differences between two surface glycoproteins in haemagglutinin (HA) and neuraminidase (NA). The HA protein is responsible for the attachment of the virus to the host and the NA facilitates release of the progeny virons (DeJong and Hien, 2006).

2.1.1. MORPHOLOGY OF AIV

Influenza A virus consists of eight different gene segments that encode at least eleven different functional proteins (Laleye et al., 2018). The virus consists of surface proteins that include hemagglutinin (HA) and neuraminidase (NA), membrane ion channel proteins (M2), internal proteins which include nucleoprotein (NP), matrix protein (M1), the polymerase complex which is composed of polymerase basic protein one and two (PB1 and PB2) and polymerase acidic protein (PA) which forms the ribonucleoprotein (RNP) complex (Swayne, 2008). The matrix protein (M1) which is incorporated within the lipid envelope plays an essential role in assembling the virus, and it has been linked with both the RNP and viral envelope (Lee and Saif, 2009). The M2 protein is integrated in the membrane protein and its function is to channel the ion particles of the virus. Both the NP and M1 proteins have a high sequence conservation that allows detection of antibody from the birds infected with Influenza A viruses (Figure 1) (Suarez et al., 2000). The virus is a pleomorphic particle that is approximately 120 nm in diameter, and the viral envelope is derived from the host cell membrane.



Figure 2.1: Schematic diagram of avian influenza

Source: http://virologydownunder.blogspot.co.za/2013/07/influenza-viruses-ifv.html

Previous studies showed that there were only 16 HA and 9 NA subtypes in birds and each virus contains one HA and NA antigen respectively (Capua et al., 2007). However recent studies conducted by Tong and colleagues identified new lineages of H17 and H18 which were detected in bat species (Tong et al., 2012).

2.1.2. ECOLOGY OF AVIAN INFLUENZA VIRUS

Influenza type A (AI) virus is a highly infectious disease that affects both wild and captive birds, it has been detected in more than 100 bird species from 26 different families. Avian Influenza virus has been isolated mostly in wild birds and waterfowl. Thus, the estimated number of AI subtype isolated from wild water birds was approximately144 avirulent viruses (De Jong et al., 2006). Wild birds are considered to be the natural host of the virus particularly waterfowl such as the *Anseriformes* (ducks, geese and swans) shown Figure 2 and *Charadriiformes* (seagulls, shorebirds and terns) depicted Figure 3 (Chen and Holmes, 2009).



Figure 2.2: *Anseriformes* bird species (Screamer, Ducks ,Geese,Cygnini anseratidae) (Source: www.dinofan.com)



Figure 2.3: *Charadriiformes* bird species (gulls,sandpiper,terns,skuas,avocet and woodpeck) (Source: <u>http://biology.ru/textbook/chapter6/section6/paragraph8/</u>).

Influenza A virus replicates in the gastrointestinal and respiratory tract of wild waterbirds thereafter it is excreted in high levels through the respiratory and fecaloral routes. The virus is typically transmitted through a contaminated water source. Direct transmission of the virus from bird to bird is complex as it depends on the strain of the virus, bird species and environmental factors (Capua et al., 2007). In wild waterfowl the virus causes mild infections that affect the intestinal tract without producing clinical signs. AIV is generally divided into two classes namely: low pathogenic avian influenza (LPAI) which causes mild subclinical diseases and high pathogenic avian influenza (HPAI) which usually causes serious illnesses and results in death. Low Pathogenic Avian Influenza can be found in numerous bird species. However, the virus is usually isolated in waterbirds where it is endemic.

2.1.3. THREAT OF AIV TO POULTRY

Movement of AIV viruses from wild birds to domestic birds is not uncommon. However the interaction between the two species rarely results in the virus becoming endemic in poultry. Therefore, the sporadic infection of AI viruses from wild birds to poultry or domesticated birds present a public health risk as a zoonotic pathogen which could eventually become pandemic (Swayne,2008).

When certain subtypes of AIV (i.e. H5 and H7) are transmitted to poultry, they can mutate from the LPAI form to the HPAI form, and cause the serious disease known as avian influenza. The ability of LPAI to switch to HPAI viruses is achieved by mutation, reassortment, deletion or insertion of an amino acid residue in the HA₀ cleavage site which facilitates systemic virus replication (Lee et al., 2009). There are number recent outbreaks of highly pathogenic avian influenza (HPAI) diseases that have increased and became more noticeable (Capua et al. 2007). Avian Influenza viruses are capable of causing severe diseases in chickens, turkeys and ostriches. The signs for this infectious virus ranges from decrease in egg production, edema of the head, excessive lacrimation, respiratory problems and neurological

symptoms; this eventually results in high mortality rate (Capua et al., 2007; Abolnik et al., 2009).

The impact of wild birds originating from LPAI mutating into HPAI after introduction and replication in poultry has been demonstrated repeatedly. In America H5 HPAI caused an outbreak among domestic poultry in 1983, 1994, 2014 and 2015 (Xu et al.,2017). High Pathogenic Avian Influenza H7 subtype viruses have already periodically been reported in poultry, there were four H7N3 outbreaks that have been reported between 2002-2016, one in Chile and two distinct outbreaks in Canada and one outbreak in Mexico (Xu et al., 2017).

2.1.4. EPIDEMIOLOGY OF AIV

Both Anseriformes and Charadriiformes are distributed around the globe except in arid regions. Therefore, AIV in wild birds has come from a long-term surveillance studies which have been performed in various parts of the world. LPAI such as the H9N2 strain has been identified and isolated on every continent of the world this virus has been isolated in both wild birds and poultry. The infection rate of H9N2 strain often decreases egg-laying rate in hens and co-infection from other viruses or bacteria causes severe morbidity and high mortality (Zhang et al., 2011).

Outbreaks of HPAI in other countries have been reported such as Hong Kong in the late 90's (Yee et al., 2009). Thereafter HPAI H5N1 spread throughout Asia to Africa and Europe. The virus strain that was isolated in China was the HPAI H5N1 which was initially recovered from a flock of sick geese. This has led to the surveillance of

Al in waterbirds between 1999-2000, in which 21 isolates of HPAI H5N1 were found in healthy ducks that showed no symptoms of the virus (Yee et al., 2009). In Japan in the late 1970s surveillance was also conducted on ducks and results showed that influenza virus subtypes were more prevalent and varied between the years and location (Olsen et al., 2006). In Siberia between 2005 and 2006 there was outbreak of HPAI H5N1 in which 27 cases were reported. The outbreak was preceded by a drop of temperature to a cold air mass that was dominating Eurasia. The drop in cold temperature resulted in crowding of wild birds into remaining unfrozen habitats thus contributing to stress and facilitating density dependent virus transmission (Takekawa et al., 2010).

Spain has demonstrated a prevalence of LPAIV that corresponded with two serological surveys that were carried out in the south of the country. Between 1990 and 1994 the average serotype prevalence varied from 6-40%, although in recent studies that were conducted 5% of the sampled wild birds from Catalonia were infected with LPAIV (Perez-Ramirez et al., 2010). With the view of HPAIV H5N1 only one case was confirmed in wild birds from Northern Spain in June 2006. Thus 8% of the wild birds that were examined in the country were found to be infected with the LPAIV (Perez-Ramirez et al., 2010).

Between 1999 and 2000, Italy experienced an outbreak of AIV H7 subtype with a mortality of two domestic ducks and two Muscovy ducks. In December 2002 there was a new outbreak of H5N1 that occurred in Hong Kong parks that caused high mortality to a large population of avian species including waterfowl. This outbreak

was the first reported incident of AIV that had caused mortality amongst waterfowl since 1961. Although between 2003-2005 domestic ducks were amongst the birds that were affected by the H5N1 epidemic, these birds were not dramatically affected as compared to the susceptible host chickens (Strum-Ramirez et al., 2005).

The H5N1 virus has been isolated from dead migratory birds in China, Mongolia, Russia and Japan (Eurasia) in 2005 when these migratory birds were on their way back from the nesting site. This resulted in an intensive AI surveillance conducted in autumn at Hakkaido Japan and Mongolia on a yearly basis. From 1996 to 2009 the subtypes that were isolated were 634 viruses of which 17 H5 viruses were isolated from fecal samples in migratory waterbirds (Yamamoto et al., 2011).

Seasonal infection pattern of AI virus have been detected with the highest prevalence occurring during late autumn and winter; this is driven by the aggregation of naïve juveniles which is followed by breeding and during migration. Therefore, is also influenced by the environmental conditions, which will determine the viral survival outside the host. (Vandergrift et al., 2010).

According to Tracey et al (2004) the Australian seasonal pattern revealed that the Anseriformes have great prevalence during the late autumn and winter and the Charadriiformes have a prevalence that peaks in spring. Movement and age of the birds also appear to be important and thus correlate with the seasonal effect. High prevalence of the virus was recorded in juvenile mallards before migrating south in the winter. The most AIV subtypes that have been detected in Australia are H1, H3, H4, H6, H11 and H12, which were found to be distinctly different from the rest of the

world (Tracey et al., 2004).

Numerous studies show that surveillance of AI viruses is necessary due to outbreaks of HPAI virus in most continents such as Asia, Africa and Europe. There is a high concern for re-emergence of HPAI H5 and H7, which are considered a notifiable strain throughout these continents. Both these strains have been isolated from waterfowl as LPAI however once they have been exposed to poultry the virus mutates and becomes pathogenic to these birds. Thus, isolating these LPAI strains will assist in evaluating the spread of the virus amongst these birds by investigating their ecological and environmental factors.

2.1.5. AIV IN SOUTH AFRICAN WILD BIRDS AND POULTRY

The first report of isolates of AIV from wild birds globally was in South Africa from Common Terns (*Sterna hirundo*) in 1953. During this period HPAI H5N3 was identified to be the cause of death for 1300 birds. It was revealed later in the 1970s that ducks and geese are the main host of the viruses (Swayne, 2008).

The sub-Saharan region constitutes a seasonal shelter for Eurasian waterbirds and these birds congregate and mix with a wide variety of Afro-tropical waterbirds. In Europe, North America and Africa AI virus has been isolated in wild duck during winter season, with LPAI detected and isolated in these regions from several major wetlands consisting of different species. This suggested that the environmental conditions in the Afro-tropical regions are favorable for the persistence and transmission of AI virus (Caron et al., 2009).

In the year 2004 and 2006 in South Africa there was an outbreak of HPAI H5N2 strain in ostrich farms within the Cape region (Eastern and Western Cape). The virus was from the wild waterfowl populations which resulted in the loss of 30 000 ostriches which represent 40% of the species in the province. Within the very same region other AI viruses have been isolated years earlier such as LP H7N1 (1991), LP H5N9 (1994), H9N2 (1995), H6N8 (1998) and H10N1 (2001) (Abolnik et al., 2009; Alexander, 2006).

According to Abolnik (2009) the extensive farming of ostriches in South Africa is of a great concern with recent HPAI H5N2 outbreaks, these wild but recently domesticated species which are free ranging and potentially in contact with wild populations has been highlighted as potential mixing vessel for AI (Abolnik et al., 2009).

CHAPTER THREE

3.1. METHODOLOGY

3.1.1. SAMPLE COLLECTION

Collection of samples took place twice monthly from February 2016 to February 2017. Sampling was undertaken in the early mornings, after the ducks had roosted overnight on the lawn banks of the study site. Only fresh wet feces were sampled. Samples were collected by dipping swabs (Carlo Roth sterile applicator-Separations) into fresh feces and storing them in a viral transport medium (VTM). The viral transport medium consisted of brain- heart infusion broth (BHI) (1000ml-Sigma Aldrich); 10%v/v glycerol and antibiotics (pennicilin-streptomycin (1ml), enroflaxin (1ml) and doxcyxycline (100mg/ml) all in 1 liter. The samples were transported on ice packs to the laboratory where they were stored at - 80°C for further extraction and analysis.

3.1.2. RNA EXTRACTION

A total of 200µl of the fecal swab fluids were transferred to a Magna pure plate containing 100µl of S.T.A.R buffer (Roche Applied Sciences) and. Samples were extracted using the Magna Pure 96 LC 2.0 instrument (Roche Applied Science) at the Onderstepoort Veterinary Research Institute. Manual extraction was also performed on positive AIV samples using TrizolTM reagent (Life Technologies TM). Briefly, 750µl of Trizol reagent was added to a sterile 1.5 ml Eppendorf tube (Merck), and 250µl of the swab fluid was added and left at room temperature for five minutes. After the incubation period, 200µl of chloroform was added and the tube was shaken until the mixture turned milky pink, followed by incubation at room temperature for 10 minutes. After the incubation period the samples were centrifuged at 13 000 rpm

for 15 minutes, thereafter the upper clear suspension of approximately 600µl was transferred into a clean Eppendorf tube and 600µl of isopropanol was added and incubated for ten minutes. The mixture was then centrifuged for 10 minutes at 13 000 rpm. After centrifugation all of the liquid was pipetted out of the tube and 700µl of 70%v/v ethanol was added, shaken briefly and centrifuged again for five minutes to wash the pelleted RNA. Thereafter all the liquid was removed from the tube and the pellet was left to dry for 10 minutes. The pelleted RNA was resuspended gently in 50µl of elution buffer, composition 10mM Tris-Cl, pH8.5 (QIAGEN-Whitehead Scientific).

3.1.3. REAL-TIME REVERSE TRANSCRIPTION PCR

Extracted nucleic acid was screened for AIV using the Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR). Detection of the viral RNA was performed using Vetmax RT-PCR kits[™] on an automated Applied Biosystems Step OnePlus instrument (Life Technologies [™]). The primers and probes for M gene detection that were used are as described by Spackman et al. 2003. The thermal cycling profile was as follows: 48°C for 10 minute; 1 cycle of 95°C for 10 minute; 40 cycles of 95°C for 15 seconds and 53°C for 45 seconds.

A similar procedure was used for the H5 and H7 subtyping assay, but the primer and probe sets that were used are those described by Slomka et al. (2009) for H7 assay and Slomka et al. (2007) for H5. Positive AI samples were further tested for H7 and H5, of which the RNA was converted to cDNA before running the samples on rRT-PCR. The cDNA was synthesized using the random primers, RNase inhibitor, M-MLUVtranscripase and nuclease free water. The thermal cycling profile was as follows: 48° of 95°C for 10 minute; 40 cycles of 95°C for 15 seconds and 53°C for 45 seconds.

3.1.4. CONVENTIONAL RT-PCR TO AMPLIFY A PORTION OF THE HA GENE

Samples that were H5 and H7 negative were tested using conventional PCR that amplified a portion of the HA gene as described by Philips et al. (2004). The specific primer pair HA-1134 F and BM-NS 890 R were used to generate the HA segment in which the amplicon produced is approximately 640 bp. Amplification was carried out in a ABI thermal cycler instrument (Life Technologies) with cycling conditions as follows: 94°C for 1 minute; 30 cycles at 94° for 1 minute; 50°C for 1 minute;72°C for 3 minute and 1cycle at 72°C for 5 minute. Polymerase Chain Reaction products were separated by gel electrophoresis using 1% (w/v) agarose gel (AEC Amersham) and stained with ethidium bromide (Life Technologist) thereafter visualized under the UV light. The product was excised and purified using the QIAquick gel extraction kit (QAIGEN, Whitehead Scientific) and the nucleic acid was sent to Inqaba Biotech (Pty) Ltd in Pretoria for Sanger sequencing.

3.1.5. VIRUS ISOLATION

Samples that tested positive for AI virus by rRT-PCR were submitted to the Department of Veterinary and Tropical Diseases at the Virology section. Karen Ebersohn and Pamela Wambulawaye performed virus isolations. Briefly, the sample supernatants, treated with antibiotics, were inoculated in the allatonic cavities of 10-day old Specific Pathogen Free (SPF) embryonated chicken eggs and incubated at 37°C for a week, with daily candling to assess embryo death. The improved method described by Tong et al 2014 for isolating viruses from fecal

samples was used, in which the embryo and allotonic fluid were homogenized and re-inoculated in the eggs.

After the incubation period the eggs were harvested by collecting the allantonic fluid and testing for the ability to hemagglutinate red blood cells, in a standard Hemagglutination Assay performed according to the OIE (World Organisation for Animal Health) procedure (World Organization for Animal Health). Positive allantonic fluid from the embryo was also submitted to the Faculty's Electron Microscopy Unit to confirm the presence of orthomyxoviruses. Negative samples were subjected to three passages.

3.1.6. GENETIC ANALYSIS

Sanger sequencing results were subjected to BLAST analysis on NCBI's webportal. The nucleic acid was subjected to Next Generation Sequencing (MiSeq Illumina) at Inqaba Biotec. The procedure followed was that similar to Abolnik and colleagues, as described in an article published on Avian Diseases (Abolnik et al., 2012)

CHAPTER FOUR

4.1. RESULTS

4.1.1. Sample collection

A total of 3674 environmental fecal samples were collected from February 2016 to February 2017 at the African Pride Irene lodge (Figure 4.1). Of the samples collected, 749 tested positive for AIV M-gene using the matrix rRT-PCR assay, with an overall prevalence of 20%. The remaining 2925 (80%) samples tested negative for the virus (Figure 4.2). The highest number of positive samples were identified between January and February 2017 with a prevalence of 81% and 98%; whilst from February to November 2016 there was a low prevalence of the virus with 0.4% being positive.

Prevalence of AIV was high in the summer season (February 2016, January and February 2017) with total of 740 samples were collected and 65% were positive for the virus. Prevalence of the virus dropped in the winter (March, April and May); autumn (June, July and August) and spring season (September, October and November), with values varying from of 0.6%, 0.6% and 0.08% respectively testing positive for the virus (Figure 4.3).



Figure 4.1: Total number of samples collected from February 2016 to February 2017.



Figure 4.2: Samples collected and tested on a weekly basis for the duration of the study period (Feb 2016 - Feb 2017), with AIV positive samples indicated.



Figure 4.3: The overall seasonal positive samples isolated during the period in which the study was conducted.

4.1.2. rRT-PCR RESULTS

Results of the rRT-PCR assays are provided in Appendix A. Samples were considered positive if the cycle threshold value(Ct) was below 40. A Ct above 40 was considered negative. A Ct value is the number of fractional cycle of which nucleic acid is formed, thus this is defined by the number of cycles required for fluorescent signal to cross the threshold. An example of an amplification plot is provided in Figure 4.4. Polymerase Chain Reaction positive samples with a Ct between 20 and 30 were submitted for virus isolation (see appendix page 35). On the 749 samples that tested positive for the AIV group based on the M-gene target, further H5 and H7 subtype rRT-PCR assays was conducted. Twenty-four percent of these samples tested positive for H7 subtype (Annex A, Figure3), and none were positive for H5. The prevalence of the H7 assay was higher between the months of January and February 2017, with an 81% - 98% H7 positive, respectively (Table 1 and Figure 4.4).



Cycle

Figure 4.4: Reverse transcriptase Real-Time PCR amplification plot with positive H7 samples reflected on graph. The reference (control) had a Ct value of 18.

Month	Total samples	Positive	Incidence (%)
February	332	6	1.8
March	201	5	2.5
April	100	0	0
May	203	0	0
June	99	0	0
July	244	3	1.2
August	457	0	0
September	514	0	0
October	313	0	0
November	406	1	0.3
January	319	259	81
February	486	475	98

Table 4.1: Total number of positive samples detected from February 2016 to February 2017.

4.1.3. VIRUS ISOLATION

Positive samples were submitted to the Department of Veterinary Tropical Diseases (DVTD) for virus isolation. From the appendix A list of virus isolated samples only one virus was isolated. The sample ID TP 430 tested positive for HA assay, the samples were further identified on an electron microscope for verification. The sample was then re-extracted and sequenced for full genome sequencing of which the virus was identified as H3N6.

4.1.4. DETECTION OF AIV GENE USING CONVENTIONAL PCR METHODS

Samples that tested negative for the H5 and H7 subtypes were tested using a conventional RT-PCR that amplifies a 600bp portion of the HA gene from most subtypes. Typical results are shown below in Fig. 6. Amplicons of the correct size were obtained for the following samples: TP3173; TP 3130; TP3076; TP 3178; TP 3194; TP

3095; TP 3014; TP 3110; TP 3193 and TP 126. The bands were observed using an Egel imager on UV light base software (Life Technologies [™]). Therefore, they were cutout cleaned up and read the concentration of the nucleic acid on the nanodrop instrumentation (Table4. 2).



Figure 4.6: AI positive PCR samples were observed on the gel, results for the detection of AIV strain using specific primers. Polymerase Chain Reaction amplificons of 640 bp were observed for the HA AI strain.

Sample ID	Well number	Nanodrop concentration (ng/µl)
TP 3014	1	10.3
TP 3076	3	8.4
TP 3095	5	7
TP 3110	9	8.8
TP 3130	13	8
TP 126	18	9.5
TP 3173	19	9.2
TP 3194	22	10.5
TP 3178	23	8.9

Table 4. 2: DNA quantification using a Nanodrop device.

4.1.5. GENETIC ANALYSIS

Column-purified amplicons described in Section 4.1.4.were submitted for Sanger sequencing the Inqaba Biotech (Pty) Ltd. The results were observed using Chromos Lite software, thereafter copied the nucleotide sequence and posted on FASTA sequencing into BLAST browser. The software used for the search was as follows:

: <u>https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch (Table 3)</u>.

Basic Local Alignment Search Tool (BLAST) is search tool that compares sequences to each other. This is accomplished by comparing a new sequence with the one that have already been stored in the database.

Of the samples from section 4.1.4 table 2, that were sequenced and put on BLAST software only one sample yielded a positive AIV identification. The sample was TP 126, which scored 98% of the similarity to of H9N2 subtype. The other remaining amplicons of similar size were determined to have 0% similarity to AIV, thus they were non-specific bacterial genome.

An overall number of samples that were isolated in the study are shown in table 4.3. There was a very high number of samples that tested positive for AIV in January and February 2017 with one AI subtype which is H7Nx was isolated, as compared to the previous year where there was a low virus isolation. However three subtype were isolated between February (H9Nx), March(H3N6) and July (H7Nx)2016 (Table 4.4).

Table 4:	Monthly	virus	isolation	table and	overall	viruses	isolated.
	withing	viiuo	100101011	tubio una	ovorun	110000	loolatoa.

Months	AIV group positive	H5/H7 positive	Virus Isolated	Test conducted
February	6	0	H9NX	Sanger sequencing
March	5	1	H3N6	Full genome sequencing
July	3	1	H7Nx	qPCR
November	1	1	H7Nx	qPCR
January	259	2	H7Nx	qPCR
February	475	178	H7Nx	qPCR

CHAPTER FIVE

5.1. DISCUSSION

A total of 3674 faecal samples were collected at the African Pride Irene lodge from February 2016 to February 2017. Twenty percent of the samples collected tested positive for AIV and 80% were negative for the virus. The virus was detected in the following months: February; March; July and November 2016, with the prevalence varying from 1.8%, 2%, 1.2% and 0.2% respectively Table 4.1. However, there was a high peak in positive cases between January and February 2017 with prevalence of 81% - 98% respectively. Between the months of April and September 2016 there were no positive samples detected, although there was a small peak of positive found in July and November of 2016.

According to the results obtained from the study the summer season has a higher prevalence of AIV compared with other seasons figure 4.3. Based on the literature review, AIV has been isolated in various seasons depending on the location, country or continent in which the surveillance study was conducted. In Eurasia (Japan, China, Mongolia and Russia) AI viruses were isolated in the autumn season whilst in North America and Africa AI has been isolated in winter in wild ducks (Yamamoto et al. 2011). In other countries such as Australia, AI is most prevalent in the autumn and spring season (Tracey et al., 2004). In contrast, in this study LPAI virus was mostly isolated in summer with a prevalence of 65% positive samples. During the winter season, isolation of LPAI dropped to a prevalence of 0.6%. Between spring and autumn very low prevalence was detected with less than < 1% of the samples testing positive for the virus.

A recent study conducted in Mali West Africa, had similar findings to the study conducted in Pretoria. The Mali study revealed that AIV circulated year-round in the afro-tropical regions throughout different seasons. According to the study the West African wetlands are exposed to higher temperature during the period when Eurasian migratory birds are absent, thus the prevalence of the virus was less than <1% when migratory birds were absent. Higher prevalence of the virus was detected when the Eurasian migrants were present, which was between January and March (Capella et al., 2011).

Twenty percent of the positive samples from this study were further tested for H5 and H7 subtype. Among the 749 AI positive samples detected,183 (24 %) tested positive for the H7(table 4.4) subtype but none tested positive for H5 subtype. Samples that tested H5 and H7 negative were further analysed for other AI subtypes. The presence of an H9 virus in the population was identified by conventional RT-PCR followed by Sanger sequencing and BLAST analysis. Other amplicons of similar size were determined to be non-specific amplification of bacterial genomes present in fecal samples. One virus was isolated and identified as H3N6 using full genome sequencing(.

In the year 1966 in Wisconsin the first isolate of AIV was H9N2 subtype which was most prevalent in ducks and shorebirds. According to Zhu et al (2014) the virus may have contributed internal genes to highly pathogenic avian influenza (HPAI) H5N1 virus in Hong Kong in 1997 and the novel H7N9 AI in mainland China 2013 (Zhu et
al., 2014).

An epidemiological surveillance was conducted in southern China were there was a high incidence of positive H9N2 which was detected in chickens and other land birds. Another surveillance was conducted in Hong Kong 1997 where H9N2 virus was second most prevalent isolate from live poultry market next to H5N1 virus. This virus was also isolated from domestic fowl from other Asian countries i.e. United Arab Emirates, Korea and India. Three H9N2 sub-lineages were circulating in poultry in several countries in Eurasia (Zhang et al., 2011).

Low precipitation was also identified as a predictor of HPAI H5N1 outbreak among wild birds, high concentration of waterbirds in fewer smaller wetlands is likely to heighten opportunity for contact with the virus prompting H5N1 outbreaks. Thus, crowding of birds in the limited wetlands may elicit a tress response, compromising the ability of the immune system to fight viral infection (Takakawa et al., 2010).

Of the positive samples from the study with low Ct value that were submitted for virus isolation, only one virus was isolated. Further optimization of virus isolation in embryonated eggs is required. One reason for the low isolation rate could be the delay in inoculating positive samples into eggs and freeze thawing of the material.

5.2. CONCLUSION

Based on the small-scale surveillance that was conducted on wild waterfowl, the results from this study provide a 12-month analysis of LPAIV from the African Pride Irene Country lodge. The results show that during certain times of the year, surprisingly in the summer, a large proportion of the resident waterfowl species may be infected with LPAI viruses. The ages, immune status and breeding cycles of the ducks and ibises in the population were not assessed, but this may have played a role in the seasonal prevalence of AIV detected.

The Irene site thus provides a good sentinel population to monitor AIV in wild ducks in Pretoria. Low Pathogenic Avian Influenza H9, H7 and H3N6 viruses were identified in the population. The isolation of viruses is important from a diagnostic perspective, where they are used as reference or control reagents in haemagglutination inhibition assays and PCR assays.

Continued surveillance at the site should be implemented as this will allow better understanding of AIV in the region and also act as an early detection system for potentially pathogenic viruses that can threaten poultry production and human health.

5. 3.REFERENCES

ABOLNIK, C., GERDES, G.H., SINCLAIR, M., GANZERVOORT, B.W., KITCHING, J.P.,BURGER, C.E., ROMITO, M., DREYER, M., SWANEPOEL, S., CUMMING, G.S. & OLIVIER, A.J. 2009a. Phylogenetic Analysis of Influenza A Viruses (H6N8, H1N8, H4N2, H9N2 and H10N7) Isolated from wild birds, ducks and ostriches in South Africa from 2007 to 2009. *Avian Diseases* 54.3.p.13-322

ABOLNIK, C., LODNT, B.Z., MANVELL, R.J., SHELL, W., BANKS, J., G, G.H., AKOL, G. & BROWN, I. A. 2009b. Charecterization of highly pathogenic influenza a virus subtype H5N2 isolated from ostriches in South Africa in 2004. *Influenza and Other Respiratory Viruses*, 3. p. 63-68.

ABOLNIK, C. AVIAN INFLUENZA IN SOUTH AFRICA: A REVIEW. Proceedings of the 9th annual congress of South Africa society for Veterinary Epidemiology and Preventive medicine.18-20 August 2010.

ABOLNIK, A., OLIVIER, A.J., GREWAR, J., GERS, S.AND

ROMOITO,M.2012. Molecular anaylysis of 2011 HPAI H5N2 outbreak in ostriches, South Africa. *Avian Diseases* 56. 865-879.

ALEXANDER, D.J. 2007. An overview of the epidemiology of avian influenza. Vaccine 25. 5637-5644.

CARON, A., GAIDET, N., DE-GRAINE-WICHATISKY, M., MORAND, S., CAMERON, E.Z.2009. Evolutionary biology, community ecology and avian influenza research. *Infection, Genetics and Evolution* 9. p. 298– 303

CARON, A., ABOLNIK, C., MUNDAVA, J., GAIDET, N., BURGER, C.E., MOCHOTHLOANE, B., BRUNZEEL, L., de-GARINE-WICHATITSKY, M. & CUMMING, G.S. 2011. Persistence of Low Pathogenic Avian Influenza Virus in waterfowl in a Southern African ecosystem. *Ecohealth.* 8. p109-115.

CAPPELLA, J., de ALMEIDA, R.S., FOFANA, B., DAKOUO, M., BALANCA, G., GIL, P., ALBINA, E. & GAIDET, N. 2011. Circulation of avian influenza viruses in wild birds in Inner Niger Delta, Mali. *influenza journal*, 240-244.

CAPUA, I. & ALEXANDER, D.J.2007. Ecology, Epidemiology and Human health implication of Avian Influenza Viruses: Why we need to share genetic data. *Zoonoses and Public health*. 55. p. 2-15

CAUSEY, D., and EDWARDS, S. 2008. Ecology of Avian Influenza Virus in Birds. *Infectious Disease*, 29-32.

CHEN, R. & HOLMES, E.C. 2009. Frequent inter-species transmission and geographic subdivision in avian influenza viruses from wild birds. *Virology Journal.* 383. p. 156–161 CUMMING, G.S., HOCKEY, P.A., BRUINZEEL, L.W. & DuPLESSIS, M.A. 2008. Wild bird movements and Avian Influenza risk mapping in Southern Africa. *Ecology & Society* vol 13

CUMMING,G.S.,CARON,A.,ABOLNIK,C.,CATTOLI,G.,BRUINZEEL.,BURGER, C.E.,CECCHETIN,K.,CHIWESHE,N.,MOCHOTLHOANE,B.,MUTUMI,G.L.& NDLOVU,M. 2011.the ecology of Influenza A in wild birds in Southern Africa.*EcoHealth* 8.p.4-13.

DeJONG, M & HIEN, T.T. 2006. Avian influenza A (H5N1). *Journal of Clinical Virology.* 35. p. 2-13.

FOUCHIER, R.A., SCHNEEBERGER, P.M., ROZENDAAL, F.W., BROEKMAN, J.M., KEMINK, S.A.G., MUNSTER, V., KIKEN, THISJ., RIMMELZWAAN, G.F., SCHUTTEN, M., vanDOOORNUM, G.J.J., KOCH, G., BOSMAN, A., KOOPMANS. M. & OSTERHAUS, D.M.E. 2004. Avian influenza A virus (H7N7) associated with human conjuctivis and fatal case of acute respiratory distress syndrome. PNAS, 1356-1361.

GUBERTI, V., SCREMIN, M., BUSANI, L., BONFANTI, L. & TERREGINO, C. 2007. A simulation model for Low pathogenicity Avian influenza Viruses in Dabbling ducks in Europe. *Avian Diseases 50*, 275-278.

HAPPOLD, J.R., BRUNHART, I., SCHWERMER, H. & STARK, D.C. 2008. Surveillance of H5 Avian Influenza Virus in Wild Birds found dead .*Avian Diseases* 52. p. 100- 105.

LALEYE, A., JOANNIS, T.J., SHITTU, I., MASEKO, C., ZAMPERIN, G., MILANI, A., ZECCHIN, B., FUSARO, A., MONNE, I. AND ABOLNIK, C. 2018. A two-year monitoring period of the genetic properties of clade 2.3.2.1c H5N1 viruses in Nigeria reveals the emergence of co-circulation of dictinct genotypes. *Infection, Genetics and Evolution* 57. p98-105.

LEE, C.W. and SAIF, Y.M. 2009. Avian Influenza virus. Science Direct. Comparative Immunology Microbiology and Infectious Diseases 32. 301-310

MUNSTER, V.J. & FOUSCHIER, R.M.A. 2009. Avian influenza virus: Of virus and bird ecology. *Vaccine* 27. p. 6340-6344.

MUZAFFAR, S. B., YDENBERG, R.C., & JONES, I.L. 2006. Avian Influenza: An Ecological and Evolutionary Perspective for Waterbird Scientists. *Journal of the Waterbird Society.* 29. p. 243-406.

OLSEN, B., MUNSTER, V., WALLESTEN, A., WALDESTROM, J., OSTERHAUS, A.D., & FOUSCHIER, R.A. 2006. Global Patterns of Influenza a Virus in Wild Birds. *SCIENCE VOL 312*, 384-388.

PEREZ-RAMIREZ, E., GERRIKAGOITIA, X., BARRAL, M., & HOFLE,

U. 2010. Detection of low pathogenic avian influenza viruses in wild birds in Castilla-La mancha (south central Spain). *Veterinary Microbiology*, 200-208.

PFITZER, S., VERWOED, D.J., GERDES, G.H., LABUSCHAGNE, A.E., ERASMUS, A. MANVE, R.J. & GRUND, C. 2000. Newcastle Disease and Avian influenza A virus in wild waterfowl in South Africa. *Avian Disease* 44. p655-660.

PHILIPS,L.P. & ESSEN, S.C. 2004.Genetic subtyping of inlfuenza A viruses using RT-PCR with a single set of primers based on conserved sequences within HA2 coding region. *Journal of Virological Methods*, 119-122

ROBERT, G., WEBSTER, W.J. BEAN, O.T., THOMAS, G., CHAMBERS, M. & KAWAOKA, Y. 1992. Evolution and Ecology of Influenza A Viruses. *Microbiological reviews.* p. 56: 1

RUSTADLER, J., HAPP, G., SLEMONS, R., SHENG, Z., GUNDLACH, N. & PETRULA, M. 2007. Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infection in ducks at Minto Flats State Game Refuge, Alaska during August 2005. *Archives of virology*, 1901-1910.

SLOMKA,M.J., PAVLIDS,T.,BANKS,J.,SHELL,W.,McNALLY,A., ESSEN,S. AND BROWN,I.H.2007.Validated H5 Eurasian Pplication in H5N1 outbreak in 2005-2006. *Avian diseases 51.* 373-377.

SLOMKA,M.K.,PAVLIDIS,T.,COWARD,V.J.,VOERMANS,J. AND KOCH,G.2009. Validated Real Time PCR methods for diagnosis and pathotyping of Eurasian H7 avian influenza viruses. *Influenza and other Respiratory Viruses* 3.151-164.

SPACKMAN, E., SENNE, D.A., MYERS, T.J., BULAGA, L.L., GARBER, L.P., PERDUE, M.L., LOHMAN, K., DAUM, L.T. & SAUREZ, D.L.2002. Development of Real-Time Reverse Transciptase PCR Assay for Type A Influenza Virus and the Avian H5 and H7 Hemagglutinin Subtype. *Journal of Clinical Microbiology*.p3256-3260.

SUAREZ, D.L. & SCHULTZ-CHERRY, S. 2000.Immunology of avian influenza virus. *A review Development and Comparative Immunology*.24. p.269-283.

STRUM-RAMIREZ, K.M; HULSE-POST, D.J., GOVORKOVA, E.A., HUMBERED, J., SEILER, P., PUTHAVATHANA, P., BURNATHAI, C., NGUYEN, T.D., CHAISINGH, A., LONG, H.T., NAIPOSPOS, T.S.P., CHEN, H., ELLIS, T.M., GUAN, Y., PEIRIS, J.S.M. & WEBSTER, R, P. 2005. Are ducks contributing to endemicity of high pathogenic influenza virus in Asia. *Journal of Virology*, 11269- 11279.

SWAYNE, D.E. 2008. Avian Influenza. 1^{sr} edition. Iowa state, Blackwell publishing Ltd.

TAKEKAWA, J.Y., PROSSER, D.J., NEWMAN, S.H., MUZZAFFAR,

S.B., HILL, N.J., YAN, B., XIAO, X., LEI, F., LI.T., SCHWARZBACH, S.E., HOWELL, J.A. Oet al. 2010. Victims and Vectors: highly pathogenic avian influenza H5N1 and the ecology of wild birds. *Avian Biology research*, 51-73.

TONG, S., LI, Y., RIVALLER, P., CONRADY, C., DANILO, A., CASTILLO, A.C., CHEN, L., RECUENCO, S., ELLISON, J.A., DAVIS, C.T., T. DAVIS, M.R. WEIL, I.A., YORK, A., TEMELLE, S., MORAND, D., ROGERS, S., SHI, M., TAO, Y., TANG, K., ROWE, L.A., SAMMONS, S., XU, X., FRACE, M., LINDBLADE, K.A., COX, N.C., ANDERSON, L.J., RUPRECHT, C.E. & DONIS. R.O. 2012. A distinct lineage of influenza A virus from bats. *PNAS*. 109p.4269-4274.

TRACEY, J.P., WOODS, R., ROSHIER, D., WEST, P. & SAUNDERS, G.R. 2004. The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective.2004. *Emu* 104. plkas109-124.

VANDERGRIFT, K.J., SOKOLOW, S.H., DASZAK, P. & KLIPATRICK, A.M. 2010. Ecology of avian influenza viruses in a changing world. *The Year in Ecology and Conservation Biology.* 1. p. 5-12.

YAMAMOTO, N., SAKADO, Y., MOTOSHIMA, M., YOSHINO, FUMI., SODA, K., OKAMATSU, M. & KIDA, H. 2011. Characterization of non-pathogenic H5N1 influenza virus isolation from migratory duck flying from Siberia in Hokkaido, Japan in October 2009. *Virology Journal*, 1-8.

YEE, K.S., CARPENTER, T.E. & CARDONA, C.J. 2009. Epidemiology of H5N1 avian influenza. Comparative Immunology. *Microbiology and Infectious disease.* 32. p. 325-340

XU, Y., RAMEY, A.M., BOWMAN, A.S., DELIBERTO, T.J., KLLIAN, M.J., KRAUS, S.N., JACQUILINE, M., TORCHETTI, M.K., REEVES. A.B., WEBBY, R.J., STALKNECHT, D. & WAN, X.F. 2017. Low-Pathogenic Influenza A Viruses in North American Diving Ducks Contribute to the Emergence of a Novel Highly pathogenic Influenza A (H7N8) Virus. *Journal of virology*.

ZHU, Y., HU, S., BAI, T., YANG, Y., WENFEI, Z., HUANG, Y., DENG, Z., ZHANG, H., BAI, Z, YU, M., HUANG, J., SHU, Y. 2014. Phylogenetic & antigenic characterization of re-assortment H9N2 avian influenza viruses isolated from wild waterfowl in East Diongting Lake wetland in 2011-2012. *Virology Journal 11:77*, 11:77

ZHANG, H., XU, B., CHEN, Q. AND CHEN, Z. 2011. Characterization of H9N2 influenza viruses isolated from Dongting lake wetland in 2007. *Virology*, 156:95-105.

APPENDIX

RT-PCR Results

Date	Sample	AI	H5/H7	Virus	Detailed Results
	ID	Results	Results	Isolated	
9-Feb-16	TP106	34.02	Negative	None	qPCR, conventional PCR and HA negative
9-Feb-16	TP126	34.07	Negative	H9N2	AI Conventional PCR and sequencing
9-Feb-16	TP143	28.11	Negative	None	Haemaglutiantion Assay and PCR
9-Feb-16	TP144	34.28	Negative	None	Haemaglutiantion Assay and PCR
9-Feb-16	TP150	36.81	Negative	None	Haemaglutiantion Assay and PCR
9-Feb-16	TP 191	30.2	Negative	None	Haemaglutiantion Assay and PCR
11-Mar-16	TP 430	34.14	Negative	H3N6	Conventional PCR and
					Sanger full genome sequencing
31-Mar-16	TP 445	36.42	36.45	H7Nx	qPCR positive and HI Negative
31-Mar-16	TP 458	37.03	Negative	None	Negative for both HI and H5/H7 subtype
31-Mar-16	TP 478	36.99	Negative	None	Negative for both HI and H5/H7 subtype
31-Mar-16	TP 483	37.38	Negative	None	Negative for both HI and H5/H7 subtype
18-Jul-16	TP 990	36.9	Negative	None	Negative for both HI and H5/H7 subtype
18-Jul-16	TP 1168	34.38	36.13	H7Nx	qPCR positive and Negative for HI virus isolation
18-Jul-16	TP 1152	36.59	Negative	None	Haemaglutiantion Assay and qPCR
29-Nov-16	TP 2748	37.06	36.13	H7Nx	qPCR positive and Negative to HI virus isolation
13-Jan-17	TP 2870	33.56	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2871	33.39	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2872	33.85	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2873	34.98	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2874	36.4	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2875	33.58	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2876	37.1	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2877	35.28	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2879	34.9	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2890	35.16	Negative	None	Negative for both HI and H5/H7 subtype

13-Jan-17	TP 2891	34.79	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2892	34.25	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2894	38.5	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2895	34.16	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2896	34.4	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2897	34.63	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2898	33.8	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2899	34.62	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2900	35.19	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2901	34.48	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2902	35.42	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2903	33.83	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2905	34.06	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2906	34.36	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2907	33.27	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2908	34.75	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2909	34.05	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2910	32.77	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2911	34.5	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2912	35.23	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2913	33.69	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2914	33.07	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2915	32.31	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2916	29.23	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2917	29.4	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2918	28.17	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2919	29.53	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2920	29.85	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2921	28.66	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2922	30.39	Negative	None	Negative for both HI and H5/H7 subtype

13-Jan-17	TP 2923	34.31	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2924	30.21	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2925	29.25	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2926	30	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2927	29.08	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2928	29.28	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2929	29.67	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2930	28.46	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2931	30.59	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2932	29.72	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2933	29.16	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2934	31.7	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2935	29.35	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2936	30.96	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2937	30.25	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2938	28.9	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2939	29.26	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2940	30.16	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2941	29.57	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2943	29.65	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2944	30.91	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2945	28.8	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2946	30.98	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2947	23.59	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2948	28.6	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2949	29.77	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2950	28.6	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2951	30.2	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2952	28.84	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2953	29.66	Negative	None	Negative for both HI and H5/H7 subtype

13-Jan-17	TP 2954	31.19	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2955	30.63	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2956	28.58	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2957	29.31	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2958	28.92	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2959	28.42	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2960	28.69	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2961	28.64	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2962	29.10	29.31	H7Nx	PCR positive and for HI negative
13-Jan-17	TP 2963	23.98	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2964	30.42	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2965	29.31	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2966	28.62	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2967	30.04	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2968	28.66	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2969	28.71	35.51	H7Nx	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2970	28.38	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2971	28.34	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2972	29.13	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2976	31.22	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2977	31.82	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2978	31.62	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2979	34.10	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2980	33.58	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2982	32.43	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2983	32.11	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2984	31.87	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2985	32.23	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2986	32.29	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2989	31.48	Negative	None	Negative for both HI and H5/H7 subtype

13-Jan-17	TP 2991	31.32	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2992	31.39	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2993	32.21	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2994	31.55	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2995	33.54	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2997	32.06	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 2998	31.91	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3000	32.22	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3001	31.66	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3002	32.03	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3003	32.22	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3004	32.27	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3005	31.95	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3006	31.29	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3007	31.86	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3008	31.88	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3009	31.47	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3010	31.73	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3011	30.92	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3012	31.63	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3013	31.55	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3014	31.81	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3015	31.73	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3017	31.35	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3018	35.86	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3019	31.86	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3020	32.2	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3021	32.18	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3023	33.74	Negative	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3027	35.85	Negative	None	Negative for both HI and H5/H7 subtype

13-Jan-17	TP 3031	34.57	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3043	30.46	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3046	35.42	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3063	36.5	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3065	38.97	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3070	30.23	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3071	33.03	Negative	I	None	Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3072	35.65	Negative	None		Negative for both HI and H5/H7 subtype
13-Jan-17	TP 3073	33.68	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3074	32.95	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3075	32.98	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3076	33.15	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3077	32.53	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3078	33.41	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3079	33.25	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3080	33.25	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3081	34.25	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3082	35.06	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3083	33.37	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3084	36.89	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3085	32.93	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3086	33.71	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3087	33.34	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3088	35.19	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3089	33.58	Negative	None		Negative for both HI and H5/H7 subtype
27-Jan-17	TP 3090	33.78	Negative	None	F	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3091	32.82	Negative	None		PCR negative, not submitted for virus isolation
27-Jan-17	TP 3092	32.6	Negative	None		PCR negative, not submitted for virus isolation
27-Jan-17	TP 3093	33.55	Negative	None		PCR negative, not submitted for virus isolation
27-Jan-17	TP 3094	33.01	Negative	None		PCR negative, not submitted for virus isolation

27-Jan-17	TP 3095	33.4	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3096	33.23	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3097	32.79	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3099	33.12	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3100	33.23	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3101	32.98	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3102	33.42	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3103	32.26	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3104	33.53	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3105	33.25	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3106	32.48	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3107	33.87	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3108	32.84	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3109	32.75	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3110	31.85	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3111	32.81	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3112	32.67	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3113	33.17	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3114	33.02	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3115	32.69	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3116	35.59	36.83	H7Nx	PCR positive, not submitted for virus isolation
27-Jan-17	TP 3118	33.69	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3119	33.93	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3120	32.99	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3121	33.03	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3122	33.63	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3123	32.79	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3124	33.14	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3125	33.9	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3126	33.9	Negative	None	PCR negative, not submitted for virus isolation

27-Jan-17	TP 3127	32.57	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3128	33.12	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3129	32.01	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3130	31.82	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3131	32.5	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3132	32.77	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3133	35.16	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3134	32.95	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3135	33.49	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3136	33.51	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3137	33.01	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3138	32.89	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3139	33.45	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3140	32.58	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3141	32.92	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3142	33.65	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3143	32.66	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3144	32.57	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3145	33.18	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3146	31.85	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3147	33.23	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3148	33.51	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3149	33.14	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3150	33.42	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3151	34.95	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3152	33.64	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3153	32.74	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3154	33.7	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3155	33.51	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3156	32.67	Negative	None	PCR negative, not submitted for virus isolation

27-Jan-17	TP 3157	32.38	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3158	33.28	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3159	32.73	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3160	31.87	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3161	31.54	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3162	33.28	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3163	33.07	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3164	33.9	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3165	32.87	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3166	32.66	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3167	32.74	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3168	33.19	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3169	32.72	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3170	33.16	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3172	32.29	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3173	32.84	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3174	32.53	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3175	33.51	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3176	32.88	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3177	33.2	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3178	32.67	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3179	31.91	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3180	32.5	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3181	35.94	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3182	32.7	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3183	33.03	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3184	32.66	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3185	33.05	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3186	32.28	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3187	32.66	Negative	None	PCR negative, not submitted for virus isolation

27-Jan-17	TP 3188	32.57	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3189	33.54	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3190	32.41	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3192	32.75	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3193	32.35	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3194	32.66	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3195	32.78	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3196	32.38	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3197	32.26	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3198	34.03	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3199	33.71	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3200	33.57	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3201	33.8	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3202	33.52	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3203	33.91	36.79	H7Nx	PCR positive, not submitted for virus isolation
27-Jan-17	TP 3204	32.61	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3205	33.41	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3206	32.72	38.35	H7Nx	PCR positive, not submitted for virus isolation
27-Jan-17	TP 3207	33.06	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3208	37.08	37.21	H7Nx	PCR positive, not submitted for virus isolation
27-Jan-17	TP 3209	34.47	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3210	32.9	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3211	34.21	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3212	33.91	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3213	33.66	Negative	None	PCR negative, not submitted for virus isolation
27-Jan-17	TP 3214	33.81	38.33	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3215	33.19	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3216	33.78	36.58	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3217	34.32	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3218	34.96	Negative	None	PCR negative, not submitted for virus isolation

17-Feb-17	TP 3219	33.23	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3220	33.44	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3221	32.79	36.41	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3222	34.05	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3223	32.73	36.93	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3224	33.21	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3225	33.25	36.69	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3226	31.47	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3227	33.58	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3228	33.54	37.73	None	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3229	34.3	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3230	34.1	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3231	33.4	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3232	32.66	34.65	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3233	32.68	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3234	33.72	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3235	33.48	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3236	33.71	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3237	33.8	37.21	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3238	32.85	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3239	33.29	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3240	31.77	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3241	33.31	37.38	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3242	32.16	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3243	33.59	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3244	33.35	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3245	35.71	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3246	33.42	34.36	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3247	33.67	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3250	34.49	Negative	None	PCR negative, not submitted for virus isolation

17-Feb-17	TP 3251	34.59	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3252	33.1	36.19	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3253	32.84	36.04	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3254	33.1	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3255	33.85	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3256	33.5	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3257	34.17	34.07	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3258	32.85	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3259	33.38	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3260	34.48	37.56	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3261	34.32	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3262	34.07	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3263	33.93	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3264	33.43	38.04	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3265	33.73	32.24	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3266	34.74	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3267	33.79	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3268	34.67	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3269	33.6	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3270	35.19	34.19	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3271	33.82	36.26	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3272	34.35	36.28	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3273	33.94	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3274	35.14	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3275	33.59	36.33	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3276	33.86	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3277	33.93	33.15	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3278	33.89	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3279	34.47	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3280	33.89	36.80	H7Nx	PCR positive, not submitted for virus isolation

17-Feb-17	TP 3281	33.79	36.64	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3282	33.55	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3283	34.11	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3284	32.21	35.54	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3285	33.89	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3286	34.4	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3287	34.28	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3288	33.91	36.61	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3289	35.4	37.86	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3290	35.3	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3291	33.92	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3292	34.31	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3293	34.2	34.99	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3294	34.59	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3295	34.11	38.01	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3296	34.29	36.88	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3297	33.69	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3298	34.73	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3299	33.85	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3300	33.58	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3301	33.95	36.84	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3302	34.62	36.49	H7Nx	PCR positive, not submitted for virus isolation
17-Feb-17	TP 3303	33.83	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3304	35.27	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3305	35.17	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3306	34.73	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3307	34.3	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3308	34.98	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3309	34.11	Negative	None	PCR negative, not submitted for virus isolation
17-Feb-17	TP 3310	34.09	Negative	None	PCR negative, not submitted for virus isolation

17-Feb-17	TP 3311	34.69	Negative	None	PC
17-Feb-17	TP 3312	34.51	Negative	None	PC
17-Feb-17	TP 3313	34.55	Negative	None	PC
17-Feb-17	TP 3314	34.45	36.25	H7Nx	PC
17-Feb-17	TP 3315	34.36	Negative	None	PC
17-Feb-17	TP 3316	34.02	Negative	None	PC
17-Feb-17	TP 331	33.41	Negative	None	PC
17-Feb-17	TP 3318	34.12	36.18	H7Nx	PCI
17-Feb-17	TP 3319	33 98	Negative	None	PCI
17-Feb-17	TP 3320	34.22	34.20	H7Ny	PCI
17-Feb-17	TP 3321	34.22	36.50	H7Nx	PCI
17-Feb-17	TP 3322	33.86	36.94	H7Nx	PC
17 Feb17	TP 3323	35.96	36.42	H7Nx	PCI
17 Feb17	TP 3324	33.65	Negative	None	PC
17 Feb17	TP 3325	30.77	Negative	None	PC
17 Feb17	TP 3327	29.73	35.08	H7Nx	PC
17 Feb17	TP 3328	31.22	36.98	H7Nx	PC
17 Feb17	TP 3329	31.06	Negative	None	PC
17 Feb17	TP 3330	32.05	Negative	None	PCI
17 Feb17	TP 3331	31.61	37.15	H7Nx	PC
17 Feb17	TP 3332	31.72	36.57	H7Nx	PC
17 Feb17	TP 3333	30.87	Negative	None	PC
17 Feb17	TP 3334	31.31	36.81	H7Nx	PC
17 Feb17	TP 3335	30	Negative	None	PCI
17 Feb17	TP 3336	29.87	36.23	H7Nx	PC
17 Feb17	TP 3337	31.55	Negative	None	PC
17 Feb17	TP 3338	30.96	Negative	None	PC
17 Feb17	TP 3339	31.45	Negative	None	PC
17 Feb17	TP 3340	31.27	Negative	None	PC

PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation

R negative, not submitted for virus isolation R positive, not submitted for virus isolation R negative, not submitted for virus isolation R negative, not submitted for virus isolation R positive, not submitted for virus isolation R positive, not submitted for virus isolation R negative, not submitted for virus isolation R negative, not submitted for virus isolation R positive, not submitted for virus isolation R positive, not submitted for virus isolation R negative, not submitted for virus isolation R positive, not submitted for virus isolation R negative, not submitted for virus isolation R positive, not submitted for virus isolation R negative, not submitted for virus isolation

17 Feb17	TP 3341 31.1	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3342 31.39	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3343 31.83	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3344 30.62	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3345 31.39	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3346 31.34	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3347 31.54	35.37	H7Nx	PCR negative, not submitted for virus isolation
17 Feb17	TP 3348 31.54	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3349 31.56	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3350 31.82	34.34	H7Nx	PCR negative, not submitted for virus isolation
17 Feb17	TP 3351 31.2	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3352 30.78	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3353 31.42	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3355 31.65	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3356 31.18	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3357 31.42	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3358 31.35	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3359 31.67	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3360 31.67	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3361 32.28	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3362 32.18	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3363 31.45	37.22	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3364 31.15	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3365 31.52	35.22	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3366 31.37	36.14	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3367 29.46	37.18	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3368 31.72	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3369 31.64	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3370 31.7	37.32	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3371 31.00	Negative	None	PCR negative, not submitted for virus isolation

17 Feb17	TP 3372 3	30.76	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3373 3	31.42	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3375 3	31.36	37.27	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3376 3	31.15	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3377 3	31.72	Negative	None	PCR negative, not submitted for virus isolation
17 Feb17	TP 3378 3	32.95	36.04	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3379 3	31.73	36.43	H7Nx	PCR positive, not submitted for virus isolation
17 Feb17	TP 3380	31.17	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3381	34.08	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3382	33.5	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3383	34.45	34.88	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3384	33.92	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3386	34.44	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3387	34.1	36.94	H7Nx	PCR negative, not submitted for virus isolation
28Feb17	TP 3388	33.31	34.84	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3389	33.5	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3390	33.65	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3391	32.9	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3392	34.58	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3393	34.66	36.40	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3394	33.83	37.04	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3395	34.95	18.72	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3396	33.74	35.82	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3397	34.28	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3398	33.83	29.05	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3399	33.23	Negative	None	PCR negative, not submitted for virus isolation
28Feb17	TP 3400	34.21	36.45	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3401	33.23	35.22	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3402	33.12	36.44	H7Nx	PCR positive, not submitted for virus isolation
28Feb17	TP 3403	33.67	Negative	None	PCR negative, not submitted for virus isolation

28Feb17	TP 3404	33.38	35.79	H7Nx	PCR posi
28Feb17	TP 3405	33.52	Negative	None	PCR nega
28Feb17	TP 3406	25.83	36.15	H7Nx	PCR posi
28Feb17	TP 3407	28.02	35.37	H7Nx	PCR posi
28-Feb-17	TP 3408	27.87	36.31	H7Nx	PCR posi
28-Feb-17	TP 3409	29.5	Negative	None	PCR nega
28-Feb-17	TP 3410	30.79	Negative	None	PCR nega
28-Feb-17	TP 3411	29.34	34.90	H7Nx	PCR posi
28-Feb-17	TP 3412	30.94	34.97	H7Nx	PCR posi
28-Feb-17	TP 3413	31.92	Negative	None	PCR nega
28-Feb-17	TP 3414	29.15	35.18	H7Nx	PCR posi
28-Feb-17	TP 3415	29.37	Negative	None	PCR nega
28-Feb-17	TP 3416	29.83	Negative	None	PCR nega
28-Feb-17	TP 3417	30.21	Negative	None	PCR nega
28-Feb-17	TP 3418	29.63	Negative	None	PCR nega
28-Feb-17	TP 3419	29.44	Negative	None	PCR nega
28-Feb-17	TP 3420	29.4	Negative	None	PCR nega
28-Feb-17	TP 3421	31.27	36.15	H7Nx	PCR posi
28-Feb-17	TP 3422	29.58	Negative	None	PCR nega
28-Feb-17	TP 3423	29.58	37.50	H7Nx	PCR posi
28-Feb-17	TP 3424	29.99	Negative	None	PCR nega
28-Feb-17	TP 3425	29.97	Negative	None	PCR nega
28-Feb-17	TP 3426	29.84	34.05	H7Nx	PCR posi
28-Feb-17	TP 3427	30.11	37.38	H7Nx	PCR posi
28-Feb-17	TP 3428	30.39	Negative	None	PCR nega
28-Feb-17	TP 3429	30.71	Negative	None	PCR nega
28-Feb-17	TP 3430	30.31	36.45	H7Nx	PCR posi
28-Feb-17	TP 3431	31.34	35.87	H7Nx	PCR posi
28-Feb-17	TP 3433	32.45	Negative	None	PCR nega
28-Feb-17	TP 3435	31.56	Negative	None	PCR nega

itive, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation itive, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation

28-Feb-17	TP 3436	29.44	Negative	None
28-Feb-17	TP 3437	32.77	Negative	None
28-Feb-17	TP 3438	32.59	Negative	None
28-Feb-17	TP 3439	32.22	36.10	H7Nx
28-Feb-17	TP 3440	31.04	34.77	H7Nx
28-Feb-17	TP 3441	31.81	35.17	H7Nx
28-Feb-17	TP 3442	32.19	Negative	None
28-Feb-17	TP 3443	31.24	36.11	H7Nx
28-Feb-17	TP 3444	32.23	36.40	H7Nx
28-Feb-17	TP 3445	30.58	36.41	H7Nx
28-Feb-17	TP 3446	31.69	Negative	None
28-Feb-17	TP 3447	33.22	Negative	None
28-Feb-17	TP 3448	32.21	37.77	H7Nx
28-Feb-17	TP 3449	30.84	36.54	H7Nx
28-Feb-17	TP 3450	34.50	Negative	None
28-Feb-17	TP 3451	30.70	Negative	None
28-Feb-17	TP 3452	31.27	34.97`	H7Nx
28-Feb-17	TP 3453	33.56	36.54	H7Nx
28-Feb-17	TP 3454	33.97	37.29	H7Nx
28-Feb-17	TP 3455	32.52	37.34	H7Nx
28-Feb-17	TP 3456	32.03	34.52	H7Nx
28-Feb-17	TP 3457	32.08	36.45	H7Nx
28-Feb-17	TP 3458	32.86	Negative	None
28-Feb-17	TP 3459	31.44	24.42	H7Nx
28-Feb-17	TP 3460	32.61	36.54	H7Nx
28-Feb-17	TP 3461	30.48	35.49	H7Nx
28-Feb-17	TP 3462	32.34	37.49	H7Nx
28-Feb-17	TP 3463	31.27	Negative	None
28-Feb-17	TP 3464	33.26	Negative	None
28-Feb-17	TP 3465	31.18	21.26	H7Nx

PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation

28-Feb-17	TP 3466	31.23	37.15	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3467	33.29	34.46	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3468	33.57	35.87	H7Nx	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3469	34.28	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3470	32.85	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3471	33.56	37.79	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3472	32.31	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3473	31.69	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3474	31.78	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3475	34.45	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3476	34.42	32.17	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3477	34.40	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3479	34.26	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3480	33.77	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3481	33.87	32.17	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3482	33.83	35.05	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3483	34.39	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3484	34.42	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3485	34.31	34.64	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3486	33.66	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3487	34.11	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3488	33.33	35.11	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3489	33.98	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3490	34.06	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3491	34.10	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3492	34.27	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3493	34.53	35.82	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3494	34.0	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3495	34.93	36.47	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3496	34.22	36.64	H7Nx	PCR positive, not submitted for virus isolation

28-Feb-17	TP 3497	33.99	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3498	33.97	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3499	34.53	36.01	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3500	34.08	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3501	33.98	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3502	34.36	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3503	34.36	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3504	33.96	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3505	34.21	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3506	34.01	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3507	33.90	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3508	33.99	36.81	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3509	33.81	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3510	34.0	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3511	34.33	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3512	33.70	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3513	34.08	37.28	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3514	33.83	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3515	33.97	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3516	33.89	35.61	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3517	33.86	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3518	33.65	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3519	33.62	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3520	34.37	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3521	34.26	35.93	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3522	34.03	35.43	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3523	33.90	35.90	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3524	34.29	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3525	34.18	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3526	34.01	Negative	None	PCR negative, not submitted for virus isolation

28-Feb-17	TP 3527	34.93	Negative	None
28-Feb-17	TP 3528	33.94	34.66	H7Nx
28-Feb-17	TP 3529	33.85	Negative	None
28-Feb-17	TP 3530	34.54	Negative	None
28-Feb-17	TP 3531	34.12	Negative	None
28-Feb-17	TP 3532	33.99	Negative	None
28-Feb-17	TP 3533	33.95	Negative	None
28-Feb-17	TP 3534	33.97	35.39	H7Nx
28-Feb-17	TP 3535	33.21	35.43	H7Nx
28-Feb-17	TP 3536	34.14	35.90	H7Nx
28-Feb-17	TP 3537	34.22	Negative	None
28-Feb-17	TP 3538	33.79	35.45	H7Nx
28-Feb-17	TP 3539	33.98	35.32	H7Nx
28-Feb-17	TP 3564	34.26	Negative	None
28-Feb-17	TP 3565	33.43	Negative	None
28-Feb-17	TP 3566	33.74	36.48	H7Nx
28-Feb-17	TP 3567	33.83	30.45	H7Nx
28-Feb-17	TP 3568	33.78	Negative	None
28-Feb-17	TP 3569	34.12	Negative	None
28-Feb-17	TP 3571	35.64	36.77	H7Nx
28-Feb-17	TP 3572	33.32	Negative	None
28-Feb-17	TP 3573	34.38	36.87	H7Nx
28-Feb-17	TP 3574	33.52	Negative	None
28-Feb-17	TP 3575	32.24	Negative	None
28-Feb-17	TP 3576	33.99	Negative	None
28-Feb-17	TP 3577	33.99	Negative	None
28-Feb-17	TP 3578	33.67	Negative	None
28-Feb-17	TP 3579	33.87	Negative	None
28-Feb-17	TP 3580	34.75	Negative	None
28-Feb-17	TP 3581	33.40	Negative	None

PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation PCR positive, not submitted for virus isolation PCR negative, not submitted for virus isolation

28-Feb-17	TP 3582	34.24	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3583	34.90	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3584	33.60	37.36	H7Nx	PCR positive , not submitted for virus isolation
28-Feb-17	TP 3585	35.62	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3586	33.93	35.83	H7Nx	PCR positive , not submitted for virus isolation
28-Feb-17	TP 3587	33.37	36.49	H7Nx	PCR positive , not submitted for virus isolation
28-Feb-17	TP 3588	33.90	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3589	34.17	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3590	34.62	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3591	34.38	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3592	33.79	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3593	33.99	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3594	33.98	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3595	33.71	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 8-Feb-17	TP 3596 TP 3597	33.95 33.42	36.28 Negative	H7Nx None	PCR positive , not submitted for virus isolation PCR negative, not submitted for virus isolation
28-Feb-17	TP 3598	33.24	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3599	33.90	35.14	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3600	34.23	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3601	34.25	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3602	33.91	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3603	33.80	36.59	H7Nx	PCR positive , not submitted for virus isolation
28-Feb-17	TP 3604	34.73	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3605	33.97	35.71	H7Nx	PCR positive , not submitted for virus isolation
28-Feb-17	TP 3606	32.85	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3607	34.75	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3608	33.65	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3609	34.22	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3610	33.56	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3611	34.28	36.83	H7Nx	PCR positive , not submitted for virus isolation

28-Feb-17	TP 3612	34.14	Negative	None	PCR nega
28-Feb-17	TP 3613	34.31	37.339	H7Nx	PCR posit
28-Feb-17	TP 3614	33.94	Negative	None	PCR nega
28-Feb-17	TP 3615	33.83	Negative	None	PCR nega
28-Feb-17	TP 3616	34.56	Negative	None	PCR nega
28-Feb-17	TP 3617	33.51	Negative	None	PCR nega
28-Feb-17	TP 3618	33.92	35.16	H7Nx	PCR posit
28-Feb-17	TP 3619	33.83	Negative	None	PCR nega
28-Feb-17	TP 3620	33.96	Negative	None	PCR nega
28-Feb-17	TP 3621	33.0	Negative	None	PCR nega
28-Feb-17	TP 3622	34.28	36.75	H7Nx	PCR posi
28-Feb-17	TP 3623	35.94	37.13	H7Nx	PCR posi
28-Feb-17	TP 3624	34.18	36.58	H7Nx	PCR posi
28-Feb-17	TP 3625	33.76	37.38	H7Nx	PCR posi
28-Feb-17	TP 3626	34.14	Negative	None	PCR nega
28-Feb-17	TP 3627	33.36	Negative	None	PCR nega
28-Feb-17	TP 3628	33.64	35.97	H7Nx	PCR posi
28-Feb-17	TP 3630	33.57	37.07	H7Nx	PCR posi
28-Feb-17	TP 3631	33.92	Negative	None	PCR nega
28-Feb-17	TP 3632	33.05	Negative	None	PCR nega
28-Feb-17	TP 3633	33.78	Negative	None	PCR nega
28-Feb-17	TP 3634	34.76	34.37	H7Nx	PCR posi
28-Feb-17	TP 3635	34.26	Negative	None	PCR nega
28-Feb-17	TP 3637	33.76	Negative	None	PCR nega
28-Feb-17	TP 3638	34.28	Negative	None	PCR nega
28-Feb-17	TP 3639	33.85	Negative	None	PCR nega
28-Feb-17	TP 3640	33.36	35.21	H7Nx	PCR posi
28-Feb-17	TP 3641	33.81	Negative	None	PCR nega
28-Feb-17	TP 3642	33.36	Negative	None	PCR nega
28-Feb-17	TP 3643	34.37	Negative	None	PCR nega

ative, not submitted for virus isolation tive, not submitted for virus isolation ative, not submitted for virus isolation tive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation itive, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation ative, not submitted for virus isolation

28-Feb-17 TP	' 3644	33.26	34.37	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP	9 3645	34.44	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP	' 3646	33.41	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP	9 3647	34.95	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP	9 3648	31.17	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP	9 3649	31.16	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP	9 3650	30.68	37.74	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP	9 3651	33.07	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP	9 3652	32.28	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 T	TP 3653	32.50	35.05	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 T	TP 3654	31.58	37.08	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 T	P 3655	30.94	34.44	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 36	656	30.78	38.26	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 36	657	33.99	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	658	31.27	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	659	29.92	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	660	30.85	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	661	30.30	35.31	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 36	662	31.35	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	663	31.60	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	664	31.41	34.84	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 3	560	33.59	36.41	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 3	562	33.94	35.05	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 3	563	33.66	37.02	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 3	570	33.19	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	665	33.92	37.30	H7Nx	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	666	33.82	38.15	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 36	667	33.14	30.45	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 36	668	33.49	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 36	669	33.73	Negative	None	PCR negative, not submitted for virus isolation

28-Feb-17 TF	P 3670	33.58	36.93	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TF	P 3671	33.47	37.06	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 3672		33.27	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 3673		33.43	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 3674		33.91	35.44	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17 TP 3675		33.74	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 3676		33.65	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17 TP 3677		33.52	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3678	33.73	38.26	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3679	33.51	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3680	33.61	35.45	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3681	33.39	36.56	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3683	33.36	35.73	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3685	33.41	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3686	33.70	36.94	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3687	33.90	34.25	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3688	33.57	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3689	32.11	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3690	33.79	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3691	33.97	36.76	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3692	33.43	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3693	33.86	37.41	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3694	33.02	13.92	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3695	33.35	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3696	33.57	36.19	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3697	33.63	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3698	33.89	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3699	33.35	36.03	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3699	33.35	36.03	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3700	33.62	Negative	None	PCR negative, not submitted for virus isolation

28-Feb-17	TP 3701	33.562	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3702	33.42	35.43	H7Nx	PCR positive, not submitted for virus isolation
28-Feb-17	TP 3703	37.38	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3704	33.98	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3705	34.31	Negative	None	PCR negative, not submitted for virus isolation
28-Feb-17	TP 3706	34.38	Negative	None	PCR negative, not submitted for virus isolation