SURVEILLANCE OF AVIAN INFLUENZA IN SOUTH AFRICA: A LOOK AT A ZOOLOGICAL MONITORING PROGRAM AND SAMPLING OF WILD BIRDS

by

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Abstract

Through this masters' field experience, I have gained exposure to many different aspects of disease surveillance, especially as it relates to avian influenza in South Africa. This report will consider three main opportunities that occurred over a ten week period. First, disease surveillance and monitoring at the National Zoological Gardens of South Africa (NZG) where I helped sample resident birds as well as some donated specimens in order to gain a significant amount of information on the prevalence of avian influenza at the zoo. Secondly, wild bird capturing and sampling to gain information on disease migration and ecology of South African wild birds. Third, proper diagnostic techniques used at the Oderstepoort Veterinary Institute laboratory for testing of avian influenza.

While avian influenza is not an immediate risk in the country of South Africa, a lot can still take place in the form of surveillance and monitoring. This was a productive experience that allowed me to learn the basics of disease control, biological pathways of disease, viral diagnostics, and field sampling. Along with this opportunity, the NZG has begun an annual avian influenza monitoring program that will continue sampling birds in the future and the Global Avian Influenza Network for Surveillance (GAINS) program was able to get information on more birds in the Strandfontein area.

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Acknowledgments

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CHAPTER 1 - Introduction

Avian influenza has had a tremendous impact around the world as the current highly pathogenic strain, H5N1, continues to spread across continents. This is a global problem that requires a global response. Since 2003, this virus has killed millions of domestic fowl and infected more than 130 persons (15). The potential for an influenza pandemic is very real and necessitates international cooperation to prevent its occurrence. By conducting field investigations in the form of surveillance and monitoring, countries that are unaffected can respond and contain any projected outbreaks before they become a severe problem to the birds of the area or the humans that reside there.

Through this masters' field experience, I have gained exposure to many different aspects of disease surveillance, especially as it relates to avian influenza in South Africa. This report will consider three main opportunities that occurred over a ten week period. First, disease surveillance and monitoring at the National Zoological Gardens of South Africa (NZG) where I helped sample resident birds as well as some donated specimens in order to gain a significant amount of information on the prevalence of avian influenza at the zoo. Secondly, wild bird capturing and sampling to gain information on disease migration and ecology of South African wild birds. Third, proper diagnostic techniques used at the Oderstepoort Veterinary Institute laboratory for testing of avian influenza.

Avian Influenza

Avian influenza is a type A influenza of the family Orthomyoxociridae and is found primarily in wild birds. Problems arise, however, since this virus can be highly contagious and infect domestic birds such as chickens, ducks, and turkeys. It also has the potential to infect humans, but cases have been rare and seem to occur only in people exposed to infected poultry. This RNA virus can be categorized as either low pathogenic avian influenza (LPAI) or high pathogenic avian influenza (HPAI). These are identified based on their components of hemagglutinin (HA) and neuraminidase (NA). There are sixteen different HA antigens, and nine different NA antigens (1). In recent years, avian influenza has made headlines as a highly pathogenic form that has struck Asia and various other countries to different degrees.

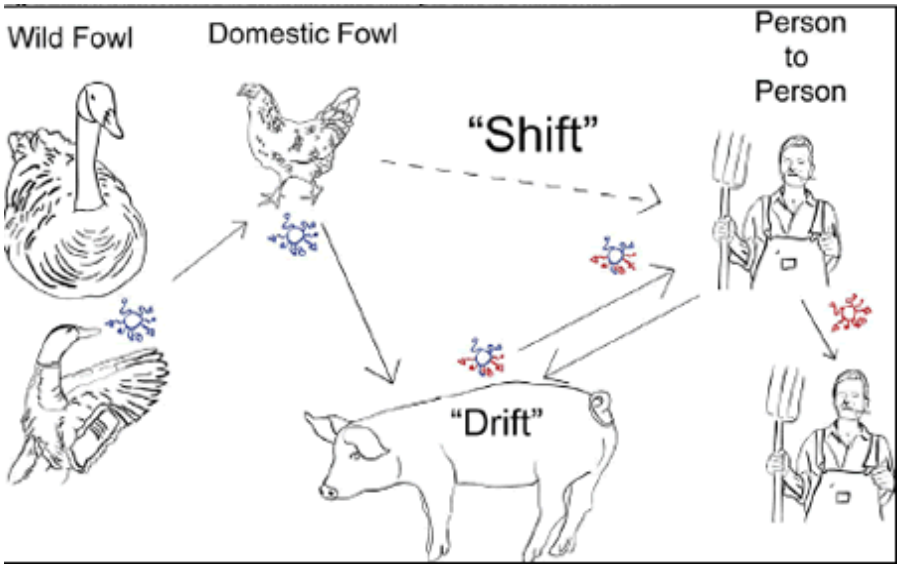
The virus is carried in saliva, nasal secretions, and feces of infected birds. Transmission occurs when birds come into contact with these secretions or contaminated surfaces (1). After exposure, the incubation period can range from two to eight days before symptoms of – diarrhea, respiratory distress, weakness, picking/scratching at themselves, poor appetite, and other behavior abnormalities present themselves (2).

According to the World Health Organization (WHO), there are three requirements to start a human pandemic; a viral strain for which there is little or no immunity in humans must emerge, it has to be able to cause serious illness in humans, and it has to spread easily from humans to humans. The current potential for a pandemic of H5N1 is that this strain has the first two of the three requirements; however, it has not successfully transferred from person to person (2). There are two methods for increasing transmissibility among humans; reassortment and adaptive mutation. Reassortment is when genetic material is exchanged between human and avian viruses by having co-infection of a human or a pig. (See fig. 1.1). Adaptive mutation is a much slower process where the virus has the capability to bind to human cells after subsequent infections of humans (2).

As the current outbreak of H5N1 spreads, we have seen that a growing amount of species are becoming infected with the virus. Many different avian species are being documented along with several mammalian species, such as tigers, leopards, domestic cats, pigs, and ferrets. This implies that the geographic and host ranges for the virus are expanding. (15) Recently, it has been discovered that ferrets can also be useful as models for measuring the pandemic potential of H5N1 (16).

In recent news, a different strain has taken headlines as a possible source of an avian influenza pandemic, H9N2. Increasing in prevalence, this strain has caused illness in at least four children of Hong Kong and can be found in humans, poultry, and pigs. It was mixed with H3N2 virus which is associated with the common cold in humans to show its ability for reassortment and then easily transmitted to ferrets. At this time, the strain is not found to be transmitted via aerosol, but can still be spread through contact with fomites (17).

Figure 1. Reassortment of Avian Influenza (3)



Should an actual pandemic of H5N1 reach the continent of Africa, it would cause major issues with the unique population that it encompasses. Based on a risk analysis conducted by the World Health Organization (WHO), an estimated 20% of the world's population would need medical care in the event of a pandemic. An estimate on the number of deaths ranges from 2 million to 7.4 million worldwide. Since the continent of Africa has major issues with HIV/AIDS, tuberculosis, and malaria, further complications and more severe infections of avian influenza are likely to arise due to the presence of many immune compromised individuals (2).

History of Disease

The most commonly known strains of avian influenza are the ones which have caused pandemics across the world. Avian influenza has been around for generations, but has only caused illness in humans during ten different outbreaks. Usually, avian influenza is a virus that stays true to the species it normally uses as a host. Of the ten outbreaks in the past, only four strains of avian influenza have been found to cause illness in humans; H5N1, H7N3, H7N7, and H9N2 (4). Most notable of these outbreaks was the pandemic of 1918 which caused 675,000 deaths in the United States and 20-50 million deaths worldwide. In 1957, another pandemic hit the globe causing 1 million deaths globally; and in 1968, another 1 million died due to an avian influenza pandemic (1).

At this time, the current outbreak of H5N1 has been going on since 1996 when it was first discovered in birds within China. It can now be found in Asia, Europe, the Near East, and in two northern countries of Africa; Nigeria and Lao People's Democratic Republic. It is thought that the H5N1 virus is now endemic in many areas of these countries and will not dissipate quickly. So far the transmission to people has been limited and sporadic, but once infected, mortality rates are very high. From 2003 to June 19, 2008, there have been 385 reported cases of H5N1 infections in humans worldwide and 243 of these have died (5).

The current situation in South Africa is that there have been three occasions where HPAI has reached the country. The first incident was in 1963 when 1,300 common terns died off the coast of the Western Cape. H5N3 was isolated from these birds, and was the first isolation of the avian influenza virus in wild birds in South Africa. The second incident was in 2004 when H5N2 was detected in ostriches of the Eastern Cape Province. The outbreak of HPAI in the ostrich population was first detected when the mortality rate suddenly increased from 5% to 44% over a five month period. To contain the spread of the virus, 26,000 ostriches were culled. Also at this time, a low pathogenic strain of H5N2 was isolated in wild Egyptian geese of the Western Cape. This suggests that the virus originated in wild birds of the Western Cape and may have mutated into a highly virulent form in ostriches of the Eastern Cape and also some ostriches of the Western Cape (6). The third outbreak in South Africa occurred in 2006 with the reemergence of HPAI H5N2 in ostriches. After extensive research, it was determined that although the two strains of 2004 and 2006 shared a common ancestor, they were not related. (12)

Although South Africa is at the end of a migratory funnel for birds, there are several factors that may be attributing to the fact that outbreaks here are so rare. First of all, there is no large-scale duck or turkey farming in the area; and although ostriches may be periodically infected, they are atypical terrestrial hosts. Another reason is that the ostrich farms and the main poultry producing areas are geographically separated. There is also such a dry climate most of the year that many of the water birds remain congregated around large bodies of water.

CHAPTER 2 - Aims of Research

Since South Africa is currently an infected country with HPAI, its goals revolve around such things as seeking access to updated information on risks, detecting clinical disease within populations, and understanding the epidemiology and ecology of avian influenza to help design effective control plans. These are large-scale plans, but there are also individual goals in place for three of the most vulnerable populations to avian influenza: zoos, wild bird populations, and poultry operations.

The surveillance of avian influenza at the NZG will hopefully be the beginning of a continual program to help create a baseline of the birds at the zoo as well as birds in the region. This report depicts the beginning of an annual winter monitoring program at the zoo that covers April 1st to mid-August. This initiative will be conducted by opportunistic sampling of birds in order to create an early warning system. Birds need to be tested regularly so that their current disease status is known. This will allow the zoo to respond quickly should a highly pathogenic strain reach the area.

The monitoring program set up by the Global Avian Influenza Network for Surveillance (GAINS) and implemented in part by the Percy Fitzpatrick Institute is set up to expand operational field capabilities, improve the understanding of viral strains and transmission of influenza viruses in wild birds, and to disseminate information to all levels of governments, international organizations, the private sector and the general public through regular sampling of wild birds and also consistent sharing of data through a globally accessible data bank (7).

The poultry industry is also an important sentinel for the spread of avian influenza. Although no data was collected during this particular study on the commercial aspects of avian influenza monitoring, it is still necessary to mention its role in surveillance. Backyard flocks of poultry and live poultry markets can be huge reservoirs for the disease; spreading it to people and other birds. These areas are also not subject to any biosecurity measures where at least the poultry industry has begun to establish protocol like the National Poultry Improvement Plan (NIPP). The NIPP is a program conducted by the United States Department of Agriculture (USDA) which includes avian influenza as one of its target diseases. Under this plan, 100% of

commercial primary breeding chickens and turkeys, 25% of game bird flocks, and 25% of mail order hatchery flocks are being tested for avian influenza as part of the nationwide surveillance in the United States. (14)

CHAPTER 3 - Surveillance at the National Zoological Gardens of South Africa

Zoos as a Sentinel for Avian Influenza

Surveillance programs in zoos across the globe can play an important role in detecting disease outbreaks such as those for HPAI. Zoos are positioned as early warning systems since they already have the facilities, staff, lab equipment, and medical history on all of the birds in their collection. Zoos are built in such a way that promote interaction and close proximity between a variety of animals and humans, including staff, veterinarians, and the public. They are sentinels for studying zoonotic diseases.

Historically, it was a zoo veterinarian who discovered the emergence of West Nile disease in the United States. In 1999, Dr. Tracey McNamara from the Bronx Zoo, began to question the death of several crows at the zoo. Based on her persistence in persuading the authorities that there was a true issue, the United States was able to begin the process of containment and protection of other animals across the country such as horses, birds, and humans (8).

Highly pathogenic avian influenza could be detrimental to zoos if the pathogen should reach their area. Zoos house very specific species of birds, some of which are threatened or endangered. Their protection is vital for the perpetuation of the species. If HPAI were to reach these areas, zoos would be forced to take them off exhibit. One of the controversies that arise with avian influenza in zoos is that the WHO requires culling of all birds infected with H5N1, but federal law prohibits the destruction of birds designated as threatened or endangered. Another issue is that modern zoos are pushing for much more natural looking habitats for their animals. This allows zoo birds to have a greater exposure to fly-in wild birds since they are out in the open or covered by a gated roof. While these structures may limit the physical contact between wild and zoo birds, feces can still enter the enclosures and infect birds that are housed inside.

Zoos of South Africa are often the host of various animals that have been confiscated at airports. At times, exotic birds are illegally traded – a source of many different diseases and

infections. Housing such birds can pose risk to the animal collection at zoos. The introduction of such birds into the hospitals should be done with extreme caution and strict quarantine to prevent the spread of H5N1 or other infections.

One of the arguments to help keep zoo birds safe is for the government to see zoos as being separate entities from poultry operations and in need of guidelines that fit their particular situation. Currently in areas with endemic cases of H5N1, all farms with an outbreak practice mass gassing of an entire poultry unit. Zoos have such a valuable and diverse collection of birds, that culling should only be used as a last resort. On the flip side of this argument, zoos also want to see that their birds receive vaccines for HPAI that are normally held on reserve for poultry operations. A balance needs to be found between protecting the birds at zoos by seeing them as a separate entity from poultry operations, but also as being high on the priority list in order to receive proper preventative measures.

Protocol

During my time at the National Zoological Gardens of South Africa, 94 birds were sampled for avian influenza. All birds that came through the veterinary hospital or the diagnostic laboratory were used as opportunistic testing samples, and more than likely these birds came to the hospital due to illness and could be potential sources of disease. Swabs from the cloaca and the choana of each available bird was taken. The swabs used were an invasive sterile collection swab with a wire shaft. According to the National Institute of Health (NIH), calcium alginate swabs or swabs with wooden sticks may contain substances that inactivate some viruses and inhibit PCR testing (9).

In order to obtain a nasopharygeal swab, a swab was first inserted into the choana. It is left in place for a few seconds to absorb secretions. By gently twisting and then withdrawing the swab a sample is collected. In order to obtain a cloacal swab, the same procedure is followed, but the swab is inserted into the cloaca. Please refer to the following chart for data on all birds collected at the National Zoological Gardens of South Africa:

Table 1. Avian Influenza Data Sheet for All Birds Sampled at the NZG

	Collection Date	Species	Sex	Live Bird	Necropsy	Choana	Cloaca	Date Taken to OVI
1	14.05.08	Cape Vulture	0.0.1		08/264	X	X	
2	15.05.08	Parrot	0.1		08/268	X	X	
3	21.05.08	King Vulture	0.1	Chick 1/08		X	X	
4	21.05.08	King Vulture	0.0.1	Chick 2/08		X	X	
5	21.05.08	King Vulture	0.0.1	Chick 3/08		X	X	21.05.08
6	22.05.08	Bald Ibis	0.0.1	PTA Zoo 14-01		X	X	
7	22.05.08	Bald Ibis	1.0		08/273	X	X	
8	27.05.08	Hadeda Ibis	0.1	Free Range		X	X	
9	24.05.05	Goliath Heron	1.0		08/251	X	X	
10	26.05.08	Rosy Flamingo	1.0		08/280	X	X	
11	26.05.08	Egyptian Goose	0.1		08/275	X	X	29.05.08
12	29.05.08	Giant Eagle Owl	1.0		08/290	X	X	
13	29.05.08	Brown Headed Parrot	0.0.1		08/288	X	X	
14	29.05.08	Black Swan	0.01		08/289	X	X	
15	30.05.08	Black Swan	0.0.1	Donation		X	X	
16	30.05.08	Egyptian Goose	0.0.1		08/283	---	X	
17	02.06.08	Hadeda Ibis	0.0.1	08/108		X	X	
18	02.06.08	Giant Eagle Owl	0.1	P 93722		X	X	
19	02.06.08	Cattle Egret	0.0.1	Don 08/89		X	X	
20	02.06.08	Spoonbill Chic	1.0		08/292	X	X	
21	03.06.08	Jandayan Conure	0.0.1	Bird 2		X	X	
22	03.06.08	Barn Owl	0.0.1	Don 08/107		X	X	
23	03.06.08	Blue and Yellow Macaw	0.0.1	Don 08/105		X	X	
24	03.06.08	Jandayan Conure	0.0.1	Bird 1		X	X	
25	04.06.08	Vulture Chic no. 1/07	1.0	913008		X	X	04.06.08
26	04.06.08	African Gray Parrot	0.1		08/296	--	X	
27	05.06.08	African Gray Parrot	0.0.1	Don 08/93		X	X	
28	09.06.08	African Gray Parrot	0.1		08/304	X	X	
29	12.06.08	Spotted Nikkop	0.1		08/308	X	X	13.06.08
30	13.06.08	Scops Owl	0.0.1	Don 07/166		X	X	
31	17.06.08	Barn Owl	0.0.1	Don 08/116-1		X	X	
32	17.06.08	Barn Owl	0.0.1	Don 08/116-2		X	X	
33	17.06.08	Barn Owl	0.0.1	Don 08/111		X	X	
34	18.06.08	Hadeda Ibis	0.0.1	Don 08/114		X	X	
35	19.06.08	Waldropp Ibis	0.0.1	H 109/08		X	X	20.06.08
36	26.06.08	Red Eyed Dove	1.0		08/318/01	X	X	
37	26.06.08	Red Eyed Dove	0.1		08/318/02	X	X	

38	27.07.08	Little Corella	1.0		08/320	No head	X	
39	02.07.08	Spoonbill Chic	0.0.1		08/330	X	X	
40	01.07.08	Coco lead Beater	1.0		08/328	X	X	
41	02.07.08	Rosy Flamingo	0.0.1	30NS		X	X	
42	02.07.08	Rosy Flamingo	0.0.1	89STB		X	X	
43	02.07.08	Rosy Flamingo	0.0.1	69BS		X	X	
44	02.07.08	Rosy Flamingo	0.0.1	58NS		X	X	
45	02.07.08	Rosy Flamingo	0.0.1	99BS		X	X	
46	02.07.08	Rosy Flamingo	0.0.1	86NS		X	X	
47	02.07.08	Rosy Flamingo	0.0.1	53BS		X	X	
48	02.07.08	Rosy Flamingo	0.0.1	65BS		X	X	
49	02.07.08	Rosy Flamingo	0.0.1	69NS		X	X	
50	02.07.08	Rosy Flamingo	0.0.1	66BS		X	X	
51	02.07.08	Rosy Flamingo	0.0.1	73NS		X	X	
52	02.07.08	Rosy Flamingo	0.0.1	36NS		X	X	
53	02.07.08	Rosy Flamingo	0.0.1	16NS		X	X	
54	02.07.08	Rosy Flamingo	0.0.1	4NS		X	X	
55	02.07.08	Rosy Flamingo	0.01	47NS		X	X	
56	02.07.08	Rosy Flamingo	0.0.1	43NS		X	X	
57	02.07.08	Rosy Flamingo	0.0.1	33NS		X	X	
58	02.07.08	Rosy Flamingo	0.0.1	26NS		X	X	
59	02.07.08	Rosy Flamingo	0.0.1	05NS		X	X	
60	02.07.08	Rosy Flamingo	0.0.1	18NS		X	X	
61	02.07.08	Rosy Flamingo	0.0.1	37NS		X	X	
62	02.07.08	Rosy Flamingo	0.0.1	62BS		X	X	
63	02.07.08	Rosy Flamingo	0.0.1	99NS		X	X	
64	02.07.08	Rosy Flamingo	0.0.1	93STB		X	X	
65	02.07.08	Rosy Flamingo	0.0.1	28NS		X	X	
66	02.07.08	Rosy Flamingo	0.0.1	91STB		X	X	
67	02.07.08	Rosy Flamingo	0.0.1	78BS		X	X	
68	02.07.08	Rosy Flamingo	0.0.1	31NS		X	X	04.07.08
69	04.07.08	Wattle Crane	0.1		08/335	X	X	
70	08.07.08	Ox Pecker	0.0.1		08/336	X	X	
71	14.07.08	Rainbow Lorikeet	1.0		08/342	X	X	15.07.08
72	16.07.08	Rainbow Lorikeet	1.0		08/362	X	X	
73	16.07.08	Rainbow Lorikeet	1.0		08/368 A	X	X	
74	16.07.08	Rainbow Lorikeet	0.0.1		08/368 B	X	X	
75	17.07.08	Red Crow	0.1	913370		X	X	
76	21.07.08	Rainbow Lorikeet	0.0.1	810LTVR		X	X	

77	21.07.08	Rainbow Lorikeet	0.0.1	70990LTVR		X	X	
78	21.07.08	Rainbow Lorikeet	0.0.1	GN01F0745		X	X	
79	21.07.08	Rainbow Lorikeet	0.0.1	No Ring		X	X	
80	21.07.08	Rainbow Lorikeet	0.0.1	112PJV		X	X	
81	21.07.08	Rainbow Lorikeet	0.0.1	83007LTVR		X	X	
82	21.07.08	Cape Vulture	1.0		08/372	X	X	22.07.08
83	23.07.08	Red Eyed Dove	0.0.1	Bird 1		X	X	
84	23.07.08	Laughing Dove	0.0.1	Bird 2		X	X	
85	23.07.08	Laughing Dove	0.0.1	Bird 3		X	X	
86	24.07.08	Red Eyed Dove	0.0.1	Bird 4		X	X	
87	24.07.08	Red Eyed Dove	0.0.1	Bird 5		X	X	
88	24.07.08	Red Eyed Dove	0.0.1	Bird 6		X	X	
89	25.07.08	Blue Crane	0.0.1		08/392	X	X	
90	29.07.08	Red Sided Electus Parrot	1.0		08/394	X	X	
91	29.07.08	Little Corella	0.1		08/400	X	X	
92	29.07.08	Little Corella	0.1		08/401	X	X	31.07.08
93	04.08.08	Black Shoulder Kite	1.0		08/406	X	X	
94	08.08.08	Greater Flamingo	0.0.1		08/420	X	X	07.08.08

**Key: Each bird sampled has an identification number given. If the bird was sampled live, it has a number corresponding to a ring number or a number given to it by the zoo. If the bird was sampled during a necropsy, it was given a postmortem number. The sex of the birds can be identified using a series of three separated numbers. The first number stands for males, the second for females, and the third for those birds whose sex is unknown. The number in that space lists the number of birds having that sex. For example, 0.0.1 indicates one bird of unknown sex.*

Once all swabs are taken from the birds, the shaft is broken off into a small collection tube containing phosphate buffered saline to be frozen at 4 degrees Celsius until it is transported to the laboratory. The testing center for all the samples from the zoo is at the Onderstepoort Veterinary Institute (OVI). Repeated freezing and thawing had to be avoided to prevent loss of infectivity, so the samples were transported to the laboratory in a Styrofoam container with ice packs (9).

Postmortems

By conducting necropsies on all dead animals at the zoo, one can gain a better understanding about disease and animal behavior. One way to recognize a disease outbreak of avian influenza is by an increased abundance of dead or dying birds, but also just as important is

the microscopic and histopathological evidence. Necropsies are done on every dead animal in the zoo for record keeping purposes. It is important that all animals receive a full necropsy even if the cause of death seems obvious. Without records of a postmortem, there is no accurate record of why the animal is no longer at the zoo, and it is necessary for discerning patterns on why groups of species may be dying off.

While working in a postmortem hall, special precautions are taken due to the high risk of zoonotic infection. Many diseases and infections can easily be spread when handling tissue, handling blood, or the travel of the pathogen through the air. In order to prevent human illness one of the precautionary measures taken is the use of a ventilation hood. All birds were processed under the hood mostly due to the risk of an air-borne chlamydial infection. Psittacosis (Chlamydophila psittaci), is of particular concern because it is found in several different bird species including parrots. All persons in the postmortem hall are required to wear rubber boots and step through a disinfectant foot bath. Gloves are to be worn when handling specimens, and masks are available in case a carcass has characteristic signs of an airborne zoonotic infection, or one is suspected.

Since wild birds can normally carry the avian influenza virus without any symptoms, a clean necropsy report is not sufficient evidence for a negative bird. It is important to swab each bird and have it tested for the virus. Birds who may show symptoms of avian influenza would present with increased nasal discharge, decrease in overall condition, a heavily soiled cloacal region, pulmonary consolidation, and possibly multifocal hemorrhage in the organs (10).

Under the tutelage of Dr. Emily Lane at the NZG, I was able to undergo training in performing bird necropsies. My experience soon included a vast amount of different bird species including black swans, ibises, parrots, and doves. None of the bird postmortems that I conducted showed any signs of disease-related causes of death. Please refer to Appendix B for a detailed protocol of avian necropsies performed at the NZG and also for an example of a necropsy report form from the zoo.

CHAPTER 4 - Sampling of Wild Birds

In conjunction with the GAINS program, Professor Graeme Cumming has implemented a monitoring program at five different sites, two of which are in South Africa, one in Botswana, one in Zimbabwe, and one in Mozambique. Each location is visited every two months to count, catch, and process birds. Of particular interest, due to their notorious labeling as one of the key wild vectors of avian influenza, are all of the duck species. Also on the rise as a commonly infected wild bird species are the swans, however, this is found more commonly in Europe. Based on a recent study published in the Emerging Infectious Diseases Journal, the mute swan is the most likely swan species to transmit HPAI. By experimentally infecting these swans, it was discovered that they are highly susceptible and can be clinically protected by pre-exposure immunity (13).

I was able to take part in sampling at one of the sites in South Africa, the Cape Flats Water Treatment Plant in Strandfontein. This site consists of a collection of 15 ponds and several channel-ways. This is a prime location for surveillance of disease based on many factors. First of all, this location is a series of ponds located between a river and the False Bay, which allows for a diverse collection of species. There is also a unique connection to humans since this is a water treatment facility very close to civilization. All of the information gathered from this site will be assimilated with the other locations to help make predictions on the prevalence of avian influenza in South Africa along with the movement of different duck species.

The sampling program set up by GAINS holds resemblance to many other monitoring efforts going on around the globe. While the goal of GAINS is for other monitoring sites to share their information at one common database, there are many countries who choose to assimilate their own information. One such effort is conducted in the Copper River Delta area of Southern Alaska, which has the highest spring shorebird concentrations in the New World. After screening for avian influenza in 1,820 samples, only one bird was positive having a H16 virus. This made the overall prevalence 0.055%. (14)

Census of Wild Birds

Counting birds at any site is essential for gaining an understanding about the population of the area. Each site is counted by sitting at consistent points and counting for 30 minute intervals for all species of birds that come within 150 meters of that area. The interaction with the environment is noted including whether the bird is foraging, not foraging, or flying over. A bird that is foraging on a pond has more of an interaction with the ecological system than a bird that is just flying over. Please refer to Appendix A for an example of a counting form used at this site.

Along with the counts, the water quality is also routinely measured in all of the ponds. The make-up of the water could be an interesting factor if avian influenza were found to be present in the ponds. Some abiotic environments at Strandfontein may prove to be more adequate for cultivating a virus than others. Noting the type of water that cultivates the virus the best can be powerful information when it comes to taking preventative measures.

South Africa is a country that has a less predictable fly pattern than the northern hemisphere largely due to its variable amounts of rainfall each year and generally warm climate. Most ducks of the area are not truly migratory. Birds find safety in water and during drier seasons can become subject to the many predators encompassing Africa, so they usually take up residence on the larger bodies of water that are at less risk of running dry. The ducks are opportunistic migrators in order to evade predation, to breed, or to molt. For this reason, it becomes more of a challenge to ascertain the movement of sick birds- or healthy birds for that matter. However, it is known that the ducks found in the Cape region have not been recorded as flying any farther north than Tanzania. This makes the movement of HPAI from countries where it is endemic to South Africa a very low risk factor, at least through migratory flying patterns. (7)

Capture of Wild Birds

In order to capture the birds at Strandfontein, traps were set at different ponds and also along three channel-ways. There were two main types of traps used, walk-in traps and mist nets. The walk-in traps were either made of chicken wire or plastic. Each has a roof of netting and either one or two funnels through which the birds enter by following bait, becoming trapped and unable to find the funnel again to get back out. Usually, it is best to place the funnels away from

the water's edge because a bird's natural instinct is to head toward water when scared. The mist nets work well when the sun is rising or just as it is setting. The light creates an illusion making it difficult for the birds to see the nets as they are flying. Inevitably, they fly right into the net and become trapped.

Surveillance of wild birds can have its challenges. One of the factors that make it difficult to capture birds in the wild is the weather. For example, there were times that it rained so much there was not much activity among the birds and the likelihood of them moving into traps was decreased. We also experienced a lot of flooding. Some of the traps we laid were soon under water due to the rising waters and had to be repositioned further up on the shore. Another factor that makes disease surveillance of wild birds a challenge is predation. Often any birds that die of illness are quickly assimilated by the environment by either being consumed or at least drug off to areas outside of the surveillance perimeters, skewing data on disease prevalence. Any search for sick or dead animals is also subject to bias since large and colorful birds are found more easily than those that blend into their surroundings. (11)

Processing Birds

During monitoring at this site, 49 birds were collected and processed. Species captured included Egyptian geese, yellow billed ducks, cape teals, red eyed doves, speckled pigeons, cape wagtails, cape shovelers, and cape gulls. Each bird is processed, which includes: ringing the birds for future identification, taking morphometrics, examining the health status, collecting feather samples, drawing blood, taking photographs, and swabbing for avian influenza. Please refer to Appendix A for proper forms used for processing live birds. After the birds have been processed, the ones that are not molting were released on site. The birds that were undergoing different stages of molt were taken back to their capture location for release.

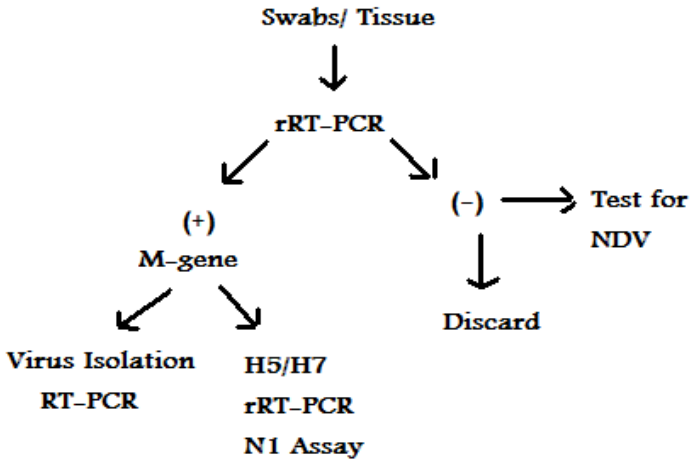
CHAPTER 5 - Processing Samples

Diagnostics

Once the swabs are all collected from avian specimens, they are transported to the ARC-OVI Laboratory where they are processed. They use either of two methods for viral extraction from the swabs; MagNA Pure Viral Isolation and Trizol RNA Isolation. The MagNA Pure method is an automated machine and is very effective in extracting large batches of samples at one time. However, the Trizol RNA Isolation method is more effective in extracting viral RNA from swabs because feces can have inhibitors that may not be adequately removed by the MagNA Pure system that adversely affects PCR. The Trizol RNA Isolation is the preferred method, but a much slower process. Please refer to Appendix C for complete procedures on these two processing methods.

After the RNA has been extracted from the samples, it must undergo rRT-PCR. In this process the RNA virus is converted to cDNA. Amplification of double stranded DNA results from the annealing of gene-specific primers and the extension of these by a DNA polymerase in a logarithmic fashion. A gene specific fluorogenic probe (Taqman chemistry) in the reaction will also anneal to the DNA template if its target sequence (i.e. avian influenza M gene) is present. If the sample is negative, it is either discarded or checked for Newcastle Disease Virus (NDV). All samples which are positive for the avian influenza M gene undergo further testing by virus isolation or H5/H7 rRT-PCR to determine the strain of the virus. Please refer to figure 5.1 for a summary of this process.

Figure 2. Summary of the Processing Procedure for Avian Influenza Samples



Results

At the NZG, 94 birds were tested and the results are shown in Table 5.1. Fifteen of the ninety four birds showed signs of being suspect positive for avian influenza M gene, but with further typing, no H5/H7 gene could be extracted and no other virus isolation was found. These results are depicted in Table 5.2.

Table 2. Sample Results

	Species	**Live Bird**	**Necropsy**	**Result Status**
1	Cape Vulture		08/264	**Suspect Positive**
2	Parrot		08/268	Negative
3	King Vulture	Chick 1/08		**Suspect Positive**
4	King Vulture	Chick 2/08		**Suspect Positive**
5	King Vulture	Chick 3/08		Negative
6	Bald Ibis	PTA Zoo 14-01		**Suspect Positive**
7	Bald Ibis		08/273	**Suspect Positive**
8	Hadeda Ibis	Free Range		Negative
9	Goliath Herone		08/251	Negative
10	Rosy Flamingo		08/280	Negative
11	Egyptian Goose		08/275	Negative
12	Giant Eagle Owl		08/290	Negative
13	Brown Headed Parrot		08/288	Negative
14	Black Swan		08/289	Negative
15	Black Swan	Donation		Negative
16	Egyptian Goose		08/283	Negative
17	Hadeda Ibis	08/108		Negative
18	Giant Eagle Owl	P 93722		Negative
19	Cattle Egret	Don 08/89		Negative
20	Spoonbill Chic		08/292	Negative
21	Jandayan Conure	Bird 2		Negative
22	Barn Owl	Don 08/107		Negative
23	Blue and YellowMacaw	Don 08/105		Negative
24	Jandayan Conure	Bird 1		Negative
25	Vulture Chic no. 1/07	913008		Negative
26	African Gray Parrot		08/296	Negative
27	African Gray Parrot	Don 08/93		Negative
28	African Gray Parrot		08/304	Negative

29	Spotted Nikkop		08/308	Negative
30	Scops Owl	Don 07/166		Negative
31	Barn Owl	Don 08/116-1		Negative
32	Barn Owl	Don 08/116-2		Negative
33	Barn Owl	Don 08/111		Negative
34	Hadeda Ibis	Don 08/114		Negative
35	Waldropp Ibis	H 109/08		Negative
36	Red Eyed Dove		08/318/01	Negative
37	Red Eyed Dove		08/318/02	Negative
38	Little Corella		08/320	Negative
39	Spoonbill Chic		08/330	**Suspect Positive**
40	Coco lead Beater		08/328	**Suspect Positive**
41	Rosy Flamingo	30NS		Negative
42	Rosy Flamingo	89STB		Negative
43	Rosy Flamingo	69BS		Negative
44	Rosy Flamingo	58NS		Negative
45	Rosy Flamingo	99BS		Negative
46	Rosy Flamingo	86NS		Negative
47	Rosy Flamingo	53BS		Negative
48	Rosy Flamingo	65BS		Negative
49	Rosy Flamingo	69NS		Negative
50	Rosy Flamingo	66BS		Negative
51	Rosy Flamingo	73NS		Negative
52	Rosy Flamingo	36NS		Negative
53	Rosy Flamingo	16NS		Negative
54	Rosy Flamingo	4NS		Negative
55	Rosy Flamingo	47NS		Negative
56	Rosy Flamingo	43NS		Negative
57	Rosy Flamingo	33NS		Negative
58	Rosy Flamingo	26NS		Negative
59	Rosy Flamingo	05NS		Negative
60	Rosy Flamingo	18NS		Negative
61	Rosy Flamingo	37NS		Negative
62	Rosy Flamingo	62BS		Negative
63	Rosy Flamingo	99NS		Negative
64	Rosy Flamingo	93STB		Negative
65	Rosy Flamingo	28NS		Negative
66	Rosy Flamingo	91STB		Negative
67	Rosy Flamingo	78BS		Negative
68	Rosy Flamingo	31NS		Negative

69	Wattle Crane		08/335	Negative
70	Ox Pecker		08/336	Negative
71	Rainbow Lorikeet		08/342	Negative
72	Rainbow Lorikeet		08/362	Negative
73	Rainbow Lorikeet		08/368 A	Negative
74	Rainbow Lorikeet		08/368 B	Negative
75	Red Crow	913370		Negative
76	Rainbow Lorikeet	810LTVR		Negative
77	Rainbow Lorikeet	70990LTVR		Negative
78	Rainbow Lorikeet	GN01F0745		Negative
79	Rainbow Lorikeet	No Ring		Negative
80	Rainbow Lorikeet	112PJV		Negative
81	Rainbow Lorikeet	83007LTVR		Negative
82	Cape Vulture		08/372	Negative
83	Red Eyed Dove	Bird 1		Negative
84	Laughing Dove	Bird 2		Negative
85	Laughing Dove	Bird 3		**Suspect Positive**
86	Red Eyed Dove	Bird 4		Negative
87	Red Eyed Dove	Bird 5		**Suspect Positive**
88	Red Eyed Dove	Bird 6		**Suspect Positive**
89	Blue Crane		08/392	Negative
90	Red Sided Electus Parrot		08/394	**Suspect Positive**
91	Little Corella		08/400	**Suspect Positive**
92	Little Corella		08/401	**Suspect Positive**
93	Black Shoulder Kite		08/406	*Pending*
94	Greater Flamingo		08/420	*Pending*

Table 3. Results for Suspect Positive Birds for the Avian Influenza M Gene

Species	Live Bird	Necropsy	Sample Type	H5/H7 gene rRT-PCR	Virus Isolation
Cape Vulture		08/264	Oropharyngeal Swab	Negative	Negative
King Vulture	Chick 1/08		Oropharyngeal Swab	Negative	Negative
King Vulture	Chick 2/08		Cloacal Swab	Negative	Negative
Bald Ibis	PTA Zoo 14-01		Oropharyngeal Swab	Negative	Negative
Bald Ibis	PTA Zoo 14-01		Cloacal Swab	Negative	Negative
Bald Ibis		08/273	Oropharyngeal Swab	Negative	Negative
Spoonbill Chick		08/330	Oropharyngeal Swab	Negative	Negative
Coco Lead Beater		08/328	Cloacal Swab	Negative	Negative
Red Eyed Dove	Bird 5		Cloacal Swab	Negative	Negative
Red Eyed Dove	Bird 6		Oropharyngeal Swab	Negative	Negative
Little Corella		08/400	Cloacal Swab	Negative	Negative
Little Corella		08/400	Cloacal Swab	Negative	Negative
Red Sided Parrot		08/394	Oropharyngeal Swab	Negative	Negative
Red Sided Parrot		08/394	Cloacal Swab	Negative	Negative
Laughing Dove	Bird 3		Oropharyngeal Swab	Negative	Negative
Little Corella		08/401	Oropharyngeal Swab	Negative	Negative

Discussion

Based on the results of the sampling conducted at the NZG, there is no conclusive evidence that there is any strain of avian influenza in the birds sampled. There are a few birds that are suspect positive for avian influenza, but many other factors could have attributed to these results such as a weakening in the primers as the PCR cycle comes to an end. The prevalence of suspect positive birds for avian influenza is 14.13%

Both the oropharyngeal and the cloacal swabs were useful in detecting the virus so one should not be favored over the other especially since only three of the suspect positive birds had both swabs pick up signs of a virus. There also does not seem to be any association between more live birds or postmortem birds sampled having a higher prevalence of disease. Future

research at the zoo could include the sampling of more water birds or ratites such as ostriches to see if these populations house more virus than the variety of other birds sampled in this report.

CHAPTER 6 - Conclusion

While avian influenza is not an immediate risk in the country of South Africa, a lot can still be done to improve surveillance and monitoring. This was a productive experience that allowed me to learn the basics of disease control, biological pathways of disease, viral diagnostics, and field sampling. Along with this opportunity, the NZG has begun an annual avian influenza monitoring program that will continue sampling birds in the future and the GAINS program was able to get information on more birds in the Strandfontein area.

After ten weeks, I have gained a wealth of knowledge in public health and also an invaluable experience in a culture different than my own. I was able to work with people from all across the globe and be immersed in their lifestyles. The things I have seen and learned in South Africa will forever affect my views and attitude toward veterinary medicine and public health.

REFERENCES

1) Centers for Disease Control and Prevention. (2008). Avian Influenza. Retrieved June 23, 2008, from: www.cdc.gov/flu/avian/.

2) World Health Organization. (2008). Influenza Pandemic Risk Assesment and Preparedness in Africa. Retrieved June 23, 2008, from: www.afro.who.int/csr/epr/ epidemic_prone_diseases/afr_avian_flu_31_10_06.pdf.

3) Correctional Service Canada. (2008). Focus on Infectious Diseases. Retrieved July 24, 2008, from: www.csc-scc.gc.ca.

4) World Health Organization. (2008). Avian Influenza Fact Sheet. Retrieved June 23, 2008, from: www.who.int/mediacentre/factsheets/avian_influenza/en/

5) World Health Organization. (2008). Cases Table. Retrieved June 25, 2008, from www.who.int /csr/disease/avian_influenza/country/cases_ table_2008_06_19 /en/index.html.

6) University of Cape Town. (2008). avian influenza. Retrieved on July 24, 2008, from www.web.uct.ac.za/depts/stats/adu/pdf/avian-flu-5-celia.pdf.

7) Global Avian Influenza Network for Surveillance. (2008). Retrieved June 24, 2008, from www.gains.org.

8) AMNH. A Medical Detective Story. Bio Bulletin. (2008).

9) National Institute of Health. (2008). Guidelines for Prevention and Control of Human Cases of Avian Influenza Disease (Bird Flu). Retrieved June 16, 2008, from: www.nih.org

10) Wilson, Mary E. Avian Influenza Moves into Zoos and Wildlife. Journal Watch. December 10, 2004.

11) Wobeser, Gary A. Essentials of Disease in Wild Animals. Blackwell Publishing. Aimes, 2006.

12) Albonik, Celia. “Molecular Characterization of H5N2 Avian Influenza Viruses Isolated from South African Ostriches in 2006.” Avian Disease. 4 (2007): 873-9.

13) Kalthoff D, Breithaupt A, Teifke JP, Globig A, Harder T, Mettenleiter TC, et al. “Pathogenicity of highly pathogenic avian influenza virus (H5N1) in adult mute swans.” Emerging Infectious Diseases 14 (2008): 1267-1270.

14) United States Department of Agriculture. (2008). National Poultry Improvement Plan. Retrieved August 2008, from: http://nsu.aphis.usda.gov/inventory/activity.faces?INVENTORY_NUMBER=100

15) Fauci, Anthony S. “Pandemic Influenza Threat and Preparedness.” Emerging Infectious Diseases 12 (2006): 73-77.

16) “New Ferret Model May Measure Pandemic Potential Of H5N1 Influenza Viruses In Humans.” Science Daily 17 July 2007, from: http://www.sciencedaily.com/releases/2007/07/070716191800.htm

17) Fox, Maggie. “New Bird Flu Threat Could Be H9N2, Researchers Say.” Reuters. Reported on August 12, 2008.

APPENDIX-A GAINS Forms

Census Form

Date (dd/mm/yy:	**Location**	**Site**	**Recorder(s)**	**Time** (real count starts and ends)	
Nearest timeslot:	6-9	9-12	12-15	15-18	18-21
			Filled in sites sampled sheet?		YES /NO

	Species	**Counts (150m)**		
		Foraging	**Not Foraging**	**Flying over**
Grebe	Great crested grebe			
	Black-necked grebe			
	Little grebe			
Cor	Reed cormorant			
	White breasted cormorant			
	African Darter			
Pel	Great white pelican			
	Pink backed pelican			
HERON	Goliath heron			
	Purple heron			
	Grey heron			
	Black headed heron			
	Yellow billed egret			
	Great white egret			
	Little egret			
	Cattle egret			
	Squacco heron			
	Black heron			
	Black crowned night heron			
	Green-backed heron			
	Dwarf bittern			
	Little bitterm			
STORK	Black stork			
	Abdim's stork			
	White stork			
	Yellow-billed stork			
	Saddle billed stork			

	Species	Counts (150m)		
		Foraging	Not Foraging	Flying over
	African openbill			
	Wooly necked stork			
Fla	Greater flamingo			
	Lesser flamingo			
IBIS	African spoonbill			
	Hamerkop			
	Hadeda ibis			
	Glossy ibis			
	Sacred ibis			

	Species	Foraging	Not Foraging	Flying over
DUCK	Spur-winged goose			
	Egyptian goose			
	South African shelduck			
	Comb duck			
	White-faced duck			
	Fulvous duck			
	African pygmy-goose			
	Southern Pochard			
	Moccoa duck			
	Yellow-billed duck			
	African black duck			
	Mallard			
	Cape Shoveler			
	Northern Pintail			
TEAL	Garganey			
	Cape teal			
	Red-billed teal			
	Hottentot teal			
COOT	Red-knobbed coot			
	Common moorhen			
	Lesser moorhen			
	African purple swamphen			
	Allen's gallinule			
	Black crake			
	African jacana			
	Lesser jacana			
	African rail			
WADERS	Black winged stilt			
	Pied avocet			
	Common ringed plover			
	Three banded plover			
	Kittlitz's plover			
	Blacksmith lapwing			
	Afr. black oystercatcher			
	Ruff			
	Curlew sandpiper			
	Little stint			
	Common sandpiper			
	Wood sandpiper			
	Common greenshank			
	Marsh sandpiper			
	African snipe			
GULLS TERNS	Cape Gull			
	Caspian Tern			
	Grey-headed gull			
	Hartlaubs gull			
	Swift tern			
	Sandwich tern			
	Common tern			
	White winged tern			
	Whiskered tern			

	Species	Foraging	Not Foraging	Flying over
Additional species	Fish eagle			
	Pied kingfisher			
	Common starling			
	Cape wagtail			
	Car disturbance	Time(s) of disturbance:		
	Person (walking)	Time(s) of disturbance:		
	Aeroplane or helicopter	Time(s) of disturbance:		
	Predator (if birds react)	Time(s) of disturbance:		
	Other (specify)	Time(s) of disturbance:		

Data entered in file? **Back-up on paper?** **File: birdcount**

Bird Capture Form

SA-GAINS: BIRD FORM Master Capture Form Sheet # ☐ Bird# ☐

Ringer:																				
Swabber:																				
Recorder:																				
Handler:												**Capture Time:**							**am/pm**	
Date:	D	D		/	M	M	/	**2**	**0**	**0**	**7**	**Ringing Time:**							**am/pm**	
Location:	BAR		CHU		GOR		MAK		NGA		STR		ZW			Other:				
Site:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Species:																				
Ring Nr:																				

Recapture?	Y	N	Trapping Source:	Walk-in trap			
Euthanised?	Y	N		Mist Net			
Other Markings?	Y	N	Details if Y (e.g., colour ring #):				

1. Metrics	Mass (g) [bird+bag - bag = mass]				
	Age	Adult ☐	Juvenile ☐	not sure ☐	
	Sex	Male ☐	Female ☐	not sure ☐	
	Forewing length (mm)				
	Moult status (Ducks P1-P10, P1 nearer body)				
	Culmen (upper bill) length (mm)				
	Head length (mm)				
	(short) Tarsal length (mm)				

2. Health	Eyes and Nostrils	Discharge	Y	N	Details if Y:	
	Trap Damage	Y	N	Details if Y:		
	Beak area/tongue	Lesions or sores	Y	N	Details if Y:	
	Cloaca	Clean ☐	Moderately soiled ☐	Heavily soiled ☐		
	Condition	1 (worst)	2	3	4	5 (best)
	Other Notes:					

3. Samples	Cloacal swabs	Vial Number (e.g., BAR0001C1)			C1
		Vial Number (e.g., BAR0001C2)	"		C2
	Tracheal Swabs	Vial Number (e.g., BAR0001P1)	"		P1
		Vial Number (e.g., BAR0001P2)	"		P2
	Blood Samples	Vial Number (e.g., BAR0001B1)	"		B1
		Vial Number (e.g., BAR0001B2)	"		B2
	Feather Samples	Label with ring number and date and tick box once collected: ☐			
	Blood smear	Slide number:	Serum Sample ID (if taken):		

4. Ectoparasites	Checked for		Present		If present and collected, label with ring number and date - write label in pencil on paper and insert paper into vial with specimen.
	Collected		Absent		

5. Photos	Camera details (owner, card#):	
	1: Profile (side view, close up) of head	
	2. Profile (side view) of whole bird, wings closed	
	3. Back view of whole bird, one wing open	
	4. Front view of whole bird, one wing open	
	5. Other (specify):	
	6. Other (specify):	
	No photos (no camera, or flat battery)	

6. Additional Notes on back of form? Yes ☐ No ☐

APPENDIX B - National Zoological Information

Necropsy Protocol

- Sample any ectoparasites (in 70% alcohol)
- Disarticulate a hip joint, expose and sample for formalin the sciatic nerve and section a long bone for bone marrow (if there is none in the femur, check the tibia or toes).
- Make a blood smear from the femoral vein if the blood is not clotted.
- Place the bird on its back and open the skin from the beak to the vent.
- Make a horizontal cut at the bottom edge of the keel bone extending on each side through the muscles and then remove the keel bone, cutting the muscle and bony attachments.
- Take samples from the air sacs and heart for histology.
- Take and ice samples from the liver for histology.
- Find the spleen at the junction of the glandular and muscular stomachs. Take a sample for histology.
- Examine the esophagus, crop, proventriculus and gizzard, intestine and pancreas; sample and place on ice for histology.
- If it is a chick, sample the bursa of fabricius for histology
- Take the thyroid glands (at the base of the neck above the heart) for histology and the thymus which runs along the neck with the prominent subcutaneous veins.
- Dissect the lungs away from the body wall and take samples for histology.
- Sample gonads (two testes in the male or one ovary in the female) which are located at the top of the kidneys; and sample adrenal glands (located just below the gonads and attached to the body next to the spine).
- Sample kidneys for histology.
- Remove a section of skull covering the brain, and place it in formalin. In small birds the brain is best exposed by a sagittal section through the skull.

Necropsy Report Form

NATIONAL ZOOLOGICAL GARDENS

NECROPSY FORM

PATHOLOGIST: Dr E Lane	DATE CONDUCTED	PM NO

ANIMAL INFORMATION

SPECIES	ID	ISIS NO
POST MORTEM CHANGE: MILD MODERATE SEVERE		BODY WEIGHT

MACROSCOPIC FINDINGS

BODY CONDITION: Excellent Good Fair Poor Emaciated

general changes
skin
lung
heart
liver
spleen
kidney
adrenal
gastrointestinal tract
intestine
testis/ovary
brain
bone/marrow
joint
lymph node
tongue
trachea
salivary gland
thymus

SAMPLES SUBMITTED

Sample	*Comment*	*Date sent*	*Technician*
Blood smear			
Faecal flotation			
WBRC			
Bacteriology			
Stored			
Dr Manger			
Photographs			

APPENDIX C - Diagnostic Procedures

MagNA Pure Isolation

To perform MagNA Pure extractions, the samples must first be prepared for diagnostics. If samples were frozen, they must be thawed to room temperature. The buffer liquid is extracted with a pipette under a ventilating hood and the liquid is placed into the MagNA Pure tray wells. The automated machine is set up with appropriate buffers and then the samples are run.

Trizol Isolation

To perform Trizol extractions the following procedure is used;

1. Add 750ul Trizol to 250ul Allantoic fluid, pipette to break cells
2. Incubate at room temperature for 5 min
3. Add 200ul chloroform and shake vigorously by hand for 15 sec
4. Incubate at room temperature for 10 min
5. Centrifuge for 15min, 13000 rpm at 4 degrees Celsius
6. Transfer upper phase to a clean tube
7. Add 500ul isopropanol
8. Incubate at room temperature for 10 min
9. Centrifuge for 10 min, 13000 rpm at 4 degrees Celsius
10. Remove supernatant
11. Wash with 1ml 75% ethanol, vortex and centrifuge for 5 min at 10,000rpm at 4 degrees Celsius.
12. Air/vacuum dry pellet; dissolve in RNAsa free water; 40ul pure water.