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IMMUNE RESPONSE AND PATHOGENICITY OF ZIKA VIRUS IN EXPERIMENTALLY INFECTED CHICKENS

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ABSTRACT

Zika Virus (ZIKV) is a member of the family *Flaviviridae* and causes neurological disease in humans characterized by irreversible brain damage in infants and peripheral nerve damage in adults. Mosquitoes transmit ZIKV, but reservoir hosts remain undefined. West Nile virus (WNV), another closely related member in the *Flavivirus* genus, is known to infect more than 300 species of birds. Similarity between ZIKV and WNV in the ability to cause neurological disease raises the question of whether ZIKV can also infect bird species such as chicken. In order to investigate the susceptibility of chicken to ZIKV and thereby potentially act as a ZIKV reservoir, we carried out experimental infection of chicken with ZIKV (strain PRVABC59). Based on ZIKV infection studies in mouse, we hypothesized that newly hatched chickens may be more susceptible to ZIKV infection than juvenile or adult birds. Three groups of different age chickens (1 day, 5 days, and 6 weeks) were infected at varying viral titers subcutaneously into lateral neck to mimic a mosquito bite. Birds were sacrificed at 2, 16, or 23 days post-infection (dpi) for day-old infected birds, 5, 10, 14, and 21 dpi for 5-day old birds, and 1, 2, 3, 5, 7, 10, and 16 dpi for 6-week old birds in order to form a complete time-course of an infection and viral clearance. Plasma, vitreous humor, and tissues including brain, crop, lung, heart, spleen, kidney, pancreas, duodenum, eye, and liver were tested for viral RNA presence using qRT-PCR. While no clinical symptoms of disease were observed, one-day-old chicks were permissible to viral infection, as virus was detected in plasma and various tissues. No virus was detected in 5-day or 6-week old birds. Anti-ZIKV antibody in serum was determined via an ELISA assay. One-day and 5-day birds seroconverted in a dose-dependent manner by 10 dpi or 2-3 weeks post-infection (wpi), while 6-week old birds failed to seroconvert by 2 wpi. Age-associated immunity may play a role in young birds failing to clear ZIKV immediately, as in 5-

day and 6-week birds. In summary, chickens were found to rapidly clear virus following subcutaneous infection with ZIKV, indicating that chickens are unlikely to serve as a natural reservoir for this virus.

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Chapter 1

Introduction

Zika Virus

Zika Virus (ZIKV) is a positive-sense RNA virus in the family *Flaviviridae*, the same family as several other vector-borne arboviruses such as Dengue (DENV), West Nile (WNV), and Yellow Fever (YFV). ZIKV is transmitted by *Aedes* mosquitos (*Aedes aegypti* and *Aedes albopictus*), which live in tropical, subtropical, or temperate climates. ZIKV can be classified into one of two lineages, African or Asian, based on phylogenetic analysis. Mature ZIKV particles have an icosahedral capsid shell and are enveloped. The translated ZIKV polyprotein makes up 3 structural segments (membrane, envelope, and capsid proteins) and 7 non-structural segments (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (1). Structural segments make up the virus particle while non-structural components are involved with viral genome replication, viral entry, and manipulation of the host-response. Notably, the NS5 protein has been discovered to agonize the Type I Interferon pathway in humans, preventing antiviral response by targeting STAT2 for proteasomal degradation (2).

History

ZIKV was first discovered in 1947, in the Zika Forest in Uganda in an infected Rhesus monkey (3). Human ZIKV infections were first detected in Africa (strain IbH 30656), but had

spread to Asia by the 1950's. Both African and Asian lineages of the virus typically have caused clinically mild symptoms. The first substantial ZIKV epidemic occurred in Micronesia in 2007. Symptoms included fever, skin rash, arthralgia, and conjunctivitis (4). Following the Micronesia outbreak, an outbreak in French Polynesia occurred and was linked to the Guillain-Barré Syndrome (GBS), the first ZIKV epidemic to cause neurological symptoms (5). However, recent ZIKV epidemics in Puerto Rico, Brazil, and Columbia in the 21st century have been linked to even more severe adverse effects, including microcephaly in infants and GBS in adults (6). The severe impact that ZIKV can have on the human nervous system is alarming, especially due to the global spread of ZIKV (Figure 1).

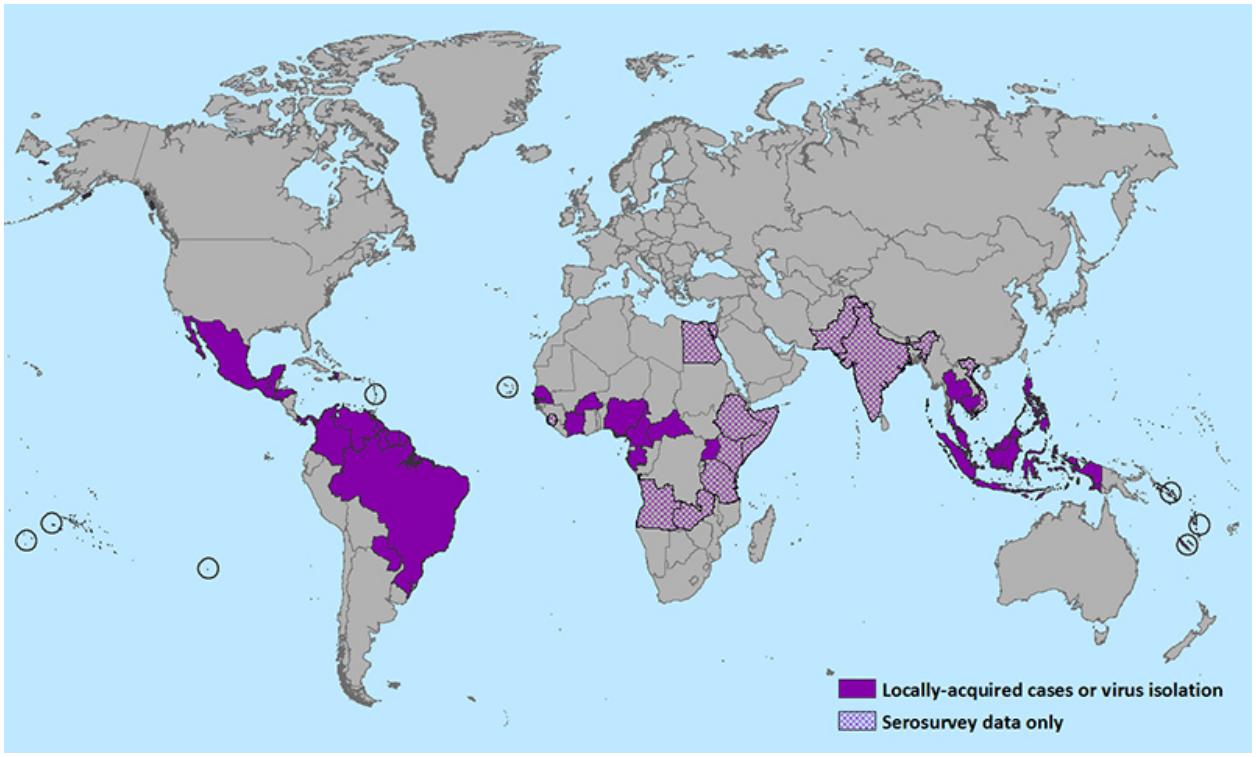

Figure 1. World map with ZIKV reported cases or serologically positive detection of ZIKV antibodies (7)

Transmission

While the typical transmission route of ZIKV is through the bite of an *Aedes* mosquito to a human or primate host, ZIKV has recently been shown to be sexually transmissible in humans as well, adding to the complications of the disease (Figure 2) (8). ZIKV RNA can be detected in blood, urine, semen, vaginal secretions, amniotic fluid, tears, and nasal discharge. While most healthy individuals clear the infection with no symptoms or a slight rash, ZIKV RNA may persist in individuals for a longer period of time. Findings have shown that ZIKV RNA is cleared in the majority of men by 3 months after infection (9), however, in some individuals the ZIKV RNA may persist for up to 6 months (10).

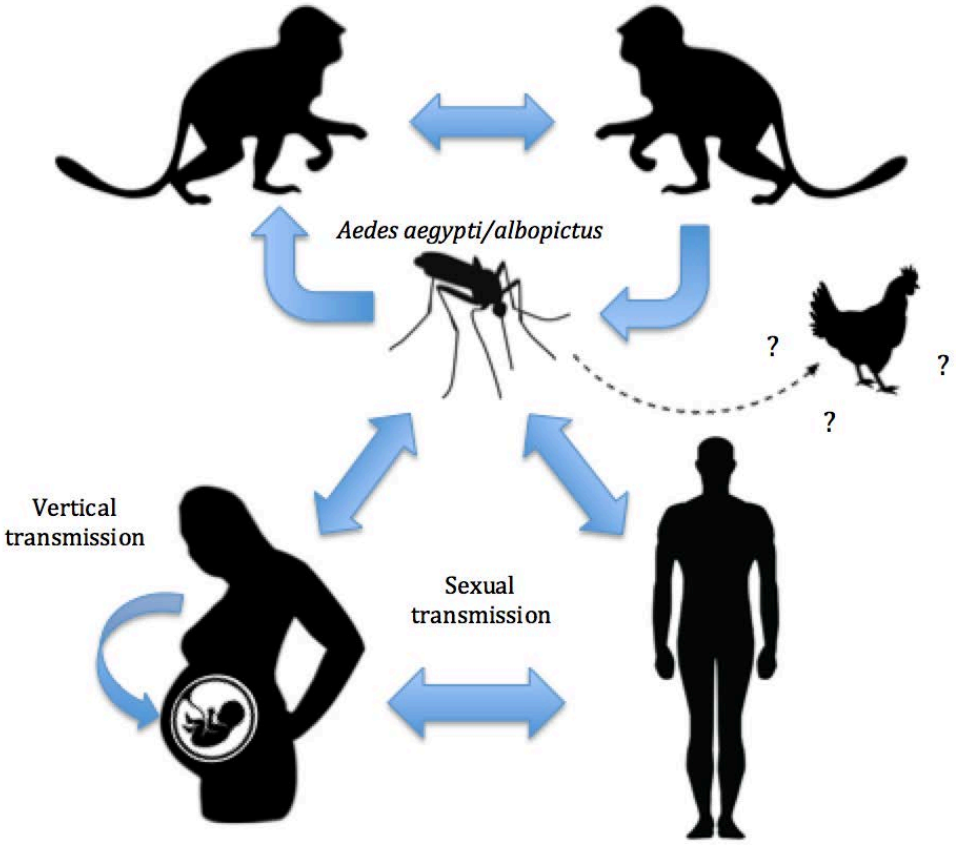

Figure 2. ZIKV Transmission Cycle

Vertical transmission of Zika Virus from infected mother to vulnerable fetus has been well documented, and can lead to the development of microcephaly and result in brain damage (11). Pregnant women are at particular risk for developing infections due to the nature of pregnancy in suppressing the immune system to allow for fetal growth (12). This natural suppression of a mother's immune system is taken advantage of by viruses, which allows for consequential spread of Zika virus to the fetus. Of the 1,673,272 cases reported during the 2015-2016 Brazil outbreak, 41,473 (2.5%) were of pregnant women. Concurrently, 1,950 cases of microcephaly were recorded during this period, an alarming increase from previously reported cases. This increase has been attributed to ZIKV infection and vertical transmission from mother to fetus (13).

The severities of such adverse health-complications such as microcephaly and Guillain-Barré Syndrome have driven the recent burst of research on Zika Virus. Due to the close living quarters of humans and animals in many parts of the world, investigating the potential viability of productive infection in wild and domestic animals as either an accidental host or as a reservoir is important to understand ZIKV, as well as any further implications that ZIKV may have.

Statement of the Problem

As ZIKV can cause severe teratogenic effects, studying the transmission and hosts of ZIKV is essential. While it is well established that ZIKV productively infects both monkeys and humans (Figure 2), there is still potential for additional animal hosts outside of the sylvatic transmission cycle. Various non-human primate models (NHPs) and murine models have been

investigated for ZIKV; however, only a few studies have been done with animals outside of NHPs and murine models to search for potential reservoirs of ZIKV (14–16).

With the close relation of ZIKV to West Nile Virus (WNV), which kills many wild birds and infects poultry, it is possible that ZIKV could infect chickens. While WNV typically does not cause clinical disease in poultry, they do serve as sentinel species for the disease. A recent study by Goodfellow *et al.* investigated the effects of infecting chicken embryos with ZIKV, and they discovered embryonic mortality at high dose infection and microcephaly at low doses. The chick embryos produced a productive infection with increasing viral replication in relation to the amount of viral particles administered (15). Due to this finding, our study expands on these previous findings to investigate ZIKV infection in juvenile and adult chickens for ZIKV infections. We hypothesized that neonates would be more susceptible to ZIKV infection in comparison to adult chickens, as their immune systems and nervous systems are underdeveloped in comparison. Investigation of clinical disease, transmissibility, and anti-viral host responses in chickens was evaluated to determine if a chicken host transmission pathway might pose a threat to both the poultry industry and human health.

Chapter 2

Methods

Test Animals

Infection of layer (white Leghorn) chickens at ages of 1 day, 5 days, and 6 weeks was performed with a subcutaneous injection into the neck to mimic the bite of a mosquito. Some intracranial injections of ZIKV were performed as well. Several trials were conducted utilizing a combination of different bird ages at time of infection, dose of virus injected, and days between injection and sacrifice. Control groups and experimentally infected groups of birds were utilized throughout each experiment (Table 1). Group A: 6 week old birds at 10^3 pfu/mL, Group B: 5 day old birds at 10^3, Group C: 6 week old birds at 10^5 pfu/mL, Group D: 5 day old birds at 10^3 and 10^5 pfu/mL, Group E: 6 week old birds at 10^5 pfu/mL, Group F: 5 day old birds, at 10^5, 10^6, or 10^7 pfu/mL, Group G: 1 day old birds at 10^5, 10^6, or 10^7 pfu/mL. Mock-infected birds were injected with DMEM. Birds were monitored daily for symptoms of clinical disease including neurological symptoms, rash, and conjunctivitis.

Table 1. Experimental groups with dosages, age of birds at inoculation, number of birds per group, and number of days post infection (dpi) at sacrifice date

Test Group	Age at Inoculation	Viral Inoculation Amount (pfu/mL)	Number of Birds Sacrificed at Days Post Infection (dpi)									
			1	2	3	5	7	10	14	16	21	23
A	6 week	10^3 *				4		5				
		mock				4		4				
B	5 day	10^3				4		5				
		mock				4		4				
C	6 week	10^5				4		4				
		mock				4		4				
D	5 day	10^3				4		4				
		10^5				4		4				
		mock				4		4				
E	6 week	10^5	3	3	3		4			4		
		mock	1	1	1		4			4		
F	5 day	10^5							3		3	
		10^6							3		3	
		10^7							3		3	
		mock							3		3	
G	1 day	10^5								3		3
		10^6								3		3
		10^7		2						3		3
		mock								3		3

*virus strain PRVABC59 was used in all cases except A, in which strain IbH30656 was used

Infection and Virus Titer

The ZIKV strain of interest used for infections was PRVABC59 (PRV). PRV is an isolate from a human ZIKV infection in Puerto Rico in 2015. Chicken infection trials were conducted using virus titers of 10^3, 10^5, 10^6, or 10^7 pfu/mL to determine if inoculation dose had an effect on viral replication, clinical symptoms, or immune response.

Blood and Tissue Collection and Processing

Immediately after euthanizing the chickens with CO_2, blood samples were collected from the test subjects by performing a cardiac puncture. Both Serum and plasma samples were collected. To yield plasma, the blood was collected into tubes coated with K2-EDTA, an anticoagulant. For serum, blood was collected into uncoated tubes. Blood collected into uncoated tubes was allowed to clot for 1 hour at room temperature. Serum and plasma were separated from other blood components by centrifugation at 1500 x g at 20°C for 5 minutes. The top liquid layer after centrifugation of each tube was collected and stored at -80°C. Liquid from K2-EDTA tubes yielded plasma, and uncoated tubes provided serum.

Tissues were collected from the liver, lung, spleen, pancreas, kidney, duodenum, brain, eye, heart, and crop of chickens. Tissues were placed in RNALater to stabilize and preserve RNA in each tissue. Tissues remained in RNALater for 24 hours, after which time RNALater was removed and all tissues were stored at -80°C.

Tracheal and Cloacal Sample Collection

Tracheal and cloacal samples were collected using sterile polyester-tipped plastic-handled swabs following the USDA procedure (17). Immediately upon collection, swabs were placed in 0.5 mL of sterile brain-heart infusion broth (product T1158 from Northeast Laboratory) and stored at 4°C until testing. Swabs were also collected on select subjects post-euthanization.

RNA Extraction

RNA extractions from tissues and blood were performed. Tissues were first placed in appropriate proportions of PBS (mass (g) x 5 → µl), and homogenized by gentleMACS™ Dissociator (M tube RNA 2.1) for RNA isolation. Tissue homogenates were then centrifuged at 1000xg for 5 minutes at 4° C, and the remaining supernatants were collected for RNA extraction. RNA was extracted from samples using Applied Biosystems® 5X MagMAX™ Pathogen RNA/DNA kit. The Whole-Blood protocol was followed for extraction of all tissue samples using 100 µl of sample. The low-cell content protocol was followed for plasma, serum, vitreous humor, and tracheal and cloacal swabs using 300 µl of sample. RNA was eluted in 90 µl elution buffer. Extracted viral RNA was stored at -80°C until used for viral RNA detection using Real Time qRT-PCR.

Real Time qRT-PCR

Detection of ZIKV RNA from extracted viral RNA from plasma, tissue samples, and swabs was performed using SuperScript® III Platinum® One-Step qRT-PCR Kit on a Thermo Fisher 7500 Fast Real-Time PCR machine . ZIKV-Dual-forward primer (5’ ATA TCG GAC ATG GCT TCG GA 3’), ZIKV-IbH-reverse primer (5’-GTTCTTTTACAGACATATTGA GTGTC-3’), and ZIKA-Dual-probe (5’ 6-FAM-TGCCCAACA/ZEN/C-AAGGTGAAGCCTAC CT-Iowa Black® FQ 3’) were used (18).

The PCR reaction master mix was created for a total volume per well of 25 µl consisting of 4.5 µl H_2O, 0.5 µl ZIKV-Dual-forward primer (40 µM), 0.5 µl ZIKV-IbH-reverse primer (40

μM), 0.5 μl ZIKV-Dual-probe (10 μM), 1 μl Rox (diluted 1:20), 12.5 μl 2X Reaction Mix, and 0.5 μl Platinum Taq DNA Polymerase with the addition of 5 μl of sample.

ZIKV Standard Curve was created by extracting PRVABC59 RNA using the Applied Biosystems® 5X MagMAX™ Pathogen RNA/DNA kit and the whole blood protocol using 100 μl of virus with $TCID_{50}$ of $4.22x10^7$ (pfu/mL of $2.954x10^7$) and eluted in 90 μl of elution buffer. After extraction, serial dilutions of 1:10 in nuclease-free water were made to create the standard curve. The standard curve was generated using SuperScript® III Platinum® One-Step qRT-PCR Kit on a Thermo Fisher 7500 Fast Real-Time PCR machine as described above for samples.

Detection of ZIKV specific antibodies in Infected chicken

Enzyme-linked immunosorbent assay (ELISA) is a commonly used method for antibody detection and quantification. As no commercial product is available for detection of anti-Zika chicken antibody, we designed and optimized an in-house assay. Antibody detection was performed via a direct ELISA using the serum of chickens. ZIKV PRVABC59 at (1:800 dilution (from $4.22x10^7$ $TCID_{50}$), 100 μl) diluted with bicarbonate/carbonate buffer (pH 9.4) was first coated to a NUNC™ MaxiSort™ 96-well plate and incubated overnight at 4°C. Plates were washed (3x for 5 minutes) with 200 μl wash buffer (Dulbecco's phosphate buffered saline and 0.2% TWEEN 20) and then blocked with blocking buffer (wash solution and 2% bovine serum albumin). Plates were incubated overnight at 4°C. Chicken serum samples were coated onto the plates (1:50 dilution, 100 μl) and incubated for 1 hr at room temperature. Plates were washed (3x for 5 minutes each) with wash solution. A secondary detection antibody, Anti-chicken rabbit antibody conjugated to alkaline phosphatase (AP), was added to plates (1:20,000 dilution, 100

μl) and allowed to incubate for 1 hr at room temperature, after which time the plates were washed (6x for 5 minutes each) to remove any unbound secondary antibody. Phosphatase substrate (5 g tablet) was added to 5 mL solution of 10% diethanolamine buffer, pH 9.8, with .01% $MgCl_2$ and 0.02% NaN_3. To each well, 50 μl of this substrate solution was added and allowed to react with the AP-conjugated antibody for 30 minutes. The presence and quantities of chicken IgY antibody against ZIKV were then detected using a plate reader at 405 nm.

Optimization of the ELISA assay involved combinations of antigen (ZIKV), serum, and secondary antibody dilutions to determine the readings that yielded greatest significant change from injected birds and control serum (Appendix 1).

Analysis

Microsoft Excel was used for creation of ZIKV Standard Curve graph, and calculation for ZIKV positive tissues were calculated in Excel. ELISA Data were analyzed for statistical significance using a two-tail t-test and graphs were made using GraphPad Prism 7.

Chapter 3

Results

Zika Virus detected in specific tissues using Real Time qRT-PCR

To determine the presence of ZIKV in chicken tissues, qRT-PCR was used. Tissues from each bird were pooled and tested for ZIKV presence. Plasma, vitreous humor, and tissue pools from each bird were tested; positive testing pools were then tested tissue by tissue. Using this method, virus was detected in the plasma, crop, liver, kidney, and brain of the two day-old birds at 2 dpi. These juvenile birds showed appreciable amounts of ZIKV RNA in their plasma, crop, liver, kidney, and brains (Table 2). Positive tissues were run with a ZIKV standard dilution curve to determine the relative amount of virus in each tissue tested. Figure 3 shows positive samples of brain, crop, liver, and kidney CT values plotted along the standard curve, presenting a logarithmic scale of virus levels in each tissue at the measurement of pfu/mL of tissue homogenate. All other samples, excluding day-old chickens at 2 dpi, were negative for ZIKV in plasma and tissues (Figure 4 and 5), as well as tracheal and cloacal swabs.

Table 2. Samples Testing Positive for Zika Virus via qRT-PCR

Test Subject	Tissue Type	CT Value
G11 (1-day old at infection, sac 2 dpi)	Plasma	36.257
	Crop	31.953
	Liver	36.236
	Kidney	40.403
G12 (1-day old at infection, sac 2 dpi)	Plasma	36.938
	Crop	34.216
	Liver	42.051

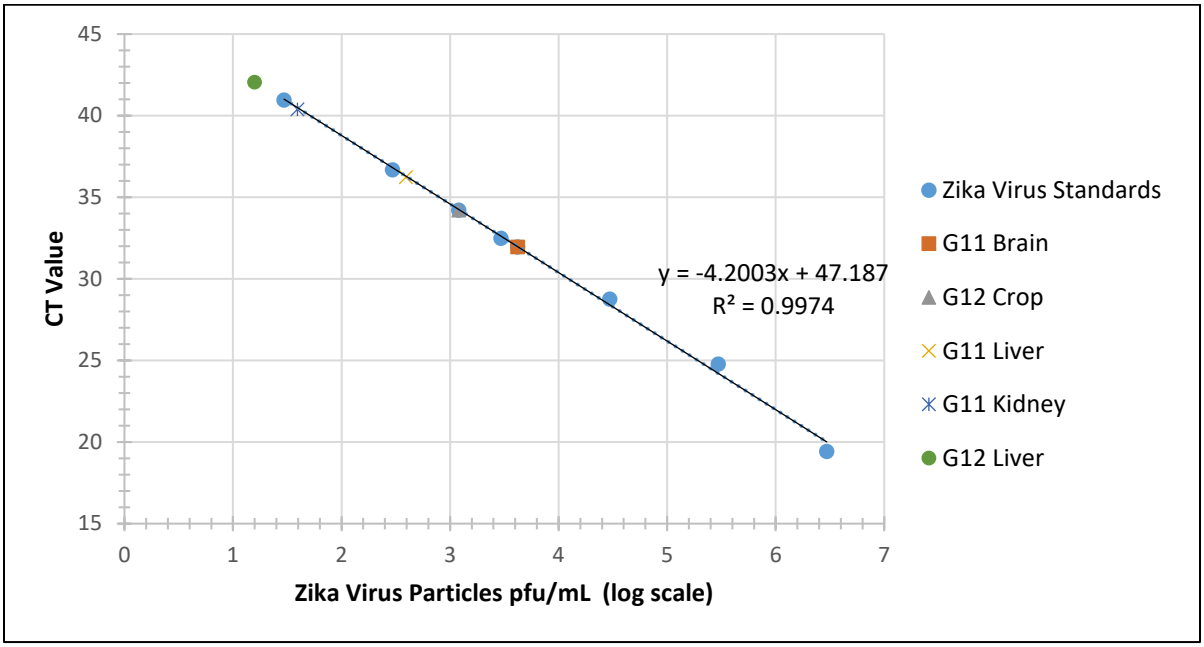

Figure 3. ZIKV (PRVABC59) Standard Curve with Positive Chicken Tissues.

Table 3. Positive qRT-PCR tissues with virus particles (pfu/g for tissues, pfu/mL per 300 µl plasma) calculated using a ZIKV Standard Curve of day-old birds at 2 dpi.

Tissue:	*Plasma*	*Crop*	*Liver*	*Brain*	*Kidney*	*Spleen*	*Eye*	*Lung*	*Heart*	*Duodenum*
G11	$4.02x10^2$	$7.38x10^4$	$5.20x10^3$	$1.45x10^3$	$8.42x10^2$	$4.71x10^2$	n.d	n.d	---	---
G12	$2.77x10^2$	$1.72x10^4$	$3.16x10^3$	n.d	---	---	n.d	n.d	n.d	n.d

*LOD=41pfu/mL (approximately 205 pfu/g for most tissue samples)

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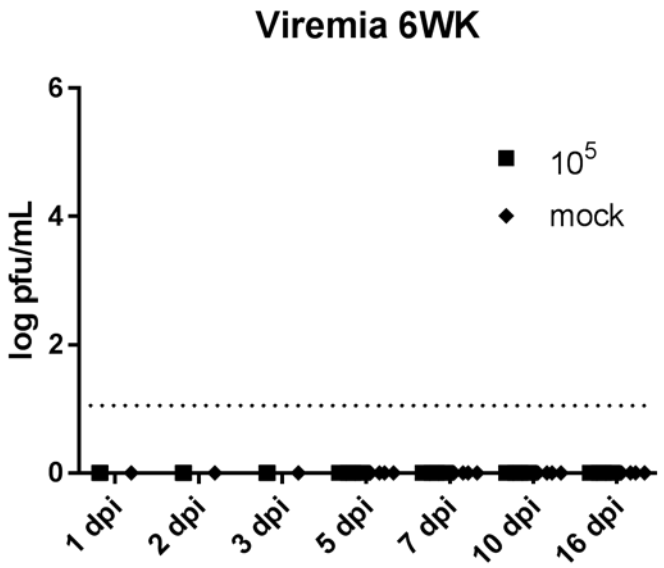

Figure 4. Viremia in 6WK birds was not detected in blood or any tested tissues.

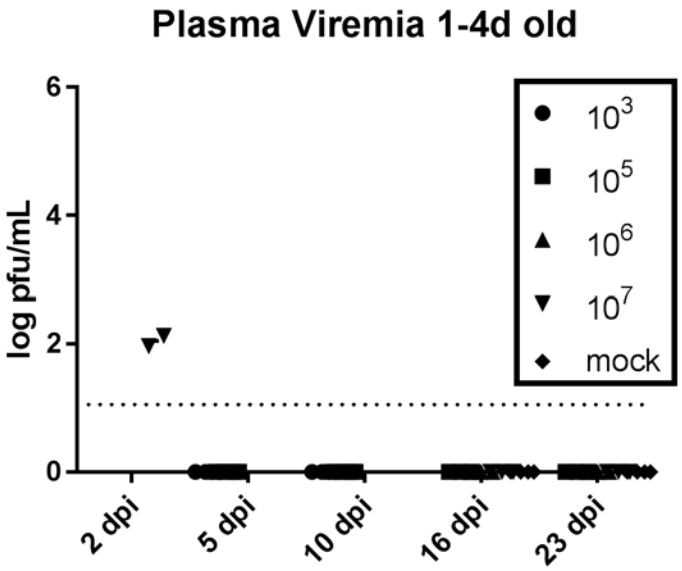

Figure 5. PCR Testing for plasma viremia was performed on all birds. Birds tested at 5, 10, 16, and 23 dpi showed no viremia in the blood. Viremia was detected in two day-old birds at 2 dpi.

ELISA

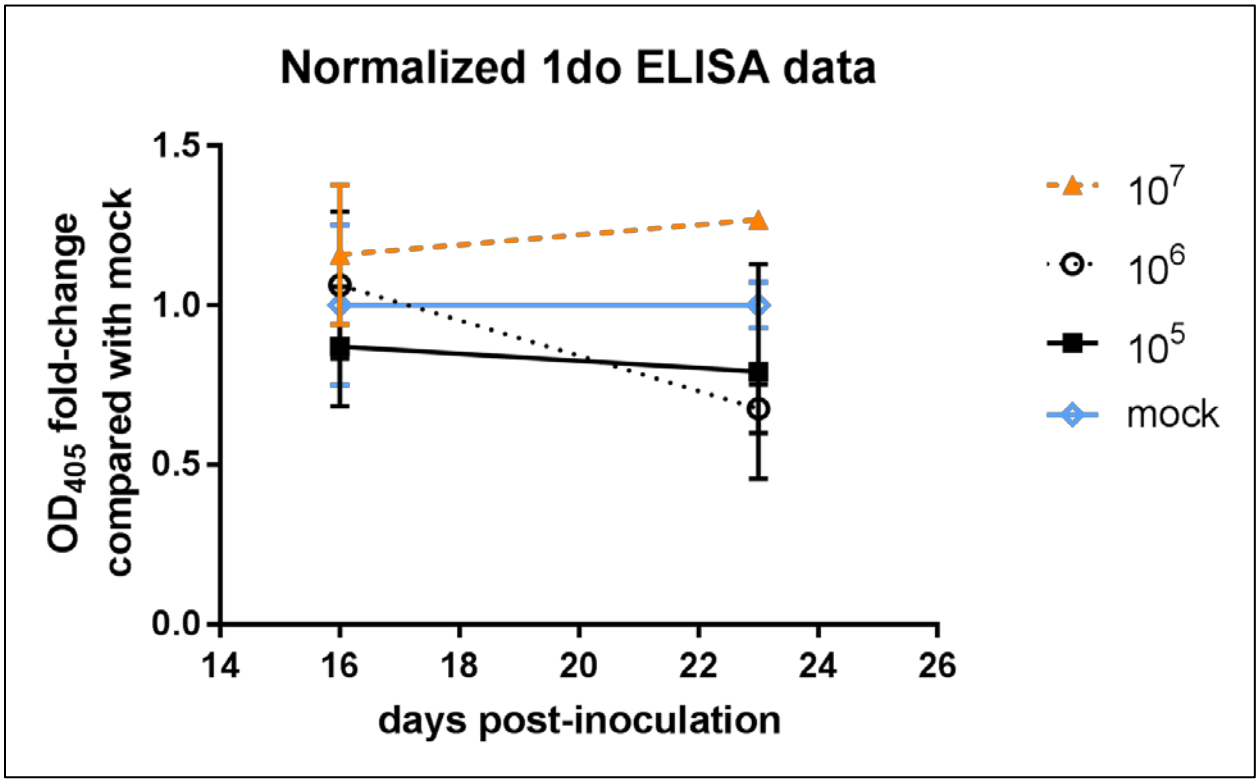

Figure 6. Normalized 1do ELISA data

Day-old birds were inoculated with varying doses of ZIKV: 10^5, 10^6 , and 10^7 pfu. Birds were sacrificed at 16 and 23 dpi. (Figure 6). A slight increase in antibody production was observed in 10^7 pfu-inoculated birds between 16 dpi and 23 dpi.

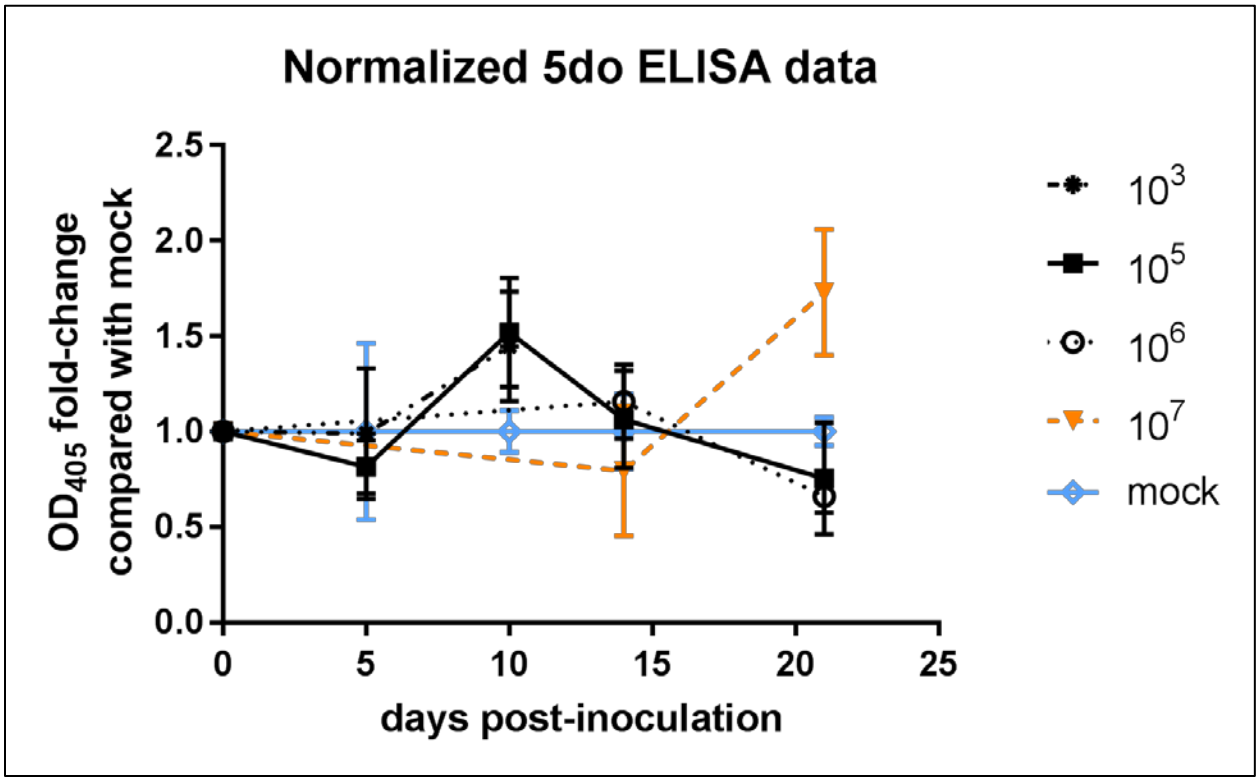

Figure 7. Normalized 5do ELISA data

Antibody response against Zika Virus in 5do chickens was seen starting at 10 dpi in 10^3 pfu- and 10^5 pfu-inoculated birds (Figure 7). Antibody response in 10^7 pfu-inoculated 5do chickens was seen in statistically significant amounts starting at 23 dpi. For 6-week birds, there was no detectable antibody generation found via ELISA.

Chapter 4

Discussion

ZIKV RNA Detection by qRT-PCR

A ZIKV Standard Curve along with positive testing qRT-PCR chicken tissue samples brain, crop, liver, and kidney from day-old infected birds at 2 dpi showing CT value (y-axis) and ZIKV pfu/mL in a logarithmic scale is shown in Figure 3. Samples run with ZIKV Standard Curve were further converted from logarithmic values of pfu/mL to pfu/g of tissue tested (calculation shown below). ZIKV particle values (pfu/g) are shown in Table 3.

Conversion of Positive PCR Samples values from pfu/mL to pfu/g :

$$\frac{\text{(pfu units per 300 µl of plasma/mL homogenate extracted) x (total homogenate volume (mL))}}{\text{tissue weight (g)}}$$

ZIKV RNA was detected in the plasma (Figure 5), brain, crop, liver, kidney, and spleen of day-old infected birds at 2 dpi (Figure 3). In 6-week old birds infected with 10^5 at 1 dpi, no virus was detected in any plasma or tissues. No virus was detected in plasma or tissues of 5-day or 6-week birds at any time-point (Figure 4), which supports the hypothesis that only young individuals with underdeveloped immune systems may be susceptible to infection, as murine studies have found (19). Age-associated immunity may play a role in very young chickens presenting virus in tissues while older birds did not have virus in blood or tissues, as they seem to clear the virus within 24 hours (Figure 4).

As the brain has been clearly linked as a site affected by Zika Virus infection in humans and primates, detection of viral RNA in the brain of chickens was expected. Zika is known to

cause microcephaly in infants infected in utero (6). Similarly, Guillain-Barré Syndrome affects the peripheral nervous system of adults. Evidence of ZIKV having neurological effects is strong. However, the mechanism by which ZIKV causes these effects has yet to be identified.

The crop is an integral part of the digestive system in birds, and serves as an extra pouch in the throat which is used for temporary storage and moistening of food (20). The crop showed an appreciable amount of viral RNA in day-old infected birds. Other studies have also detected Zika RNA in the crop of chickens (15). Presence of ZIKV in the crop might be attributed to the mucous secretions from the mouth and esophagus. However, the injection site was sub-cutaneous in the neck of chickens, which may play a role in the high levels of ZIKV in the crop due to the close proximity to the injection site.

Decreased liver function has been associated with some human ZIKV cases (21). ZIKV has been detected in the livers of infected mice as well, and ZIKV was found to cause liver inflammation (22) . There is a possibility that the liver contained ZIKV in young birds due to blood processing in the liver, as we did not remove blood from tested tissues. However, as other experiments have discovered ZIKV particles in the liver, this finding is most likely legitimate.

ZIKV RNA persists for a long time in human urine and is detectable for months after infection, explaining the ZIKV detection in the kidneys of chickens (9). While in this experiment we did not detect viral RNA in the pancreas, the pancreases of birds sacrificed after 16 dpi demonstrated notable redness compared to mock birds, which may be explainable by a systemic inflammatory response.

Viral Mechanisms for Evading the Innate Immune Response

Using human cell *in vitro* studies, researchers have been able to determine that when ZIKV infects human cells, the virus is able to evade the innate immune response by escaping the interferon antiviral response. The NS5 non-structural protein of ZIKV inhibits the induction of the Type I IFN signaling by impairing JAK/STAT signaling (23). Zika virus NS5 induces STAT2 degradation in humans, but does not induce degradation in mice. This explains why Type I IFN deficiency is required for ZIKV to cause infection in mice (19). We speculate that the reason why chickens did not become infected may because of this same mechanism, that chicken STAT2 is not recognized by the NS5 and thus does not prevent the Type I IFN antiviral response.

Antibody Detection via ELISA

Utilizing an ELISA to determine antibody generation in injected birds over time, it was determined that there may be a direct relationship between dose and time to antibody generation. In 5 day-old birds injected with 10^5, statistically significant antibody was detected at 10 dpi (Figure 7). After the peak at 10 dpi, antibody levels steadily decline. However, birds infected with 10^7 did not show antibody production at the earliest tested timepoint (16 dpi), and instead antibody levels peaked at 23 dpi. Peak antibody response of 10^7 birds was statistically significant at 23 dpi. We believe this relationship may be caused by the high levels of virus particles overwhelming the chicken's immune system, increasing the time to full adaptive response and antibody generation. The adaptive antibody response may not be developed as early as in birds receiving lower doses, as the birds may require more time for the innate immune response to

control the amount of virus. While 5 day-old birds and 1 day-old birds produced antibody against Zika Virus, 6 week-old birds did not. Taking into account the lack of detectable viral RNA in any tissue samples of 6 week-old birds, it is likely that the virus is quickly sequestered and cleared by innate immune cells and systems. If the virus is cleared quickly, there is no biological need for an adaptive immune response because the virus is neither infecting tissues nor causing clinical symptoms.

The lack of antibody formation against Zika Virus and undetectable virus levels even one day post-infection in 6 week-old birds (Figure 4) points to a clear difference between the immune responses of juvenile and adult chickens. Viral RNA is detectable in 1 day-old birds at 2-dpi, and injections of 10^5, 10^6, and 10^7 pfu generate adaptive antibody response in 1 day-old birds as well as 5 day-old birds.

This study was a preliminary study to determine the ability of chickens to become infected with ZIKV and their antibody response to the virus. A second study will be performed to confirm and to further characterize the interaction between ZIKV and day-old chickens. Gene expression of IFNα/β, IFNγ, MX1, OAS1, RIG-I, MDA5, IL-6, and IP-10 in infected and mock birds will be examined to determine up-regulation or down-regulation of anti-viral signaling pathways.

Chapter 5

Conclusion

While ZIKV is a clinically significant disease in humans and primates, we found that ZIKV caused no clinical symptoms in chickens in 1-day, 5-day, or 6-week old birds. ZIKV was detected in two day-old birds sacrificed at 2 dpi in plasma, brain, crop, liver, kidney, and spleen tissues using qRT-PCR. Virus was undetectable in both 5-day and 6-week old birds at all timepoints (5 – 21 dpi). Antibody detection by ELISA showed that 6-week old birds did not generate detectable antibody against ZIKV. Day-old and 5-day old birds generated antibody against ZIKV in a dose-dependent manner, with higher doses requiring longer time periods to highest detection level of antibody. Future studies will seek to confirm our preliminary findings, as well as determine upregulation and downregulation of innate immune system pathways that may play a role in chicken immunity to ZIKV. Chickens do not appear to have the potential to play a role in the ZIKV transmission cycle, as either a reservoir or accidental host. Additionally, ZIKV does not appear to possess a health threat to chickens or the poultry industry. However, there is much to be learned about the mechanism of ZIKV as well as anti-viral host responses through chicken studies.

Appendix 1

ELISA Optimization

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.195	0.144	0.166	0.148	0.139	0.092	0.167	0.186	0.176	0.174	0.091	0.127
B	0.236	0.19	0.194	0.171	0.149	0.115	0.188	0.189	0.17	0.163	0.167	0.109
C	0.316	0.29	0.27	0.254	0.186	0.125	0.228	0.245	0.222	0.206	0.171	0.137
D	0.396	0.373	0.304	0.272	0.215	0.143	0.35	0.253	0.248	0.206	0.244	0.244
E	0.724	0.642	0.595	0.471	0.408	0.266	0.57	0.555	0.506	0.45	0.422	0.173
F	1.163	1.232	0.954	0.908	0.823	0.522	1.104	1.114	0.692	0.774	0.686	0.608

Appendix Figure 1. ELISA Optimization absorbance values read at 405 nm. Dilutions were made using varying viral and antibody concentrations to determine optimal dilutions for the assay.

Columns 1-6 received G17 serum (infected day-old bird at 24 dpi) and Columns 7-12 received G46 serum (mock infected day-old bird at 24 dpi). Varying antigen dilutions (ZIKV) are done by column and varying antibody dilutions by row.

Row: Antibody Dilution	Column: Antigen Dilution (ZIKV)
A: 1:50,000	1 & 7: 1:1,600
B: 1:40,000	2 & 8: 1:800
C: 1:30,000	3 & 9: 1:400
D: 1:20,000	4 & 10: 1:200
E: 1:10,000	5 & 11: 1:100
F: 1:5,000	6 & 12: 1:10

Absorbance values from G17 were compared to G46 to determine the best combination of antigen/antibody dilutions. Using this optimization, we decided to use the 1:800 Ag dilution with the 1:20,000 Ab dilution for serum testing. This optimization was done with 1:10 diluted serum. A second optimization was performed to determine optimal concentration of serum to be tested, in which the chosen dilution was 1:50.

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ACADEMIC VITA

Margo Keller

EDUCATION

The Pennsylvania State University — **University Park, PA**
College of Agricultural Science, Schreyer Honors College — Graduated May 2018
- B.S. in Immunology & Infectious Disease
- Minors in Spanish and Biology

Spanish School for Foreigners — **Ronda, Spain**
- Studied Spanish language, culture, and conversation in Ronda — May 2016 – July 2016
- Experienced different cultures from various regions of Spain

Harrisburg Area Community College — **Lebanon, PA**
- Trained as a Certified Nursing Assistant — Graduated August 2015
- Pennsylvania State CNA Certification (#10076174) obtained Sep 9, 2015

Eastern Lebanon County High School — **Myerstown, PA**
- Class of 2014 — Aug 2010 - June 2014

LEADERSHIP & ACTIVITIES

Schreyer Honors College Student Council — **University Park, PA**
Primary THON Chair, Council Member — *Sept 2014 – May 2018*
- Raised support and awareness for THON, the largest student-run philanthropy in the world, which raised over $13 million to help fund treatment for children with pediatric cancer
- Served as an ambassador to the Honors College by giving tours to prospective honors students
- Participated in community service events including blood drives, Stop Hunger Now, and Arboretum Trail Cleanup
- Danced in THON 2018

Spanish Immersion Club — **University Park, PA**
Active Member — *Sept 2015 – May 2018*
Focuses on utilizing the Spanish language and immersing yourself in the language to help achieve a greater level of fluidity in learning speakers

Mission Trips to Costa Rica — **Cartago, Costa Rica**
Team Member — *June 2010 & July 2012*
Traveled to Costa Rica to help teach the English Language within both rural and suburban school communities, while additionally providing physical services to the community through painting houses

RESEARCH AND WORK EXPERIENCE

Undergraduate Research — **University Park, PA**
Laboratory Research, Dr. Suresh Kuchipudi Laboratory — *Sept 2016 – May 2018*
- Conducting scientific research centering around the virulence factors of flu strains

Nursing Assistant, WellSpan Good Samaritan Hospital, PRN — **Lebanon, PA**
- 2E Medical/Surgical Unit — *May 2017- Jan 2018*
- Performed nursing assistant duties by taking vitals, checking blood sugars, and aiding in activities of daily living

Dietary Aide — **Myerstown, PA**
- Part-Time Position — *Aug 2013- Aug 2014*
- Responsibilities include assisting with the preparation and service of resident meals.

Tasks include plating of food, delivery of trays or carts, operating the dishwasher, stocking of inventory items, and the cleaning of the kitchen and kitchen equipment.