Acknowledgments

My path toward higher education has been focused and efficient, but I would have never been successful without the love and support of my family. Since an early age, my parents taught me that any goal could be achieved, and by their example I chose to enter graduate studies at Virginia Tech. Their support and encouragement was valuable during times of frustration. My wife, Heather, never hesitated to assist me when I needed advice or help, although she was occupied with her studies in veterinary school during much of the past five years. It was her help that enabled me to make decisions about my future path without hesitation or regret.

The work performed in the laboratory was complex, confusing, and often frustrating. Without the help of skilled technicians such as Mary Mainous, Denis Guenette, Kay Carlson, and Gretchen Berg, such experimentation would never have been successful. The help provided by Lori Settle and other students enabled me to better handle the final stages of my research. Finally, without input and encouragement from my committee members, I would never have made it this far. They made me believe in myself even when I did not feel that I was accomplishing anything.

Thanks to all of the people who helped me and encouraged me to keep moving in the right direction. Now I have many decisions to make and many paths to choose from, but I will proceed with confidence knowing that I am ready to make a difference in the world.

Table of Contents

		Page
Abstract		ii
Acknowledge	ements	iv
List of Figure	s	ix
List of Tables	S	xi
List of Abbre	viations	xiii
Chapter 1: F	Review (of Literature
1.1	Scope	of the Problem
1.2	Adend	oviruses
	1.2.1	Taxonomy Overview
	1.2.2	General Mechanisms
	1.2.3	Common Genes
	1.2.4	Genus Siadenovirus 8
1.3	Turke	y Hemorrhagic Enteritis Virus
	1.3.1	Morphology9
	1.3.2	Characteristics of HE in Turkeys
	1.3.3	Strain Differentiation
	1.3.4	Virulent and Avirulent Strains
	1.3.5	Serologic Differences
	1.3.6	Sequence Differences
	1.3.7	Diagnostic Methods
1.4	Persis	tent and Latent Infection
1.5	Staten	nent of Hypothesis40
1.6	Refere	ences

Chapter 2: P	CR Methods for the Detection of Turkey Hemorrhagic Enter	ritis Virus
DNA		
2.1	Abstract	63
2.2	Introduction	64
2.3	Materials and Methods	65
	Standard PCR	66
	Competitive PCR	68
	Competitor Molecule Design	68
	Real-time Quantitative PCR	71
2.4	Results	72
2.5	Discussion.	75
2.6	References	80
Chapter 3: C	Complete Genome Sequence of the Virginia Avirulent Strain of	of Turkey
Hemorrhagio	c Enteritis Virus	
3.1	Abstract	82
3.2	Introduction	83
3.3	Materials and Methods	85
3.4	Results	87
3.5	Discussion	90
3.6	References	99
Chapter 4: Se	equence Comparison of ORF1, E3 and Fiber Genes from Tw	elve
Different Isol	lates of Turkey Hemorrhagic Enteritis Virus	
4.1	Abstract	104
4.2	Introduction	105
4.3	Materials and Methods	
4.4	Results	
4.5	Discussion.	115

4.6	References	124
Chanter 5: S	Sequence Comparison of Turkey Hemorrhagic Enteritis Viru	s and Frog
-	1: Prediction of Genetic Features of the Genus Siadenovirus	s and Frog
5.1	Abstract	128
5.2	Introduction	
5.3	Materials and Methods	
5.4	Results/Discussion.	
5.5	References	
Chanter 6: I	Persistent Infection of Turkeys with an Avirulent Strain of Tu	ırkev
-	ic Enteritis Virus	irkey
6.1	Abstract	150
6.2	Introduction	
6.3	Materials and Methods	
6.4	Results	
	Experiment 1	156
	Experiment 2	
	Experiment 3	
6.5	Discussion	
6.6	References	180
Chapter 7: (General Conclusions, Discussion, and Future Research	
7.1	Summary	183
7.2	Future Research	184
	Full-length Infectious DNA Clone	185
	Recombinant Live-virus Vaccine	187
	Transcriptional Mapping	192
	Comparison of Viral mRNA Levels	

7.3	Conclusions	194
7.4	References	195
Appendix A	A	
PCR Primer	Sequences	
Appendix B	3	
Creation of a	a Full Length Infectious DNA	
Clon	ne of THEV – Preliminary Work	198
Curriculum	ı Vitae	202

List of Figures

<u>Figure</u>	<u>Page</u>
Chapter 1	
1-1 N	Major Structural Features of the Adenoviridae
Chapter 2	
2-1	Agarose Gel from a Competitive PCR
2-2 (Competitor Molecule Creation
2-3 \$	Standard PCR Efficiency Calculation
2-4 (Comparison of cPCR and qPCR
I	Estimates of Genome Copy Number
Chapter 3	
3-1	Genetic Organization of the Virginia Avirulent Strain of THEV
Chapter 4	
4-1 I	Mutations in the Putative ORF1 Gene
4-2 I	Mutations in the Putative E3 Gene
4-3 I	Mutations in the Fiber-knob Domain
4-4 I	Phylogenetic Relationship Between Strains Sequenced
Chapter 5	
5-1 (Comparison of Sequences at the Genome Termini
5-2 \$	Spatial Layout of the Major Late Promoter
5-3 \$	Spatial Layout of the E3 Promoter
5-4 I	Features of the Fiber Protein
5-5 I	Features of the Putative E3 Protein
5-6 I	Features of the Putative ORF1 Protein
5-7 I	Features of the Putative ORF8 Protein

Chapter 6 6-1 Experiment One Percentage of PCR Positive 6-10 Experiment Two Percent PCR Positive 6-11 Experiment Two Effect of Dexamethasone Treatment Chapter 7 7-1 Alternative Method for the Creation of

X

Appendix B

List of Tables

<u>Table</u>	Page
Chapter 2	
2-1 PCR Primer Sequences.	67
2-2 Basic Thermocycler Program	67
2-3 Quantification of Viral Genomes in HE Vaccines	74
Chapter 3	
3-1 Sequencing PCR Primers	88
3-2 Siadenovirus Genome Comparison	89
3-3 Sequence Differences	91
Chapter 4	
4-1 Viruses Sequenced	107
4-2 Sequencing PCR Primers	109
4-3 Sequence Differences	111
Chapter 5	
5-1 Genetic Features of the Members of the Genus Siadenovirus	ıs 132
5-2 Comparison of Promoter Elements	136
Chapter 6	
6-1 PCR Primers	153
6-2 PCR Thermocycler Program	153
6-3 Experiment One PCR Results	157
6-4 Experiment Two PBL PCR Results	167
6-5 Experiment Two Tissue PCR Results	
6-6 Experiment Three Spleen-Body Weight Ratios	171

6-7 Experiment Three PCR results	
Appendix A	
PCR Primer Sequences	187
Appendix B	
B-1 PCR Primers with Restriction Endonuclease Sites	199

List of Abbreviations

aa – Amino acid

AAS – Aviadenovirus type II associated splenomegaly of chickens

AASV – Aviadenovirus type II associated splenomegaly virus

AGID – Agar gel immunodiffusion

bp – DNA base-pair

BTC – Basal transcription complex

cPCR – Competitive polymerase chain reaction

CsA – Cyclosporine A

DBP – DNA binding protein

Dex – Dexamethasone

DNA – Deoxyribonucleic acid

dpi – Days post-inoculation

E3 – Early region three (promoter); Siadenovirus-specific ORF

FrAdV-1 – Frog adenovirus 1

GFP – Green fluorescent protein

HE – Hemorrhagic enteritis

IBDV – Infectious bursal disease virus

IBV – Infectious bronchitis virus

INI – Intranuclear inclusion

INR – Initiator

IP – Intraperitoneal

IPA – Immunoperoxidase assay

ISH – *In situ* hybridization

ITR – Inverted terminal repeat

IV – Intravenous

IVS – Israel virulent strain (THEV)

kb – Kilobase-pair (DNA)

RP-19 – Chicken lymphoblastoid cell line used for THEV propagation in vitro

MLP – Major late promoter

mRNA – Messenger ribonucleic acid

MSD - Marble spleen disease of pheasants

MSDV – Marble spleen disease virus

MSV - Marble spleen vaccine

NDV – Newcastle disease virus

NOIF – Nitric oxide inducing factor

nt – nucleotide

ORF – Open reading frame

ORF1 – Siadenovirius-specific ORF, known as sialidase

PBS – Phosphate buffered saline

PCR – Polymerase chain reaction

pHEV1 – Plasmid HEV 1, containing 400 bp fragment of hexon gene

pHEV2 – Plasmid HEV 2, competitor molecule

pi – Post-inoculation

qPCR – Quantitative polymerase chain reaction

RNA – Ribonucleic acid

RT-PCR – Reverse transcriptase polymerase chain reaction

TBP – TATA binding protein

TCID₅₀ – Tissue culture infectious dose 50%

THEV – Turkey hemorrhagic enteritis virus

THEV-A – Avirulent turkey hemorrhagic enteritis virus

THEV-V – Virulent turkey hemorrhagic enteritis virus

TID₅₀ – Turkey infectious dose 50%

T_m – Melting temperature

TNF – Tumor necrosis factor

VAS – Virginia avirulent strain (THEV)

VVS – Virginia virulent strain (THEV)