THE PENNSYLVANIA STATE UNIVERSITY
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IDENTIFICATION OF THE EFFECTS OF SE AND VITAMIN E DEFICIENCY ON THE SEVERITY OF COCCIDIOSIS AND CONCURRENT BOVINE VIRAL DIARRHEA AND CRYPTOSPORIDIUM INFECTIONS IN CATTLE

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ABSTRACT

Coccidiosis is an infection characterized by the excretion of watery feces that is common in young calves between the ages of 1-2 months and 1 year. The clinical disease is not usually seen within the first three weeks of life. In cattle, this disease can be caused by three different types of *Eimeria* species - *E. zuernii, E. bovis,* and *E. auburnensis*. Pathogenic coccidia often cause enteritis in the large intestine. However, they can damage the mucosa of the lower small intestine, colon, or cecum. This calf diarrhea, also known as calf scours, is a major cause of economic and productive loss to cattle producers worldwide. Even more, coccidia have often shown resistance to many anti-coccidial drugs. Thus, the search for new control methods is important for improvements in both agriculture and veterinary medicine. In previous studies, selenium supplementation has been shown to have some control of parasites such as *E. tenella* in chickens. In addition to coccidia, bovine viral diarrhea virus (BVDV) is an immunosuppressive, enteric pathogen that can cause watery diarrhea in young cattle. Another parasite, *Cryptosporidium parvum* is another possible etiologic agent for calf scours. The objective of this study is to examine the influence of liver mineral levels – with emphasis on selenium – on the severity of coccidiosis in calves. While this study suggests that vitamin E supplementation may be an important factor for the prevention of coccidiosis in calves, definitive relationships between coccidiosis severity and mineral levels were not established. In addition, no correlations were found between coccidosis severity and concurrent *Cryptosporidium* or bovine viral diarrhea virus infections. This study provides information that could be imperative to improving the prevention of coccidiosis in calves. Yet, future studies must be conducted in order to better understand these relationships.

TABLE OF CONTENTS

LIST OF FIGURES iv

LIST OF TABLES v

ACKNOWLEDGEMENTS vi

Chapter 1 Introduction 1

- Coccidiosis 1
- Selenium and Other Mineral Interactions and Functions 1
- Bovine Viral Diarrhea Virus 3
- Cryptosporidiosis 3

Chapter 2 Materials and Methods 5

- Review of Pennsylvania Animal Diagnostic Laboratory System (PADLS) Records 5
  - Signalment and Anamnesis 5
  - Pathological Examination 5
  - Past Diagnostic Results 6
- Collection of Samples 6
  - Feces Collection 6
  - Tissue, Serum, and Whole Blood Samples Collection 7
- Data Retrieval from the Laboratory Information Management System 8
- Detection and Quantification of Coccidia 8
  - Zinc Sulfate Solution Preparation 9
  - Fecal Flotations 9
  - McMaster Egg Counting Technique 9
- Cryptosporidium Antigen Detection ELISA 10
  - Storage Conditions 10
  - Wash Preparation 11
  - In-Tube Dilutions 11
  - Sample Loading and Washing Procedures 11
  - Reading the Results 13
- One-Step Real-Time Reverse Transcriptase-Polymerase Chain Reaction for Detecting Bovine Viral Diarrhea Virus Types I and II 13
  - RNA Extraction 14
  - Amplification 17
  - Procedural Improvements 19
- Nutritional Screening Methods 21
- Statistical Analysis 22

Chapter 3 Results 23

Coccidia Quantification and Detection 23
Coccidia Severity Relationships to Vitamin E and Mineral Levels 25
Cryptosporidium Antigen Detection ELISA 35
Coccidiosis and Cryptosporidiosis Co-infection 36
Bovine Viral Diarrhea Virus Types I and II Polymerase Chain Reaction 39

Chapter 4 Discussion 43

Overview 43
Coccidiosis and Vitamin and Mineral Interdependence 43
Concurrent Cryptosporidiosis 46
Concurrent Bovine Viral Diarrhea Virus 47
Future Experiments and Opportunities 47

Appendix A Cryptosporidium Antigen Detection ELISA Templates 49

Appendix B Real-Time Reverse Transcriptase Polymerase Chain Reaction Graphical Representations 50

BIBLIOGRAPHY 53

Academic Vita 55

LIST OF FIGURES

Figure 1. Normal Reference Ranges for Bovine Serum and Liver Mineral Concentrations. ..28

Figure 2. Relationships Between Coccidia Count and Selenium Levels. 29

Figure 3. Relationships Between Coccidia Count and Copper Levels. 30

Figure 4. Relationships Between Coccidia Count and Iron Levels.....31

Figure 5. Relationships Between Coccidia Count and Vitamin E Levels.....33

Figure 6. Simple Linear Regression Model Comparing Coccidia Count and Vitamin E Levels. 34

Figure 7. Analysis of Variance and Simple Linear Regression Model Summary for Coccidia Count vs. Vitamin E Levels. 35

Figure 8. Comparisons Between Coccidia Count and the Presence of *Cryptosporidium* Antigen.38

Figure 9. One-Way Analysis of Variance for Coccidia Count versus Cryptosporidium Antigen Presence. 38

Figure 10. Comparisons Between Coccidia Count and the Presence of Bovine Viral Diarrhea Virus Presence.....41

Figure 11. One-Way Analysis of Variance for Coccidia Count versus Bovine Viral Diarrhea Virus Presence.....42

Figure 12. Bio-Tek Model ELX808 Absorbance Plate Reader Worksheet. 49

Figure 13. Real-Time RT-PCR for BVDV Types I and II from October 10, 2017. 50

Figure 14. Real-Time RT-PCR for BVDV Types I and II from October 19, 2017. 51

Figure 15. Real-Time RT-PCR for BVDV Types I and II from October 31, 2017. 52

LIST OF TABLES

Table 1. Cryptosporidium ELISA 96-Well Plate Template.....12

Table 2. Tissue Samples Obtained from Necropsy for PCR Analysis.....14

Table 3. Extraction Plate Template.....16

Table 4. Amplification Plate Template. RNA from sample on the extraction plate was added to a 96 -Well Plate.....18

Table 5. Coccidia Quantification from Fecal Flotations for Collected Samples. 24

Table 6. Serum Biological Fluid Mineral Screen for Collected Samples. 26

Table 7. Liver Nutritional Mineral Screen for Collected Samples. 27

Table 8. Coccidia Quantification and Vitamin E Levels for Liver Samples.....32

Table 9. Coccidia Quantification in *Cryptosporidium* Antigen Positive Cases. 36

Table 10. Coccidia Quantification in Bovine Viral Diarrhea Type I or Type II Positive Cases. 40

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Chapter 1 Introduction

Coccidiosis

Coccidiosis is a protozoal disease characterized by the excretion of watery feces. This disease is common in young calves between the ages of 1-2 months and 1 year. The clinical disease is not often seen within the first three weeks of life. However, infection during this time is possible. In cattle, coccidiosis can be caused by three different types of *Eimeria* species - *E. zuernii*, *E. bovis*, and *E. auburnensis*. Pathogenic coccidia often cause enteritis in the large intestine. However, they can also damage the mucosa of the lower small intestine, colon, or cecum.[1] This diarrheal disease, also known as calf scours, is a major cause of economic and productive loss to cattle producers worldwide.[2] Even more, coccidia have often shown resistance to many anti-coccidial drugs.[3] Thus, the search for new control methods is important for improvements in both agriculture and veterinary medicine.

Selenium and Other Mineral Interactions and Functions

In short, an optimal immune system and health status are derived from the ideal nutritional state of the animal. Both selenium and copper deficiencies have been generally associated with disease in cattle. Selenium is an important trace element with invaluable antioxidant properties. In cattle, selenium is effectively transferred from the cow to the fetus through the placenta and to the newborn through the colostrum.[4] According to a previous study by Petrie *et al*., supplementation of selenium in cattle is beneficial for both humoral and cellular immune responses. Supplementation of selenium induced expression of natural killer cells in the spleen.[5] In another study, Salles *et al.* demonstrated that high serum selenium concentrations stimulated the phagocytic activity of macrophages and an increase in

immunoglobulin levels in calves.[6] However, the supplementation of selenium must be carried out using extreme caution and finesse. Excess levels of selenium have been associated with detrimental effects on the function of the immune system and the production of harmful reactive oxygen species.[7]

It is imperative to note that the antioxidant functions of selenium are directly linked to the functions of vitamin E. This direct link between vitamin E and selenium can play a distinct role in the fertility, growth, and disease status of an animal.[4] Research conducted by Levander *et al.* has detailed the effects of selenium and vitamin E interdependence on the ability of the host to resist acute infection. This research has exhibited that selenium and vitamin E may play a role in control of parasites. The study suggests that dietary fish oil exerts pro-oxidative stress on malarial parasites. The study also shows a decreased number of caecal lesions in chicks that were infected with *E. tenella*, a coccidial parasite, and fed flaxseed or fish oil.[3]

Finally, a study conducted by Ahmed *et al*. describes the associations between coccidia infection and trace mineral levels in buffalo calves. Calves infected with coccidia exhibited decreased copper, iron, and selenium levels. These calves also exhibited increased free radical concentrations and depleted antioxidant system. The results of this study suggest that these mineral deficiencies may have played a role in host disease susceptibility, and there is a strong association between coccidiosis and a disrupted oxidant/antioxidant status.[8]

There is currently no established link between selenium and vitamin E concentrations and the severity of coccidia in calves.[3] However, the determination of a significant association between these two factors could be imperative to improving diagnostic procedures and the prevention of coccidiosis. The objective of this study is to examine the influence of liver mineral levels – with emphasis on selenium – on the severity of coccidiosis in calves. The number of coccidia oocysts per gram of feces characterizes the severity of the disease. This study provides information that could be imperative to improving diagnostic procedures and the prevention of coccidiosis in calves.

Bovine Viral Diarrhea Virus

In addition to coccidia, bovine viral diarrhea virus (BVDV) is an enteric pathogen that can cause watery diarrhea in young cattle. Transmission of this disease can occur vertically or horizontally. Fetuses that survive *in utero* infections may be born as BVDV-infected calves. These calves will remain infected throughout their life and continuously shed the virus in their feces. In cattle, a transitory BVDV infection can lead to immunosuppression. The immunosuppressive nature of this pathogen may increase susceptibility to other infections, the severity of other diseases, and the virulence of specific pathogens.[9] Many cases of BVDV have also exhibited moderate to severe leukopenia. The aforementioned immunosuppression may induce a subclinical *Eimeria* infection to become clinical. Thus, it is anticipated that one would see a more severe form of coccidiosis with a coexisting BVDV infection. Additionally, recent studies have found that BVDV infection in calves is correlated to a vitamin E deficiency.[10] Since Se and vitamin E are interrelated, it is anticipated that calves infected with BVDV and *Eimeria* species will exhibit deficiencies in liver vitamin E and selenium levels.

Cryptosporidiosis

Another parasite, *Cryptosporidium parvum* is a possible etiologic agent for calf scours.[2] Cryptosporidiosis can be either asymptomatic or characterized by the presence of watery and profuse diarrhea, inappetance, and dehydration. Calves between the ages of 1 to 4 weeks are most commonly infected. The characteristic diarrhea is caused by one of two mechanisms. Foremost, the pathogen can cause loss of enterocytes and the blunting of villi, leading to malabsorption. Thus, this mechanism is also characterized by decreased water and nutrient absorption. A second mechanism involves the stimulation of prostaglandins to induce the secretion of chloride and carbonate ions into the intestinal lumen. This causes more water to flow into the lumen, leading to diarrhea. The severity of cryptosporidiosis has been directly linked to the state of the host's immune system, the infective dose, and the presence of concurrent

infections.[11] *Cryptosporidium* species have the ability to cause debilitating damage to the gastrointestinal tract. Additionally, these protozoan parasites may be more likely to cause infection in a host with a weakened immune system.[2] Therefore, it is hypothesized that there will be a direct correlation with the presence of cryptosporiosis and an increasing severity in coccidiosis.

Chapter 2 Materials and Methods

All materials and instruments were obtained from the Animal Diagnostic Laboratory at the Pennsylvania State University. The methods used in these tests were guided by the standard operating procedures set in place by the Animal Diagnostic Laboratory. Each test was monitored and reviewed by a supervisor of that department.

Review of Pennsylvania Animal Diagnostic Laboratory System (PADLS) Records

Records of calves with scours that were submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University for necropsy between May 23rd, 2017 and October 10th, 2017 were examined. Records of farm-raised deer, sheep and goats exhibiting signs of scours that were submitted to the Animal Diagnostic Laboratory for necropsy during the same time period were also examined. All animals were between the ages of 0 days and 1 year old.

Signalment and Anamnesis

The signalment was recorded for each animal specified above. This included the species, sex, age, and, if applicable, production type. The date submitted, home address, accession number, owner, and veterinarian were also noted for each animal. Due to the author's status as an undergraduate researcher, any further information regarding the history of each animal was not released for review.

Pathological Examination

The pathologists of the Animal Diagnostic Laboratory performed pathological examinations on animals submitted from clients across the United States. These analyses included gross necropsy, histo-

pathological examination of tissues, and/or diagnostic testing on collected samples. Any internal or external abnormalities found by the pathologists during these examinations were documented. In addition, the final diagnoses made by the case coordinator were noted.

Past Diagnostic Results

All diagnostic results obtained by the Animal Diagnostic Laboratory at the Pennsylvania State University were reviewed. These included results obtained from the following departments: Mammalian Pathology, Histopathology, Microbiology, Parasitology, and Mammalian Virology. In some cases, toxicology results were obtained from diagnostic tests run by the University of Pennsylvania's New Bolton Center at the request of the Penn State Animal Diagnostic Laboratory.

Collection of Samples

Faculty and staff members of the Animal Diagnostic Laboratory at the Pennsylvania State University assisted in the collection of all samples. Each sample collected was labeled with accession number.

Feces Collection

Some of the fecal samples were collected from animals submitted to the Animal Diagnostic Laboratory at The Pennsylvania State University for necropsy between May and October 2017. The samples were collected from calves between the ages of 0 days and 1 year that have a history of severe scours. Fecal samples were also collected from sheep, goats, and deer submitted to the Animal Diagnostic Laboratory for necropsy. Like the calves, these animals were between the ages 0 days and 1

year. In addition, these species exhibited signs of severe scours prior to their death. Fecal samples were also obtained from live cattle. These samples were not directly collected by the undergraduate researcher or members of the Animal Diagnostic Laboratory. The fecal samples were collected by the client and sent to the Animal Diagnostic Laboratory for examination.

All fecal samples collected for coccidia detection were refrigerated at 2-8°C until further examination. Fecal samples collected for the *Cryptospiridium* Antigen Detection ELISA were stored in the freezer at ≤ -15°C.

Tissue, Serum, and Whole Blood Samples Collection

Representative samples of liver, lymph node, and spleen were collected from each animal at necropsy. Due to technical circumstances, both types of lymphoid tissue were not collected from every animal. Collected lymph node and spleen samples were no larger than 250 mg and 0.5 cm in width. These samples were stored in 2.5 ml of RNA*later*™ solution. Each sample was stored 2-8°C overnight and then transferred to the freezer to be stored at ≤ -15°C. Liver samples were collected as unfixed tissues. Approximately 100 to 200 mg of liver tissue was collected from each animal and frozen at ≤ -15°C.

In some cases, clients collected serum and whole blood samples from live animals. The clients submitted these samples to the Animal Diagnostic Laboratory for further analysis. Serum samples were collected in Vacutainer tubes containing no additives or anticoagulants. Each serum sample was separated in the centrifuge, transferred to a new tube, and frozen at ≤ -15°C. Whole blood samples were collected in Vacutainer tubes containing anticoagulant. These samples were stored in the refrigerator at 2-8°C.

Data Retrieval from the Laboratory Information Management System

Due to limitations in the results obtained from the samples collected, additional data was obtained from the Laboratory Information Management System (LIMS) used by the Pennsylvania Animal Diagnostic Laboratory System. Approval to review this data was received from the director of the Animal Diagnostic Laboratory at the Pennsylvania State University. These results were used to evaluate possible relationships between coccidiosis and bovine viral diarrhea virus infection or cryptosporidiosis. A query search was conducted by Animal Diagnostic Laboratory faculty using the Laboratory Information Management System. Data collected for cases submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University between 2006 and October 2017 were reviewed. The search was further refined to include cases occurring in calves between the ages of 0 days and 1 year. Cases that had been determined positive for the presence of coccidia were analyzed. Those cases that were also positive for *Cryptosporidium* antigen and/or bovine viral diarrhea virus type I or II were further evaluated. This data was not directly released to the author. Only the results reported in this study were provided to the student by Animal Diagnostic Laboratory faculty.

Detection and Quantification of Coccidia

Before performing this procedure, the undergraduate researcher on this project was trained in the technical concepts of this assay by members of the Animal Diagnostic Lab Parasitology Department. The fecal samples used in this assay were those collected from calves, farm-raised deer, sheep, and goats at necropsy, as well as those submitted by clients from live cattle.

Zinc Sulfate Solution Preparation

Zinc sulfate solution was prepared by adding 579 grams of zinc sulfate ($ZnSO_4$) to 1500 ml of deionized water. The solution was mixed thoroughly. A hydrometer was used to check the specific gravity of the solution. In order to obtain a specific gravity between 1.18 and 1.2, deionized water and zinc sulfate were added as needed. The solution was stored at room temperature (20-24°C).

Fecal Flotations

Collected fecal samples were thoroughly mixed. The following steps were completed for each feces sample. One gram of feces was added to 29 ml of zinc sulfate solution and mixed thoroughly. A 3 ml disposable pipette was used to stir the mixture, while simultaneously being filled. The full pipette was then used to fill the chambers of the McMaster slide. The entire chamber was filled, not just the area under the grid. If any air bubbles were present, the fluid was removed and the air chambers were refilled. The slides were set aside for 10 minutes to allow any eggs present to float to the top of the chambers.

McMaster Egg Counting Technique

Each McMaster slide was placed on the stage of the Reichert Microstar IV microscope. All slides were first focused using a 10X lens. Each slide was then observed using a 40X lens. All eggs were counted in the marked area of both chambers. The engraved lines of the marked area were used to guide the examination. The columns were read from top to bottom, starting from the top right corner of the marked area in the first chamber. It is important to note that eggs floated to the top of each chamber. Thus, the engraved lines were focused on in order to have the eggs in the plane of focus. The fine focus was adjusted throughout observation to more clearly observe each egg. The egg count was tracked using a Hand Tally Counter. Only coccidia eggs were counted during the examination of each slide.

Each egg count was multiplied by 100 to get the number of eggs per gram., as denoted by the following equation: $\#\ of\ eggs\ observed\ \times\ 100 = total\ eggs\ per\ gram\ of\ feces$.

Samples that did not contain any coccidia eggs were reported as negative at a sensitivity of 100 eggs/gram. The total number of eggs per gram of feces entered for these negative samples was zero. Any samples containing coccidia eggs were reported as positive at a sensitivity of 100 eggs/gram. The total egg count per gram of feces was reported with each positive sample.

Cryptosporidium Antigen Detection ELISA

The following sections outline the procedure for performing an *in vitro* immunoassay for the qualitative determination of *Cryptosporidium* antigen in non-human fecal material. This method used the IVD Research Inc. *Cryptosporidium* Antigen Detection Microwell ELISA Kit Lot LW1396. Negative and positive controls were supplied with the test kit. Before performing this procedure, the undergraduate researcher on this project was trained in the technical concepts of this assay by members of the Animal Diagnostic Lab Parasitology Department.

Storage Conditions

Reagents, test strips, and bottled components were stored at 2-8°C. The squeeze bottle containing the diluted wash buffer was stored at room temperature. Fecal samples were stored at -15°C or lower until examination. Before use, all reagents and samples were set aside until they reached room temperature (15-25°C).

Wash Preparation

The 20X Wash Concentrate enclosed in the ELISA kit contained concentrated buffer with detergent and thimerosal. The concentrate was diluted by added the contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of reagent grade DI water. The wash solution was mixed by swirling.

In-Tube Dilutions

Each reagent and sample was mixed before use. Reagents were mixed by inversion. Test strips containing a total of 30 microwells were removed from the kit and placed in the 96-well strip holder. Each microwell contained anti-*Cryptosporidium* antibodies conjugated to horseradish peroxidase with thimerosal. Microcentrifuge tubes were labeled with sample identification numbers. For each sample, 0.1 g of the sample and 0.7 ml of Dilution Buffer were added to the appropriate microcentrifuge tube. This combination was mixed thoroughly. The Dilution Buffer was composed of a buffered protein solution with thimerosal.

Sample Loading and Washing Procedures

The following steps were completed using a micropipette. The pipette tips were changed before transferring each sample. 100 µl of negative control was added to well #2, and 100 µl of positive control was added to well #1. The positive control contained diluted *Cryptosporidium* positive formalinized stool supernatant. The negative control contained a buffered protein solution with thimerosal. Additionally, 100 µl of diluted sample was added to the each corresponding well. The template in Table 1 below depicts the exact placement of the controls and samples on the 96-Well Plate. Columns are denoted by

numbers 1 through 12, while rows are denoted by letters A through H. Each sample on the plate is denoted by an accession number.

Table 1. Cryptosporidium ELISA 96-Well Plate Template. Fecal samples, as well as positive and negative controls, were added to a 96-Well Plate as previously described. Each sample is denoted by an accession number. Enzyme Conjugate, wash solutions, Chromogen, and Stop Solution were added to each well in appropriate quantities.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos Cntrl.	17941	6	14								
B	Neg Cntrl.	17962	7	15								
C	15962	20861	8	16								
D	16762 Calf 1A	22477	9	17								
E	16762 Calf 1B	1	10									
F	17097	2	11									
G	17579	3	12									
H	17779	4	13									

The wells were incubated for 60 minutes at room temperature (15-25°C). All wells were then washed five separate times by filling each well to overflow with diluted wash buffer and decanting contents. Bubble formation in the wells was avoided during this process, in order to avoid effects on the results. After the fifth wash, the wells were tapped on a clean absorbent towel to remove excess wash buffer. Two drops of Enzyme Conjugate were added to each well. The wells were incubated for 30 minutes at room temperature and then washed five separate times using the same procedure as before. Two drops of Chromogen, which contained tetramethylbenzidine and peroxide, were added to each well. The wells were incubated for 10 minutes at room temperature (15-25°C). Two drops of Stop Solution were added to each well. The Stop Solution contained 5% phosphoric acid solution. The well were mixed by gently tapping the side of the strip holder with an index finger for about 15 seconds. The reaction was

read within 5 minutes after adding the Stop Solution. The results were read visually and with an ELISA plate reader.

Reading the Results

The results were interpreted using two methods. Using visual interpretation, each well was classified as positive or negative based upon the color of the well. Any sample well that was clearly more yellow than the negative control well was classified as positive. Any sample well that was not clearly more yellow than the negative control well was classified as negative. The visual read card in the ELISA kit was used to confirm color comparisons.

The results were also interpreted using a Bio-Tek Model ELX808 Absorbance Plate Reader. The reader showed a value of zero on air. All wells were read using a bichromatic reading with filters at 450 nm and 620-650 nm. All wells that had an absorbance reading of 0.08 OD and above were samples that were considered to contain *Cryptosporidium* antigen. These samples were classified as positive. All wells that had an absorbance reading of less than 0.08 OD were samples that did not have detectable levels of *Cryptosporidium* antigen. These samples were classified as negative. Please note that all control wells needed to yield the expected results for the test to be valid.

One-Step Real-Time Reverse Transcriptase-Polymerase Chain Reaction for Detecting Bovine Viral Diarrhea Virus Types I and II

This procedure was conducted under the supervision and guidance of the Virology Services Manager. All materials were certified RNase free or cleaned with RNase ZAP or Molecular Grade water before use.

Tissue, whole blood, and serum samples were used for this assay. Lymph node and/or spleen samples were collected from the animals that were sent to the Animal Diagnostic Laboratory at The

Pennsylvania State University for necropsy. Details of the types of tissues used for each animal are detailed in Table 2. Every sample is denoted by an accession number. Serum and whole blood samples were obtained from live calves and sent into the Animal Diagnostic Laboratory by clients for analysis.

Table 2. Tissue Samples Obtained from Necropsy for PCR Analysis. Lymphoid tissues were collected from animals sent to the Animal Diagnostic Laboratory at The Pennsylvania State University for necropsy. These samples were all collected from animals exhibiting signs of scours. Each case from which samples were collected is denoted by an accession number. The type of lymphoid tissue(s) collected from each case is described.

Accession Number	Type of Sample
P1715962	Spleen and Lymph Node
P1716762 Calf 1A	Spleen and Lymph Node
P1716762 Calf 1B	Spleen and Lymph Node
P1717097	Spleen
P1717579	Spleen
P1717779	Spleen and Lymph Node
P1717941	Lymph Node
P1717962	Spleen and Lymph Node
P1720861	Spleen and Lymph Node
P1722477	Spleen and Lymph Node

RNA Extraction

The following steps were completed using a 5X MagMAX™ Pathogen RNA/DNA Kit Lot 00424843. A single buffer control was set up per extraction plate. The worksheets used to calculate the component volumes for the Lysis/Binding Solution and Bead Mix automatically took pipetting error into account. The Lysis/Binding Solution was prepared for each appropriate sample type using the PS WS

PCR EXT-002 MagMax Lysis/Binding Solution Preparation. All serum samples were classified as low cell content samples, and tissue and whole blood samples were classified as whole blood samples. The Positive Extraction Control (PEC) and the Negative Extraction Control (NEC) were classified as low cell content samples. The low cell content Lysis/Binding Solution was prepared for 18 reactions at 300 µl sample volumes. 6930 µl of lysis/binding solution concentrate, 39.6 µl of carrier RNA, 39.6 µl of xeno RNA, 39.6 µl of xeno DNA, and 6930 µl of 100% isopropanol were combined and mixed by vortexing. The whole blood Lysis/Binding Solution was prepared for 27 reactions at 100 µl sample volumes. 5720 µl of lysis/binding solution concentrate, 57.2 µl of carrier RNA, 57.2 µl of xeno RNA, 57.2 µl of xeno DNA, and 5720 µl of 100% isopropanol were combined and mixed by vortexing. The Bead Mix was prepared for all sample types using the PS WS PCR EXT-001 MagMax BEAD MIX for RNA-DNA Extractions. The Bead Mix was made for 44 total reactions. First, the nucleic acid binding beads were mixed by vortexing. Next, 10 µl of nucleic acid binding beads and 10 µl of lysis enhancer were combined on ice and vortexed well.

Four MME-96 Deep Well Plates were used to prepare wash plates. Into two of the MME-96 Deep Well Plates, 300 µl of Wash Solution 1 was added to every well that would contain a sample or a control. 450 µl of Wash Solution 2 was added to the remaining two MME-96 Deep Well Plates in the same manner. The program 4462359_DW_HV was started on the MagMax Express-96 Magnetic Particle Processor. A MME-96 Deep Well Tip Comb was placed into a standard plate. All four MME-96 Deep Well Plates, as well as the plate containing the Deep Well Tip Comb, were loaded into the MagMax Express-96 Magnetic Particle Processor.

Samples were prepared before loading onto the extraction plate. Low cell content samples were prepared first. These samples were thawed and vortexed briefly. Each tissue sample was transferred from a tube containing RNAlater to 3 ml of phosphate-buffered saline at pH 7.4. Tissues were homogenized using a Power Gen 125 homogenizer. The homogenizer and scalpels were cleaned with RNase Zap wipes and rinsed twice with Molecular Grade water prior to use and subsequent to the homogenization of each

sample. These instruments were cleaned using the following sequence: two rinses in 0.5M NaOH, then two rinses in 10% bleach, and then two rinses in Molecular Grade water. Each homogenized tissue sample was spun in the centrifuge for 3 min at 9000 rpm. The whole blood samples did not need any further preparation before loading.

Table 3. Extraction Plate Template. Following RNA extraction, serum, whole blood, and tissue samples were added to a MME-96 Deep Well Plate. Each sample is denoted by an accession number. Fetal Bovine Serum (FBS) was used as a positive extraction control (PEC). Phosphate-buffered saline was used as a negative extraction control (NEC).

		1	2	3	4	5	6	7	8	9	10	11	12
Serum	**A**	1	2	3	4	6	7	8	9	10	11	12	13
Serum	**B**	14	15	16	17	FBS PEC	NEC						
Whole Blood	**C**	1	2	3	4	5	6	7	8	9	10	11	12
Whole Blood	**D**	13	14	15	16	17							
Tissues	**E**	15962	16762 Calf 1A	16762 Calf 1B	17097	17579	17779	17941	17962	20861	22477		
	F												
	G												
	H												

Extraction plate preparation was carried out in accordance with the sample type. 20 µl of prepared Bead mix was added to each well of a MME-96 Deep Well Plate. Fetal Bovine Serum (FBS) was used as a Positive Extraction Control (PEC), and phosphate-buffered saline was used as a Negative Extraction Control (NEC). As previously stated, each control was treated as low cell content sample. 300 µl of each low cell content sample was added to the appropriate well. 700 µl of Lysis/Binding Solution

was added to each well containing a low cell content sample. 100 µl of each whole blood sample was added to the appropriate well. 400 µl of Lysis/Binding Solution was added to each well containing a whole blood sample. The positions of each sample and control on the extraction plate are detailed in Table 3 above. Samples are denoted by accession numbers.

Next, 90 µl of Elution Buffer was added to a standard plate. Both the plate containing the Elution Buffer and the extraction plate were placed in the MagMax Express Magnetic Particle Processor. Once all plates were added, the extraction program 4462359_DW_HV was run on the Particle Processor. Once the extraction was completed, the plate containing the total nucleic acid was sealed with a MicroAmp® Clear Adhesive Film and was placed in the freezer at ≤ 15°C until use for the amplification.

Amplification

All samples and controls were run in duplicate. First, a Real-Time RT-PCR reaction mix was prepared for BVDV detection. Enough reaction mix was prepared for 50 µl reactions using 2 µl of template RNA for each sample, a negative control, two positive controls and two additional reactions to account for pipetting error. The BVDV Real-time reaction mix was prepared using the PS WS PCR 014-001 BVDV Real Time Worksheet for a total of 96 reactions. Pipetting error was automatically calculated into the total volume of each component.

The primer set used in this reaction mix included two Pesti Primers with an amplicon size of 106 bp. The Pesti-F primer was composed of the sequence 5'-CTAGCCATGCCCTTAGTAG 3'. The Pesti-R primer was composed of the sequence 5'-CGTCGAACCAGTGACGACT 3'. Two probes, FAM and Texas Red, were used in the reaction mix. This was a 102/208 bp product. The BVD Type I probe was composed of the sequence 5'-FAM-TAGCAACAGTGGTGAGTTCGTTGGATGGCT-BHQ3'. The BVD Type II probe was composed of the sequence 5'-TxR-TAGCGGTAGCAGTGAGTTCGTTGGATGGCC-BHQ3'.

The BVDV Real-time reaction mix was prepared by combining 1742.4 µl of Diethylpyrocarbonate-treated (DEPC) water, 2640 µl of reaction mix, 211.2 µl of Pesti-F primer, 211.2 µl of Pesti-R primer, 52.8 µl of RNase Inhibitor, 105.6 µl of SSIII RT Taq Mix, 52.8 µl of Type I probe (FAM), and 52.8 µl of Type II probe (Texas Red). Both the reaction mix and the SSIII RT Taq Mix used in this preparation were obtained from the SuperScriptIII Platinum One Step qRT PCR Kit.

Table 4. Amplification Plate Template. RNA from sample on the extraction plate was added to a 96 - Well Plate. Each sample is denoted by an accession number and the type of sample – serum, whole blood, or tissue. Both positive and negative controls were also added, along with positive amplification controls (PAC) for BVDV types I and II. All samples and controls were run in duplicate. BVDV Real-time reaction mix was also added to each well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Serum 1	Serum 2	Serum 3	Serum 4	Serum 6	Serum 7	Serum 8	Serum 9	Serum 10	Serum 11	Serum 12	Serum 13
B	Serum 1	Serum 2	Serum 3	Serum 4	Serum 6	Serum 7	Serum 8	Serum 9	Serum 10	Serum 11	Serum 12	Serum 13
C	Serum 14	Serum 15	Serum 16	Serum 17	FBS PEC	NEC	NTC	WB 13	WB 14	WB 15	WB 16	WB 17
D	Serum 14	Serum 15	Serum 16	Serum 17	FBS PEC	NEC	NTC	WB 13	WB 14	WB 15	WB 16	WB 17
E	WB 1	WB 2	WB 3	WB 4	WB 5	WB 6	WB 7	WB 8	WB 9	WB 10	WB 11	WB 12
F	WB 1	WB 2	WB 3	WB 4	WB 5	WB 6	WB 7	WB 8	WB 9	WB 10	WB 11	WB 12
G	15962	16762 Calf 1A	16762 Calf 1B	17097	17579	17779	17941	17962	20861	22477	BVD I PAC	BVD II PAC
H	15962	16762 Calf 1A	16762 Calf 1B	17097	17579	17779	17941	17962	20861	22477	BVD I PAC	BVD II PAC

Upon completion, the BVDV Real-time reaction mix was mixed by vortexing. 48 µl of BVDV Real-time reaction mix was added to each well of a 96-well plate. 2 µl of template RNA, including positive and negative controls, was then added to the appropriate wells. All samples and extraction controls were obtained from the extraction plate containing the total nucleic acid that was previously sealed and stored. DEPC water was used for the No Template Control (NTC). Two separate positive amplification controls (PAC) were also added in volumes of 2 µl each. Each control was composed of RNA extracted from cell cultures. The first amplification control was BVDV type I Singer strain NVSL 140-BDV. The second amplification control was BVDV type II Strain 125 NVSL 145-BDV. The positions of each sample and control on the plate are detailed in Table 4.

The plate was sealed with a Microseal® 'B' Film. Using a mini plate spinner, the sealed plate was spun for approximately five seconds to make sure that both the template and reaction mix were at the bottom of the well. The plate was then placed in a Bio-Rad CFX96 C1000™ Thermal Cycler. The Real-time RT-PCR reaction was then run in accordance with the following reaction parameters: 30 minutes at 50°C, 15 minutes at 95°C, and then 40 cycles at 94°C for 15 seconds and then 60°C for one minute. Once the reaction was complete, the results were evaluated on the computer under the log phase view. The computer-derived value for the threshold was adjusted to the middle of the log phase of the amplification curve for the positive controls. Samples demonstrating normal curve parameters with Ct values less than or equal to 36.9 were considered positive for BVD Type I under the FAM probe or Type II under the Texas-Red probe. Samples with normal curve parameters and Ct values greater than 37 were considered very weak positive samples.

Procedural Improvements

In order to improve the quality of the results, amendments were made to the procedure. To obtain these results, various runs were conducted in the presence or absence of specific reagents.

The first two runs that were conducted showed a considerable amount of background noise. Additionally, the positive amplification controls for both BVDV Types I and II were not amplifying as expected. A third run was conducted using only those samples that exhibited suspicious and inconclusive results in the previous runs. These "positive" samples, along with positive and negative extraction and amplification controls, were analyzed. For this run, a Xeno primer probe was added to the BVDV Real-time reaction mix to serve as an internal positive control. However, even with the presence of the Xeno probe, only 3 of the questionable samples amplified. In addition, the positive extraction control did not amplify. Thus, it was decided to complete the RNA extraction process once again.

After re-extraction, another run was conducted using the Bio-Rad CFX96 C1000™ Thermal Cycler. While the positive extraction controls amplified, there was a significant amount of background noise run seen with the FAM probe. Upon further evaluation of the results, the fifth run was conducted using the Applied Biosystems™ 7500 Real-Time PCR Systems. It was suspected that there might have been issues with the Bio-Rad CFX96 C1000™ Thermal Cycler. Running the real-time RT-PCR reaction using the Applied Biosystems™ 7500 Real-Time PCR Systems did not improve the quality of the results.

The final run was conducted using the Bio-Rad CFX96 C1000™ Thermal Cycler. All positive extraction and amplification controls amplified as expected during this run. Additionally, all negative controls did not amplify, yielding Ct values of 0. The threshold had to be set higher in order to eliminate some late background noise. However, the ranges achieved for each of the controls directly mirror the expected values.

Nutritional Screening Methods

Toxicology results were obtained from diagnostic tests run by the University of Pennsylvania's New Bolton Center at the request of the Penn State Animal Diagnostic Laboratory. Collected samples were shipped to the PADLS New Bolton Center Toxicology Laboratory, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA. The samples were analyzed for metals using a Nexion 300D (Perkin Elmer, Shelton, CT). The analytical standards were purchased from SCP (Champlain, NY) and trace metal grade nitric acid was purchased from Fisher Scientific (Pittsburgh, PA). All dilutions were done using in-house deionized water (≥18 MΩ) obtained from a water purification system (EMD Millipore, Billerica, MA). The dried bovine liver sample (1577 C) used as reference material was obtained from NIST (National Institute of Standards and Technology, Gaithersburg, MD). Bovine serum was used as a reference material for the serum samples and was purchased from Sigma Aldrich (St. Louis, MO).

The serum and liver samples were weighed into Teflon PFA vials (Savillex, Minnetonka, MN). The serum samples were digested overnight with twice the quantity (weight/volume) of 100% nitric acid and tissue samples were digested overnight with 20 times the quantity (weight/volume) of 70% nitric acid, both at 70°C. A 0.15 mL portion of each digested serum sample and 0.1 mL portion of each digested liver sample were individually mixed with 0.05 mL of 2 ppm internal standard containing Ge (germanium), In (indium), Tb (terbium), and Y (yttrium) and each mixture was diluted with deionized water to a final volume of 5 mL for analysis. The concentration of each metal in the submitted sample was measured using a calibration curve of aqueous standards prepared at four different concentrations of each metal. Selenium concentration was analyzed by mixing 0.25 mL of digested serum sample or 0.1 mL of digested liver sample with 0.05 mL of 2 ppm internal standard and each mixture was diluted to 5 mL using a 1% methanol solution (Fisher Scientific, Pittsburgh, PA). The concentration of selenium was measured using a calibration curve of aqueous/methanol standards prepared at five different concentrations of selenium.

The accuracy of the results was monitored by analyzing reference material (NIST 1577C) with known values and bovine serum with in-house established values of metals of interest with each batch of samples. All results are reported in ppm on a wet weight basis.

Statistical Analysis

The program Minitab Express™ was used in order to conduct statistical analyses on the data obtained from the various diagnostic tests. Scatterplots were created in order to determine any correlations between coccidia counts and vitamin E and mineral levels. The strength of these correlations was determined using the Pearson correlation method. In terms of vitamin E levels, the strength of correlations were also analyzed using a Simple Linear Regression model. The relationships between *Cryptosporidium* antigen or bovine viral diarrhea virus presence and coccidia counts were evaluated using side-by-side boxplots and analysis of variance tables.

Chapter 3 Results

A majority of the subsequent results were obtained using the instruments and materials available at the Animal Diagnostic Laboratory at the Pennsylvania State University. The methods used in each assay were based upon the standard operating procedures set in place by the Animal Diagnostic Laboratory. Each test was monitored and reviewed by a supervisor of the overseeing department. Other results included in this section were obtained from the Laboratory Information Management System (LIMS) used by the Pennsylvania Animal Diagnostic Laboratory System. Approval was received from the director of the Animal Diagnostic Laboratory at the Pennsylvania State University in order to review the aforementioned data.

Coccidia Quantification and Detection

Following the conduction of fecal flotation tests, only eight cases were determined positive for the presence of coccidia. The severity of the infection was directly correlated to the quantity of eggs present in each fecal sample. Members of the Parasitology Department in the Penn State Animal Diagnostic Lab reviewed the results below. The fecal samples used in these assays were collected from calves, farm-raised deer, sheep, and goats at necropsy, as well as those submitted by clients from live calves. This data does not encompass cases that were reviewed and acquired from the Laboratory Information Management System. The quantification of coccidia in each fecal sample is detailed in Table 5 below. Samples that contained 180,000 eggs per gram of feces or more were classified as too numerous to count (TNTC).

Table 5. Coccidia Quantification from Fecal Flotations for Collected Samples. Each fecal sample is denoted by an accession number. The species of the animal from which the sample was collected is also specified. The quantity of coccidia in each fecal sample was determined via fecal flotation. Samples that contained 180,000 eggs per gram of feces or more were classified as too numerous to count (TNTC). Only eight of the cases examined were positive for the presence of coccidia.

Accession Number	Species	Coccidia count (eggs per gram of feces)
P1715962	Bovine	0
P1716762 Calf 1A	Bovine	0
P1716762 Calf 1B	Bovine	0
P1717097	Cervine	46,800
P1717579	Cervine	TNTC
P1717779	Caprine	TNTC
P1717941	Ovine	83,800
P1717962	Bovine	0
P1720861	Bovine	53,200
P1722477	Bovine	0
1	Bovine	0
2	Bovine	0
3	Bovine	0
4	Bovine	0
5	Bovine	0
6	Bovine	0
7	Bovine	0
8	Bovine	0
9	Bovine	2400
10	Bovine	0

11	Bovine	0
12	Bovine	1800
13	Bovine	0
14	Bovine	200
15	Bovine	0
16	Bovine	0
17	Bovine	0

Coccidia Severity Relationships to Vitamin E and Mineral Levels

Toxicology results were obtained from diagnostic tests run by the University of Pennsylvania's New Bolton Center at the request of the Penn State Animal Diagnostic Laboratory. Previously, case number 5 had been determined negative for the presence of coccidia, *Cryptosporidium* antigen, and bovine viral diarrhea virus. Thus, samples for this case were not submitted to the University of Pennsylvania's New Bolton Center for mineral screening. Table 6 below details the serum Ca, Cu, Fe, Mg, Se and Zn levels for serum samples collected between May and October 2017. In comparison, Table 7 details the liver Ca, Cu, Fe, Mg, Se and Zn levels for liver samples collected during the same time period.

Table 6. Serum Biological Fluid Mineral Screen for Collected Samples. Serum samples were submitted to the University of Pennsylvania's New Bolton Center for mineral screening. Each sample is denoted by an accession number. All samples exhibited elevated calcium levels, which may be result of over-supplementation of dietary calcium or dehydration. Abnormally elevated levels of iron and selenium were also observed in a significant portion of cases.

		Serum Mineral Levels (ppm)					
		Calcium	**Copper**	**Iron**	**Magnesium**	**Selenium**	**Zinc**
Accession Number	**1**	134**	0.537*	1.32	22.5	0.067	1.08
	2	123**	0.712	1.69	20.1	0.062	1.16
	3	121**	0.869	1.41	20.8	0.075	1.04
	4	127**	0.555*	1.32	22.3	0.066	1.19
	6	129**	0.705	1.12	22.8	0.081**	1.15
	7	131**	0.722	3.03**	22.1	0.064	1.09
	8	127**	0.643	1.69	23.6	0.082**	1.16
	9	120**	0.761	3.02**	18.8	0.082**	1.14
	10	125**	0.622	1.51	21.6	0.080	0.963
	11	135**	0.629	3.13**	26.8	0.092	1.28
	12	130**	0.731	5.24**	27.3	0.094	1.20
	13	127**	0.780	3.30**	26.7	0.095	1.36
	14	130**	0.623	2.38	26.7	0.082	1.23
	15	133**	0.710	2.13	24.7	0.068	1.03
	16	133**	0.906	3.17**	21.9	0.073	0.927
	17	126**	0.616	2.26	23.6	0.068	1.04

* Serum mineral deficiencies according to the reference ranges provided by the University of Pennsylvania's New Bolton Center.

** Serum mineral toxicities according to the reference ranges provided by the University of Pennsylvania's New Bolton Center.

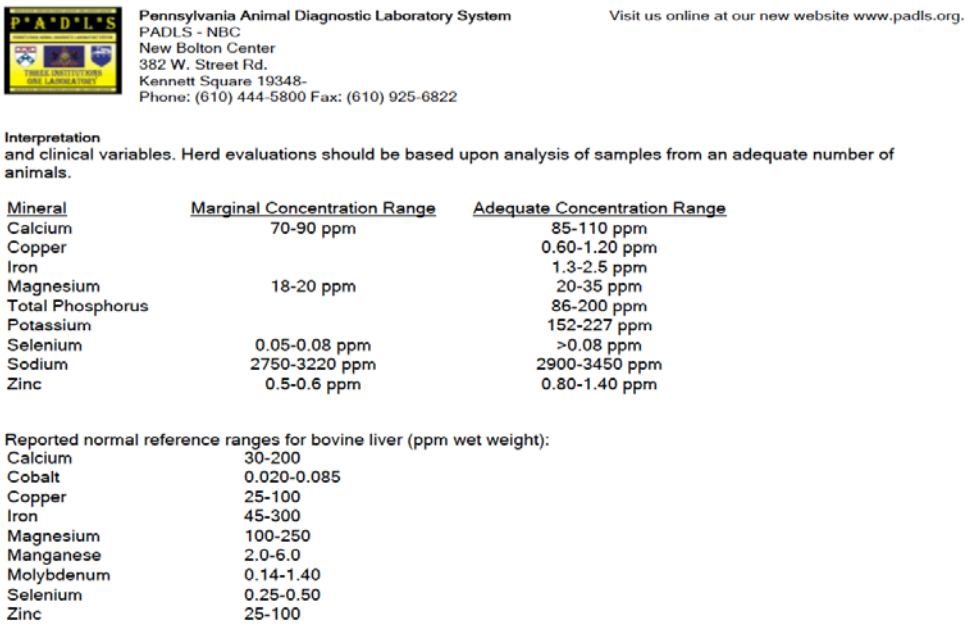
Table 7. Liver Nutritional Mineral Screen for Collected Samples. Liver samples were submitted to the University of Pennsylvania's New Bolton Center for mineral screening. Each sample is denoted by an accession number. Abnormal levels of iron, copper, and selenium were observed in a significant portion of cases. A majority of the selenium abnormalities were excesses. In contrast, abnormalities noted in copper and magnesium levels were a mixture of excesses and deficiencies.

		Liver Mineral Levels (ppm)					
		Calcium	**Copper**	**Iron**	**Magnesium**	**Selenium**	**Zinc**
Accession Number	**P1715962**	55.7	129**	71.0	168	0.829**	182**
	P1716762 Calf 1A	142	91.9	140**	132	2.28**	108
	P1716762 Calf 1B	95.9	127	44.1	180	3.06**	133
	P1717097	49.1	59.2	205	185	1.05	87.9
	P1717579	41.8	87.5	233	171	0.853	69.5
	P1717779	33.4	20.4*	17.6*	155	1.04**	41.0
	P1717941	57.4	2.04*	75.1	141	<0.15*	31.1
	P1717962	55.4	112**	47.9	177	0.541**	140**
	P1720861	69.0	90.1	313**	168	0.466	80.3
	P1722477	81.5	4.84*	24.5*	151	0.297	35.4

* Serum mineral deficiencies according to the reference ranges provided by the University of Pennsylvania's New Bolton Center.

** Serum mineral toxicities according to the reference ranges provided by the University of Pennsylvania's New Bolton Center.

Abnormalities in mineral levels were determined based upon normal physiological reference ranges for cattle, sheep, goats, and deer are provided by the University of Pennsylvania's New Bolton Center. Bovine mineral ranges are depicted in Figure 1.



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Visit us online at our new website www.padls.org.

Interpretation
and clinical variables. Herd evaluations should be based upon analysis of samples from an adequate number of animals.

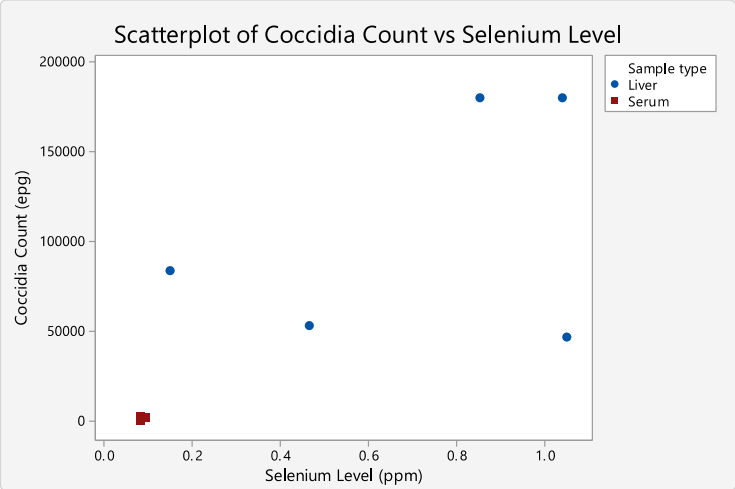
Mineral	Marginal Concentration Range	Adequate Concentration Range
Calcium	70-90 ppm	85-110 ppm
Copper		0.60-1.20 ppm
Iron		1.3-2.5 ppm
Magnesium	18-20 ppm	20-35 ppm
Total Phosphorus		86-200 ppm
Potassium		152-227 ppm
Selenium	0.05-0.08 ppm	>0.08 ppm
Sodium	2750-3220 ppm	2900-3450 ppm
Zinc	0.5-0.6 ppm	0.80-1.40 ppm

Reported normal reference ranges for bovine liver (ppm wet weight):

Calcium	30-200
Cobalt	0.020-0.085
Copper	25-100
Iron	45-300
Magnesium	100-250
Manganese	2.0-6.0
Molybdenum	0.14-1.40
Selenium	0.25-0.50
Zinc	25-100

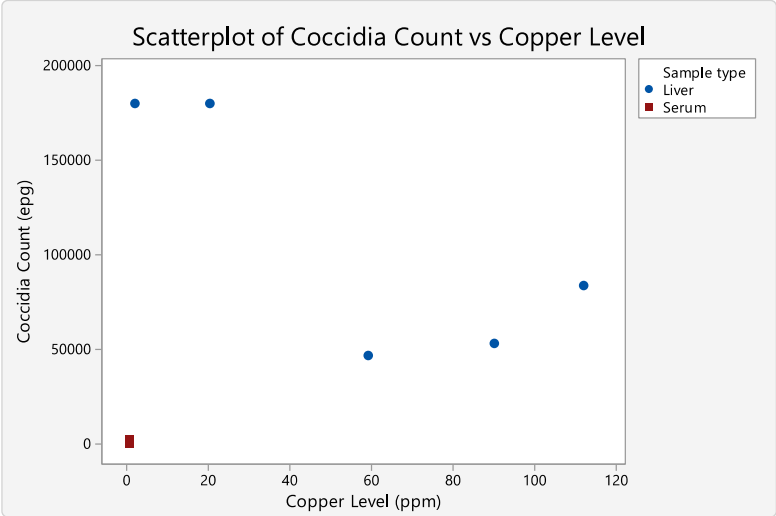
Figure 1. Normal Reference Ranges for Bovine Serum and Liver Mineral Concentrations. The normal physiological reference ranges for serum and liver mineral concentrations in cattle were provided by the University of Pennsylvania's New Bolton Center. Each mineral concentration is reported on a parts per million (ppm) basis. These ranges were used to determine abnormalities in serum and liver mineral levels in the samples examined in this study.

The following figures exhibit possible relationships between coccidia quantification and liver mineral levels for the cases described in Tables 5, 6, and 7. Upon reviewing the serum and liver mineral levels obtained for each case, it was determined that a majority of the mineral deficiencies and toxicities were seen with selenium, copper, and iron. The levels of these three minerals in each of the cases exhibiting the presence of coccidia eggs were further analyzed. For coccidia counts that were classified as TNTC, the number of 180,000 epg was used for statistical analysis. Figures 2, 3, and 4 display the relationships between coccidia count in eggs per gram and selenium, copper, and iron levels, respectively.



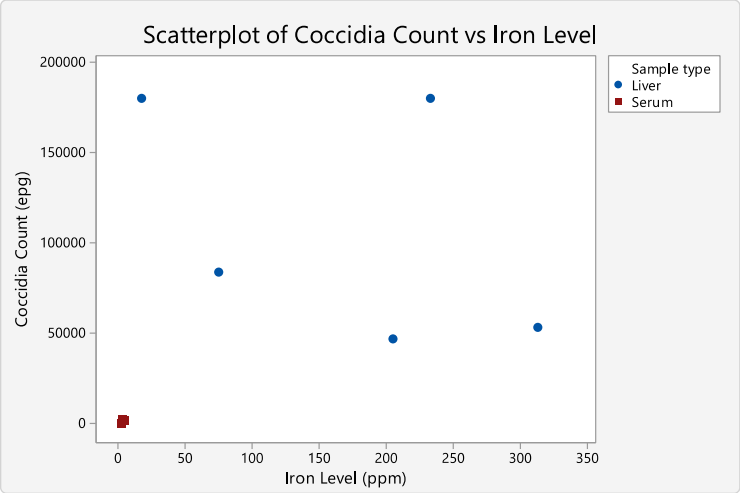
86000 0.006928 0.082000 0.094000

Figure 2. Relationships Between Coccidia Count and Selenium Levels. The quantity of coccidia in each coccidia-positive sample was plotted against the reported serum or liver selenium level for each respective case. Previous tests had shown that only eight of the collected samples were positive for the presence of coccidia. The shape of the scatterplot suggests a positive correlation between coccidia counts in coccidia-positive samples and liver selenium levels. The relationship to serum selenium levels is difficult to assess due to the highly limited number of samples.



8 0.62300 0.76100

Figure 3. Relationships Between Coccidia Count and Copper Levels. The quantity of coccidia in each coccidia-positive sample was plotted against the reported serum or liver copper level for each respective case. Previous tests had shown that only eight of the collected samples were positive for the presence of coccidia. The shape of the scatterplot suggests a negative correlation between coccidia counts in coccidia-positive samples and liver copper levels. The relationship to serum copper levels is difficult to assess due to the highly limited number of samples.



0 2.3800 5.2400

Figure 4. Relationships Between Coccidia Count and Iron Levels. The quantity of coccidia in each coccidia-positive sample was plotted against the reported serum or liver iron level for each respective case. Previous tests had shown that only eight of the collected samples were positive for the presence of coccidia. The shape of the scatterplot suggests a negative correlation between coccidia counts in coccidia-positive samples and liver iron levels. The relationship to serum iron levels is difficult to assess due to the highly limited number of samples.

Toxicology results related to vitamin E liver levels were also obtained from diagnostic tests run by the University of Pennsylvania's New Bolton Center. Due to monetary limitations, vitamin E screenings were not conducted on samples collected by the undergraduate researcher. The following cases were obtained from query searches using the Laboratory Information Management System. These searches looked for cases submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University between 2006 and October 2017. The search was further refined to include cases occurring in calves between the ages of 0 days and 1 year. Cases that had been determined positive for the presence of coccidia and exhibited vitamin E deficiencies were further evaluated. Table 8 details coccidia quantification as well as liver vitamin E levels for such cases.

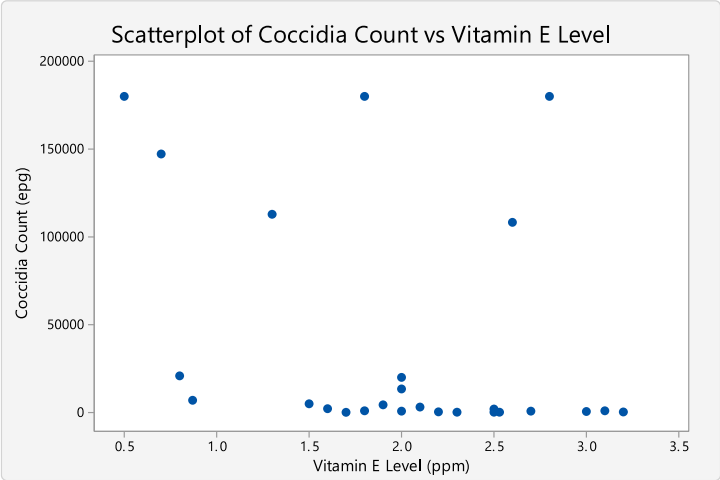
Table 8. Coccidia Quantification and Vitamin E Levels for Liver Samples. Each fecal sample is denoted by an accession number. The vitamin E level for each sample is also specified. All of these cases exhibit vitamin E deficiencies, as determined by the normal reference ranges for liver vitamin E levels provided by the University of Pennsylvania's New Bolton Center. The quantity of coccidia in each fecal sample was determined via fecal flotation. Samples that contained 180,000 eggs per gram of feces or more were classified as too numerous to count (TNTC). All samples were positive for the presence of coccidia.

Accession Number	Vitamin E Level (ppm)	Coccidia count (eggs per gram of feces)
P0700124	2.0	20,000
P0703967	2.7	800
P0826201	2.53	200
P0935652	2.0	13,400
P1006041	2.5	200
P1013329	0.87	7,000
P1116550	1.7	100
P1117430	2.2	400
P1122535	2.0	800
P1129446	2.8	TNTC
P1130021	3.2	300
P1209567	0.7	147,200
P1210863	1.8	TNTC
P1215330	2.6	108,300
P1314911	3.1	1,000
P1323438	1.5	5,000
P1323843	1.3	112,900
P1414516	3.0	600
P1504844	1.9	4,400
P1509236	1.8	1,000
P1604325	0.5	TNTC

P1612949	0.8	20,900
P1620360	1.6	2,200
P1623009	2.1	3,100
P1626435	2.3	200
P1628416	2.5	2,000

Vitamin E deficiencies were determined based upon normal physiological reference ranges for cattle provided by the University of Pennsylvania's New Bolton Center. The normal reference range for vitamin E in adult bovine liver is 4.0 to 9.0 ppm wet weight.

The following figure utilizes data from the cases described in Table 8 above. For coccidia counts that were classified as TNTC, the number of 180,000 epg was used for statistical analysis. Figure 5 exhibits the relationships between coccidia count in eggs per gram and vitamin E levels for liver samples.



80000

Vitamin E Level	26	2.0000	0.7429	0.5000	3.2000

Figure 5. Relationships Between Coccidia Count and Vitamin E Levels. The quantity of coccidia in each coccidia-positive sample was plotted against the reported liver vitamin E level for each respective case. All of these samples were previously determined to be deficient in vitamin E. The shape of the scatterplot suggests no correlation between coccidia counts in coccidia-positive samples and liver vitamin E levels.

The strength of the correlations for vitamin E levels was further analyzed using a simple linear regression model. The original model created exhibited large residuals (R) for three different cases: P1129446, P1210863, and P1215330. Based upon these results, those cases were determined to be outliers. Once the outliers were removed, another simple linear regression model was created, which is depicted in Figure 6. The analysis of variance table corresponding to the simple linear regression model is shown in Figure 7.

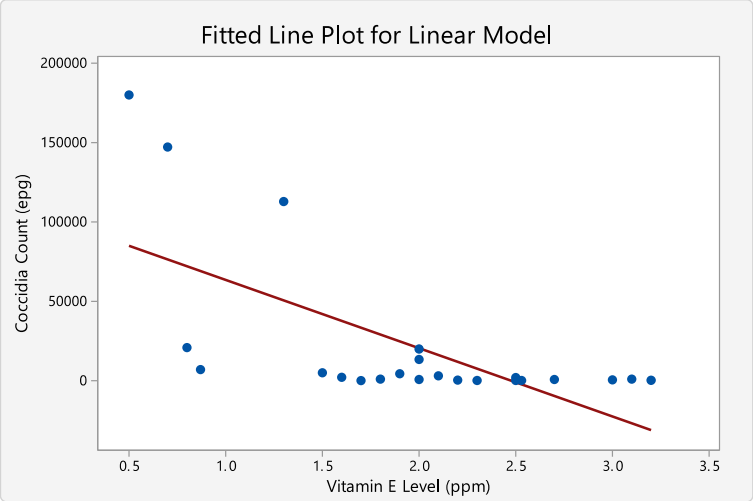


Figure 6. Simple Linear Regression Model Comparing Coccidia Count and Vitamin E Levels. Modeling of the relationship between coccidia counts and vitamin E levels via linear regression highlighted three outliers. Once those samples were removed from the analysis, the linear regression model demonstrated a negative correlation between coccidia count in coccidia-positive samples and vitamin E level.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	23468016458	23468016458	15.14	0.0008
Error	21	32554012238	1550191059		
Total	22	56022028696			

Model Summary

S	R-sq	R-sq(adj)
39372.5	41.89%	39.12%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value
Constant	106516	23037	4.62	0.0001
Vitamin E Level	-42995	11050	-3.89	0.0008

Regression Equation

Coccidia Count = 106516 – 42995 Vitamin E Level

Figure 7. Analysis of Variance and Simple Linear Regression Model Summary for Coccidia Count vs. Vitamin E Levels. Further statistical examination of the variation in the data was carried out. The calculated P-value for this relationship was much lower than the established significance level of 0.05. The reported R^2 value suggests that the model explains almost 42% of the variability of the coccidia count around its mean.

Cryptosporidium Antigen Detection ELISA

Each of the cases presented below were analyzed using an *in vitro* immunoassay for the qualitative determination of *Cryptosporidium* antigen in non-human fecal material. Cases that yielded an absorbance reading of 0.08 OD and above were samples that were classified as positive. Cases that yielded an absorbance reading of less than 0.08 OD were classified as negative.

Coccidiosis and Cryptosporidiosis Co-infection

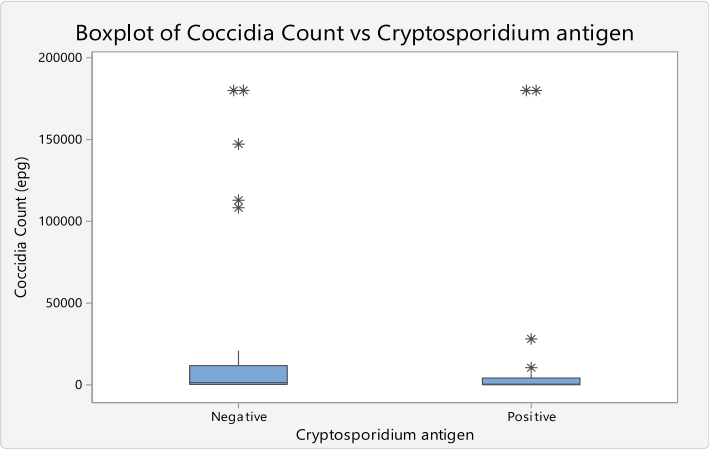
None of the bovine cases from which samples were collected between May and October 2017 were determined positive for both the presence of coccidia and *Cryptosporidium* antigen. In order to still explore possible relationships between coccidiosis and cryptosporidiosis, a query search was conducted using the Laboratory Information Management System. Data collected for cases submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University between 2006 and October 2017 were reviewed. The search was further refined to include cases occurring in calves between the ages of 0 days and 1 year. Cases that had been determined positive for the presence of coccidia were analyzed. Those cases that were also positive for *Cryptosporidum* antigen were further evaluated. Coccidia counts for *Cryptosporidium* antigen positive cases are described in Table 9 below.

Table 9. Coccidia Quantification in *Cryptosporidium* Antigen Positive Cases. Each fecal sample is denoted by an accession number. The quantity of coccidia in each fecal sample was determined via fecal flotation. Samples that contained 180,000 eggs per gram of feces or more were classified as too numerous to count (TNTC). All samples were previously determined positive for the presence of *Cryptosporidium* antigen.

Accession Number	Coccidia count (eggs per gram of feces)
P0731343 calf 2	TNTC
P0804636 calf 2	100
P0805112 calf 2	200
P0823124	200
P0824201	3,400
P0824306 calf 1	6,600
P0824306 calf 2	1,230
P0824306 calf 3	10,500
P0824785	500
P0826637 calf 4	1,200
P0827219-182	200

P0827219-235	100
P0828598	1,200
P0830539	100
P1301096	100
P1314746	28,100
P1321125	4,200
P1415391	100
P1416237	300
P1518891	500
P1715962	0
P1717579	TNTC

The relationships between *Cryptosporidium* antigen presence and coccidia counts were evaluated using side-by-side boxplots and analysis of variance tables – depicted in Figures 8 and 9 below. These statistical analyses also took into account cases that were positive for the presence of the coccidia and negative for the presence of *Cryptosporidium* antigen. Information regarding these cases – which were submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University between 2006 and October 2017 – was retrieved from the Laboratory Information Management System.



					0	180000	(600, 4601)
Positive	23	0	100	500	4200	180000	(145, 2428)

Figure 8. Comparisons Between Coccidia Count and the Presence of *Cryptosporidium* Antigen. The relationship between coccidia count and the presence or absence of *Cryptosporidium* antigen was evaluated by constructing a boxplot. The median value for coccidia count was much higher for cases determined to be negative for *Cryptosporidium* antigen.

Method

Null hypothesis	H_0: All means are equal
Alternative hypothesis	H_1: At least one mean is different

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Cryptosporidium antigen	2	Negative, Positive

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cryptosporidium antigen	1	731803822	731803822	0.26	0.6123
Error	53	149258773353	2816203271		
Total	54	149990577175			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
53067.9	0.49%	0.00%	0.00%

Means

Cryptosporidium antigen	N	Mean	StDev	95% CI
Negative	32	25609	54213	(6793, 44426)
Positive	23	18214	51411	(-3980, 40409)

Pooled StDev = 53067.9

Figure 9. One-Way Analysis of Variance for Coccidia Count versus Cryptosporidium Antigen Presence. Further statistical examination of the variation in the data was carried out. The calculated P-value for this relationship was much higher than the established significance level of 0.05. The reported R^2 value suggests that the model explains only 0.49% of the variability of the coccidia count around its mean.

Bovine Viral Diarrhea Virus Types I and II Polymerase Chain Reaction

Each of the cases presented below were analyzed via a one-step real-time reverse transcriptase-polymerase chain reaction for detecting bovine viral diarrhea virus types I and II. Samples demonstrating normal curve parameters with Ct values less than or equal to 36.9 were considered positive for BVDV Type I under the FAM probe or Type II under the Texas-Red probe. Samples with normal curve parameters and Ct values greater than 37 were considered very weak positive samples. The bovine cases from which samples were collected between May and October 2017 were all determined negative for Bovine viral diarrhea virus types I or II. Each case yielded a Ct value of 0.

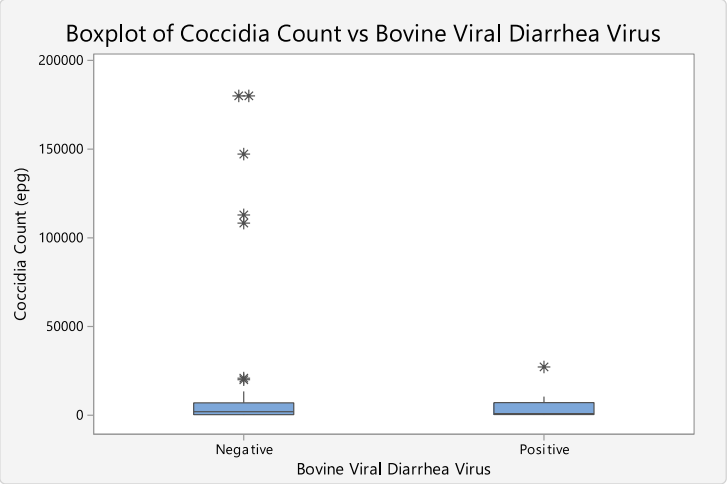
In order to explore possible relationships between coccidiosis and bovine viral diarrhea virus infection, a query search was conducted using the Laboratory Information Management System. Data collected for cases submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University between 2006 and October 2017 were reviewed. The search was further refined to include cases occurring in calves between the ages of 0 days and 1 year. Cases that had been determined positive for the presence of coccidia were analyzed. Those cases that were also positive for bovine viral diarrhea virus type I or II were further evaluated. Coccidia counts for bovine viral diarrhea type I or type II positive cases are described in Table 10 below.

Table 10. Coccidia Quantification in Bovine Viral Diarrhea Type I or Type II Positive Cases. Each fecal sample is denoted by an accession number. The quantity of coccidia in each fecal sample was determined via fecal flotation. Samples that contained 180,000 eggs per gram of feces or more were classified as too numerous to count (TNTC). All samples were previously determined positive for BVDV type I or type II.

Accession Number	Coccidia count (eggs per gram of feces)
P0725818	300
P0726084	400
P0824306 calf 1	6,600
P0824306 calf 2	1,230
P0824306 calf 3	10,500
P0901388	100
P0915931	27,200
P0921425	8,600
P0921980	600
P0928651	500
P0935383	200
P1614145	400
P1631061	1,600
P1721187	1,300

.

The relationships between bovine viral diarrhea virus infection and coccidia counts were evaluated using side-by-side boxplots and analysis of variance tables – depicted in Figures 10 and 11 below. These statistical analyses also took into account cases that were positive for the presence of the coccidia and negative for either type of bovine viral diarrhea virus. Information regarding these cases – which were submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University between 2006 and October 2017 – was retrieved from the Laboratory Information Management System.



							80000	(630, 4539)
Positive	14	100	375	915	7100		27200	(395, 6703)

Figure 10. Comparisons Between Coccidia Count and the Presence of Bovine Viral Diarrhea Virus Presence. The relationship between coccidia count and the presence or absence of BVDV type I or type II infection was evaluated by constructing a boxplot. The median value for coccidia count was significantly higher for cases determined to be negative for BVDV type I or II.

Method

Null hypothesis	H_0: All means are equal
Alternative hypothesis	H_1: At least one mean is different

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Bovine Viral Diarrhea Virus	2	Negative, Positive

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bovine Viral Diarrhea Virus	1	3805564903	3805564903	1.92	0.1723
Error	47	93123876836	1981359082		
Total	48	96929441739			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
44512.5	3.93%	1.88%	0.00%

Means

Bovine Viral Diarrhea Virus	N	Mean	StDev	95% CI
Negative	35	23760	52132	(8624, 38896)
Positive	14	4252	7449	(-19680, 28185)

Pooled StDev = 44512.5

Figure 11. One-Way Analysis of Variance for Coccidia Count versus Bovine Viral Diarrhea Virus Presence. Further statistical examination of the variation in the data was carried out. The calculated P-value for this relationship was much higher than the established significance level of 0.05. The reported R^2 value suggests that the model explains only 3.93% of the variability of the coccidia count around its mean.

Chapter 4 Discussion

Overview

Throughout this project, there were various challenges in terms of collecting an adequate number of samples in order to evaluate the desired relationships. Due to the lack of bovine samples submitted to the Animal Diagnostic Laboratory, a small portion of the samples collected was from ovine, cervine, and caprine species. Although a limited number of samples collected were by the undergraduate researcher, many of the aforementioned relationships were still evaluated. A large portion of these analyses used data from the Laboratory Information Management System. While the author did not directly collect these samples, the data provided from previous cases submitted to the Penn State Animal Diagnostic Laboratory was imperative to exploring the effects of various mineral and vitamin levels on the severity of coccidiosis, as well as the relationships between coccidiosis and concurrent diarrheal diseases.

Coccidiosis and Vitamin and Mineral Interdependence

In the present experiment, the presence of coccidia was determined in only eight of the 27 fecal samples collected. These samples were all collected from animals exhibiting signs of scours. The etiologic agents for calf scours include but are not limited to coccidia, *Cryptosporidium* spp., bovine viral diarrhea virus, bovine coronavirus, and bovine rotavirus. Therefore, the absence of coccidia, *Cryptosporidium* antigen, or bovine viral diarrhea virus in one of the samples examined in this project does not indicate a complete absence of scours. In terms of serum mineral levels, there were a surprising number of cases showing excess of certain minerals. All of these cases exhibited elevated serum calcium levels. The maximum range for serum calcium, courtesy of the University of Pennsylvania's New Bolton

Center, is denoted as 110 ppm. These cases were ten to twenty ppm higher than the reference maximum. There is a slight possibility that these high calcium levels were indicative specific mineral interactions with coccidia. However, all of these cases were from the same herd. In addition, none of the liver samples collected for the other cases exhibited abnormally high calcium levels. Therefore, it concluded that the widespread increase in serum calcium levels in cases 1 through 17 is consistent with chronic over-supplementation of dietary calcium. In contrast, these elevated serum calcium levels could be the result of severe dehydration. Significant gastrointestinal fluid loss due to diarrhea may have resulted in transient hypercalcemia.[12] Thus, over-supplementation and dehydration are both possible justifications for the elevated serum calcium levels reflected in the results of this study.

On the contrary, a large quantity of cases exhibited abnormal copper, iron, and selenium levels. These trends were seen with both serum and liver samples. Eight of the 27 total cases exhibited excess in selenium. However, only three of the eight total cases positive for the presence of coccidia displayed abnormal selenium levels. The scatterplot in Figure 2 suggests a positive correlation between coccidia counts in coccidia positive samples and liver selenium levels. The Pearson correlation value of 0.384012 denotes a weak positive correlation between the coccidia count in eggs per gram and selenium levels in ppm. However, the P-value of 0.5234 is much higher than the significance level of 0.05. Therefore, the weak positive correlation is not statistically significant.

In addition, five of the 27 total cases showed a deficiency in copper, while two cases showed an excess of copper. However, only two of the eight total cases positive for the presence of coccidia displayed abnormal copper levels. The scatterplot in Figure 3 suggests that there is negative correlation between coccidia counts in coccidia positive samples and liver copper levels. The Pearson correlation value of -0.805462 denotes a strong negative correlation between the coccidia count in eggs per gram and selenium levels in ppm. However, the P-value of 0.0999 is higher than the significance level of 0.05. Therefore, the strong negative correlation is not statistically significant.

Finally, eight of the 27 total cases exhibited excess in iron. Yet, only four of the eight total cases positive for the presence of coccidia displayed abnormal iron levels. Upon evaluation, the scatterplot in Figure 4 suggests a negative correlation between coccidia counts in coccidia positive samples and iron levels. The Pearson correlation value of -0.441015 denotes a moderate negative correlation between the coccidia count in eggs per gram and selenium levels in ppm. However, the P-value of 0.4573 is much higher than the significance level of 0.05. Therefore, the moderate negative correlation is not statistically significant.

On another note, the scatterplot depicted in Figure 5 suggests that there is no correlation between coccidia counts in positive samples and vitamin E levels. Once outliers were identified via the simple linear regression model, the Pearson correlation value was determined without these outliers present. The Pearson correlation value of -0.647230 suggests a moderate negative correlation between the coccidia count in eggs per gram and vitamin E levels in ppm. In addition, the P-Value of 0.0008 is much lower than the significance level of 0.05. Thus, the moderate negative correlation can be considered statistically significant. Further evaluation using the simple linear regression model suggests that 41.89% of the variation in coccidia count is explained by vitamin E level. According to these data, for each additional 1 ppm of vitamin E present in the liver tissue, the coccidia count in the fecal sample will decrease by 42,995 eggs per gram. Therefore, one can conclude that there is a negative correlation between vitamin E levels and the severity of coccidiosis infections. As vitamin E levels increase, one can expect to see a decreased quantity of coccidia eggs in the calves' feces. These results support the findings of previous studies, suggesting that vitamin E plays an important role in the disease status of an animal.[4] There is an imperative dependence between selenium and vitamin E, which influence susceptibility of the host to acute infections. [3] Since the results of this study suggest that vitamin E plays a large role in controlling coccidia infections, then selenium must also play a role in this process. Further research is required to determine the presence of this role and related mechanisms.

Overall, relationships seen between coccidia counts and selenium, copper, and iron levels were not considered significant. Additionally, some of the correlations that were depicted in these analyses did not support the original hypotheses. These discrepancies are in part largely due to the limited sample size used. Only eight of the collected samples contained coccidia. Thus, only these samples could be used for analysis. This left a very limited number of cases to evaluate in terms of mineral relationships. The small sample size does not provide an overarching and representative sample of infected cattle. Therefore, further studies must be conducted in order to clearly define the relationships between these minerals and coccidiosis severity.

Concurrent Cryptosporidiosis

There appears to be no correlation between the severity of coccidiosis and the presence of a concurrent *Cryptosporidium* infection. This is not consistent with the hypotheses formulated based upon the results of previous studies. The severity of cryptosporidiosis has been directly linked to the state of the host's immune system, the infective dose, and the presence of concurrent infections.[11] *Cryptosporidium* species cause debilitating damage to the gastrointestinal tract, and these protozoan parasites may be more likely to cause infection in a host with a weakened immune system.[2] Thus, it was hypothesized that there would be a direct link between coccidiosis severity and the presence of cryptosporidiosis. However, side-by-side boxplots revealed the median number of coccidia eggs per gram was much higher for cases that were determined to be negative for *Cryptosporidium* antigen. Further analysis of variance indicated that the only a minimal amount of 0.49% of the variation in coccidia count is explained by the presence or absence of *Cryptosporidium* antigen. Additionally, the P-value of 0.6123 was much higher than the significance level of 0.05. Therefore, it was concluded that there is not a significant correlation between the severity of coccidiosis and concurrent cryptosporidiosis. Discrepancies in the data may have occurred due to varying effects on the immune system that may have affected host susceptibility to disease. The

effects of coccidiosis on the immune system and the relationships between coccidiosis and cryptosporidiosis must be explored in future studies.

Concurrent Bovine Viral Diarrhea Virus

In a similar vein, there appears to be no significant correlation between the severity of coccidiosis and the presence of a bovine viral diarrhea virus type I or type II infection. This is not consistent with the hypotheses formulated based upon the results of previous studies. Previous studies suggest that bovine viral diarrhea virus increases susceptibility to other infections, the severity of other diseases, and the virulence of specific pathogens.[9] Yet, side-by-side boxplots revealed the median number of coccidia eggs per gram was much higher for cases that were determined to be negative for bovine viral diarrhea virus. Further analysis of variance indicated that the only 3.93% of the variation in coccidia count is explained by the presence or absence of bovine viral diarrhea virus infection. Also, the P-value of 0.1723 was higher than the significance level of 0.05. The data suggests that there is not a significant correlation between the severity of coccidiosis and concurrent bovine viral diarrhea virus Infection. While the virus may have made the host more immunocompetent, it did not increase the virulence of the coccidia pathogen. These results suggest that bovine viral diarrhea virus infections increase the susceptibility of the host to coccidia infections. Yet, the presence of the BVDV infection does not increase the intensity and severity of the coccidia infection once it is acquired.[9] The details of the effects of coccidiosis on the immune system and the relationships between BVDV and coccidiosis must be explored in future studies.

Future Experiments and Opportunities

In all, the results suggest that vitamin E may play a significant role in the susceptibility of the host to coccidiosis and the severity of coccidiosis. This suggests that vitamin E supplementation may be

an important factor for the prevention of coccidiosis in calves. However, more research must conducted to understand the mechanisms by which vitamin E is capable of reducing this type of infection. Although definitive relationships between coccidiosis severity and mineral levels were not established in this study, future studies must further explore these possibilities. Previous studies have suggested that deficiencies in iron, selenium, and copper are associated with coccidiosis.[8] Yet, the mechanisms behind these possible associations and correlations have not yet been explored. Larger sample sizes must be used in future studies to more effectively represent the calf population and elucidate these possible relationships.

In terms of concurrent infections, this study was unable to clarify the relationships between coccidiosis and cryptosporidiosis or bovine viral diarrhea infections. Discrepancies in the data may be due to underlying mechanisms regarding the roles of bovine viral diarrhea virus or *Cryptosporidium* species that have not been thoroughly explored. While the presence of these infections was not associated with increasing severity of coccidiosis, it is possible that these diseases did play a large role in the susceptibility of the host to coccidia infections. Thus, the relationships between coccidia and these other pathogenic agents must continue to be explored.

Appendix A Cryptosporidium Antigen Detection ELISA Templates

Software Version 2.07.17

Experiment File Path: R:\Animal_Diagnostic_Lab\AvianSerology\2017 ELISA reports\Experiment1.xpt
Protocol File Path:

Plate Number	Plate 1
Date	10/6/2017
Time	3:17:28 PM
Reader Type:	ELx808
Reader Serial Number:	Unknown
Reading Type	Reader

Procedure Details

Plate Type	96 WELL PLATE
Read	Absorbance Endpoint
	Full Plate
	Wavelengths: 450
	Read Speed: Normal

Results

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.841	0.038	0.041	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
B	0.035	0.033	0.038	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
C	1.264	0.035	0.039	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
D	0.037	0.032	0.033	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
E	0.050	0.040	0.039	1.097	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
F	0.035	0.036	0.040	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
G	0.170	0.033	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450
H	0.041	0.049	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	450

Figure 12. Bio-Tek Model ELX808 Absorbance Plate Reader Worksheet. The quantity reported in each well of the 96-well plate represents an absorbance value for a sample or control. A positive control was placed in well A1, while a negative control was placed in well B1. All samples with an absorbance reading of 0.08 OD or greater were classified as positive. Any samples with an absorbance reading of less than 0.08 OD were classified as negative. The samples present in wells E4 and F4 were eliminated from this study due to lack of other necessary data.

Appendix B Real-Time Reverse Transcriptase Polymerase Chain Reaction Graphical Representations

BIO-RAD

admin_2017-10-10 12-31-31_CC004157.pcrd

10/12/2017 1:38 PM

Report Information

User: BioRad/admin
Data File Name: admin_2017-10-10 12-31-31_CC004157.pcrd
Data File Path: C:\Users\Public\Documents\Bio-Rad\CFX\Users\admin\RT PCR\PESTI BVD\2017 BVD\Oct
Well Group Name: All Wells
Report Differs from Last Save: No

Run Setup

Run Information

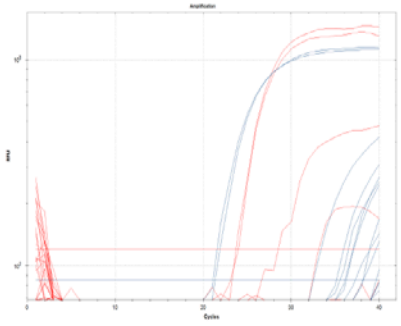
Run Date: 10/10/2017 12:31 PM
Run User: admin
Run Type: User-defined
Plate File: BVD plate.pltd
ID:
Notes:
Sample Volume: 50
Temperature Control Mode: Calculated
Lid Temperature: 105
Base Serial Number: CC004157
Optical Head Serial Number: 785BR2178

Protocol

1: 50.0°C for 30:00
2: 95.0°C for 15:00
3: 94.0°C for 0:15
4: 60.0°C for 1:00
Plate Read
5: GOTO 3, 39 more times

Quantification

Step #: 4
Analysis Mode: Fluorophore
Cq Determination: Single Threshold
Baseline Method:
FAM: Auto Calculated
Texas Red: Auto Calculated
Threshold Setting:
FAM: 84.99, User Defined
Texas Red: 120.22, User Defined



Quantification Data

Well	Fluor	Targer	Content	Sample	Cq	Cq Mean	Cq Std. Dev
A01	FAM		Unkn-01	Serum 1	N/A	0.00	0.000
A02	FAM		Unkn-02	Serum 2	N/A	0.00	0.000
A03	FAM		Unkn-03	Serum 3	N/A	0.00	0.000
A04	FAM		Unkn-04	Serum 4	N/A	0.00	0.000
A05	FAM		Unkn-05	Serum 6	N/A	0.00	0.000
A06	FAM		Unkn-06	Serum 7	N/A	0.00	0.000
A07	FAM		Unkn-07	Serum 8	N/A	0.00	0.000
A08	FAM		Unkn-08	Serum 9	N/A	0.00	0.000
A09	FAM		Unkn-09	Serum 10	38.17	38.17	0.000
A10	FAM		Unkn-10	Serum 11	N/A	0.00	0.000
A11	FAM		Unkn-11	Serum 12	N/A	0.00	0.000
A12	FAM		Unkn-12	Serum 13	N/A	0.00	0.000
B01	FAM		Unkn-01	Serum 1	N/A	0.00	0.000
B02	FAM		Unkn-02	Serum 2	N/A	0.00	0.000
B03	FAM		Unkn-03	Serum 3	37.24	37.24	0.000
B04	FAM		Unkn-04	Serum 4	N/A	0.00	0.000
B05	FAM		Unkn-05	Serum 6	N/A	0.00	0.000
B06	FAM		Unkn-06	Serum 7	N/A	0.00	0.000
B07	FAM		Unkn-07	Serum 8	36.08	36.08	0.000
B08	FAM		Unkn-08	Serum 9	N/A	0.00	0.000
B09	FAM		Unkn-09	Serum 10	N/A	0.00	0.000
B10	FAM		Unkn-10	Serum 11	N/A	0.00	0.000
B11	FAM		Unkn-11	Serum 12	N/A	0.00	0.000

Figure 13. Real-Time RT-PCR for BVDV Types I and II from October 10, 2017. The first run yielded a significant amount of background noise. Also, the positive amplification controls for both BVDV types I and II did not amplify as expected. This yielded inconclusive results that could not be adequately evaluated.

BIO-RAD

admin_2017-10-19 12-56-21_CC004157.pcrd

10/19/2017 3:18 PM

Quantification

Step #: 4
Analysis Mode: Fluorophore
Cq Determination: Single Threshold
Baseline Method:
VIC: Auto Calculated
FAM: Auto Calculated
Texas Red: Auto Calculated
Threshold Setting:
VIC: 113.02, Auto Calculated
FAM: 60.66, User Defined
Texas Red: 146.87, User Defined

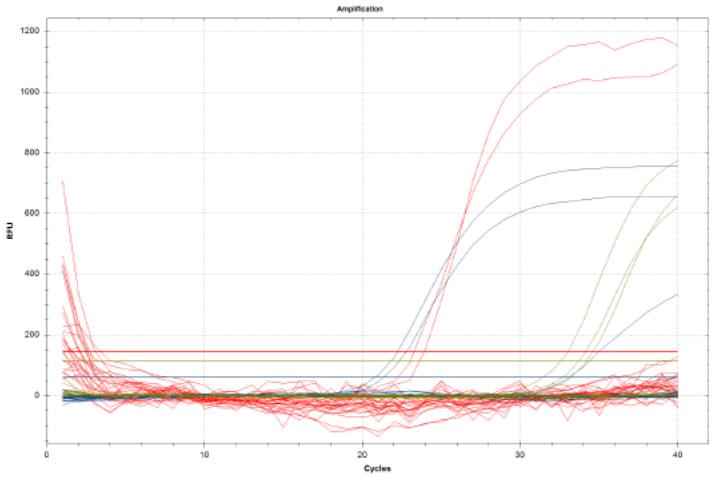
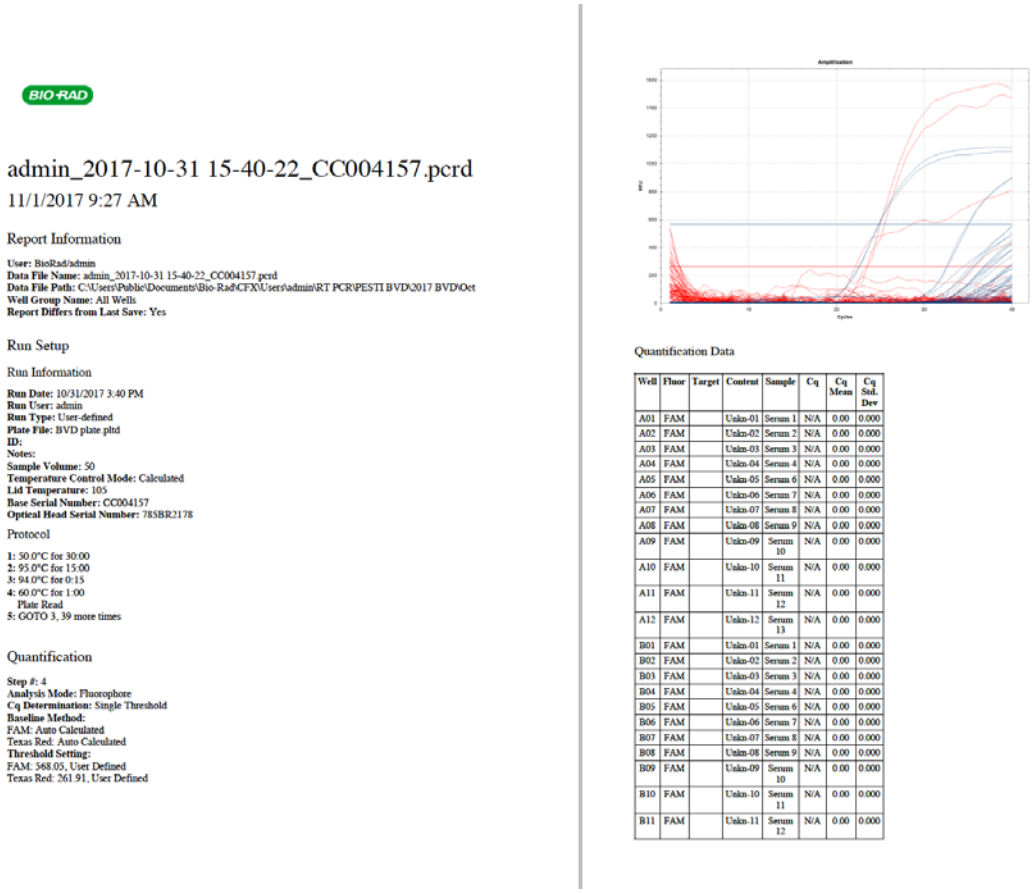


Figure 14. Real-Time RT-PCR for BVDV Types I and II from October 19, 2017. Samples that were suspected to be positive in the previous run were re-evaluated, along with positive and negative extraction and amplification controls. A Xeno primer probe was added to the BVDV Real-time reaction mix to serve as an internal positive control. Only three of the suspected samples amplified, and the positive extraction control did not amplify at all.



BIO-RAD

admin_2017-10-31 15-40-22_CC004157.pcrd
11/1/2017 9:27 AM

Report Information

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Data File Name: admin_2017-10-31 15-40-22_CC004157.pcrd
Data File Path: C:\Users\Public\Documents\Bio-Rad\CFX\Users\admin\RT PCR\PESTI BVD\2017 BVD\Oct
Well Group Name: All Wells
Report Differs from Last Save: Yes

Run Setup

Run Information

Run Date: 10/31/2017 3:40 PM
Run User: admin
Run Type: User-defined
Plate File: BVD plate pltd
ID:
Notes:
Sample Volume: 50
Temperature Control Mode: Calculated
Lid Temperature: 105
Base Serial Number: CC004157
Optical Head Serial Number: 785BR2178

Protocol

1: 50.0°C for 30:00
2: 95.0°C for 15:00
3: 94.0°C for 0:15
4: 60.0°C for 1:00
Plate Read
5: GOTO 3, 39 more times

Quantification

Step #: 4
Analysis Mode: Fluorophore
Cq Determination: Single Threshold
Baseline Method:
FAM: Auto Calculated
Texas Red: Auto Calculated
Threshold Setting:
FAM: 568.05, User Defined
Texas Red: 261.91, User Defined

Quantification Data

Well	Fluor	Target	Content	Sample	Cq	Cq Mean	Cq Std. Dev
A01	FAM		Unkn-01	Serum 1	N/A	0.00	0.000
A02	FAM		Unkn-02	Serum 2	N/A	0.00	0.000
A03	FAM		Unkn-03	Serum 3	N/A	0.00	0.000
A04	FAM		Unkn-04	Serum 4	N/A	0.00	0.000
A05	FAM		Unkn-05	Serum 6	N/A	0.00	0.000
A06	FAM		Unkn-06	Serum 7	N/A	0.00	0.000
A07	FAM		Unkn-07	Serum 8	N/A	0.00	0.000
A08	FAM		Unkn-08	Serum 9	N/A	0.00	0.000
A09	FAM		Unkn-09	Serum 10	N/A	0.00	0.000
A10	FAM		Unkn-10	Serum 11	N/A	0.00	0.000
A11	FAM		Unkn-11	Serum 12	N/A	0.00	0.000
A12	FAM		Unkn-12	Serum 13	N/A	0.00	0.000
B01	FAM		Unkn-01	Serum 1	N/A	0.00	0.000
B02	FAM		Unkn-02	Serum 2	N/A	0.00	0.000
B03	FAM		Unkn-03	Serum 3	N/A	0.00	0.000
B04	FAM		Unkn-04	Serum 4	N/A	0.00	0.000
B05	FAM		Unkn-05	Serum 6	N/A	0.00	0.000
B06	FAM		Unkn-06	Serum 7	N/A	0.00	0.000
B07	FAM		Unkn-07	Serum 8	N/A	0.00	0.000
B08	FAM		Unkn-08	Serum 9	N/A	0.00	0.000
B09	FAM		Unkn-09	Serum 10	N/A	0.00	0.000
B10	FAM		Unkn-10	Serum 11	N/A	0.00	0.000
B11	FAM		Unkn-11	Serum 12	N/A	0.00	0.000

Figure 15. Real-Time RT-PCR for BVDV Types I and II from October 31, 2017. The final run conducted using the Bio-Rad CFX96 C100 Thermal Cycler, all positive amplification and extraction controls amplified. All negative controls did not amplify and yielded Ct values of 0. Late background noise was eliminated by raising the threshold, but the ranges achieved for all control directly mirrored the expected values. All samples yielded Ct values of 0 and were determined negative for BVDV types I and II.

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Academic Vita

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EDUCATION

The Pennsylvania State University *University Park, PA* *8/14 – 5/18*

- Schreyer Honors College
- BS Veterinary and Biomedical Sciences

HONORS

- Gamma Sigma Delta: The Honor Society of Agriculture
- The President's Freshman Award

VETERINARY AND ANIMAL EXPERIENCE

Ritchie Veterinary Hospital *State College, PA* *6/17 – Present*
Veterinary Assistant

Restrained animals for physical exams and sample collection, including dogs, rabbits, and cats. Provided routine husbandry and care for the animals boarded in and admitted to the hospital. Managed the reception area, communicated with clients, and filled prescriptions. Cleaned the hospital and the exam rooms. Conducted various diagnostic tests using blood, fecal and urine samples. Prepared paperwork and samples for shipment to IDEXX Laboratories, Quest Diagnostics, and Antech Diagnostics for additional testing.

Elmwood Park Zoo *Norristown, PA* *5/15 – 8/15*
Animal Care Intern

Prepared animal diets and maintained exhibits. Created enrichment materials that induced natural animal behaviors. Directed injection trainings involving Chacoan Peccary and bobcats, which simplified the process of vaccine administration. Educated the public on various species and the importance of conservation. Communicated with zoo visitors and answered any questions that they presented. Restrained various types of birds, including Mute Swans and Sun Conures.

UNDERGRADUATE TEACHING EXPERIENCE

The Pennsylvania State University – ANSC201 *University Park, PA* *8/16 – Present*

Instructed an animal science laboratory of 30 members. Educated students on animal and poultry science, as well as genetic, physiological, nutritional, and health factors in food production. Demonstrated lab animal handling and the dissections of goats, pigs, and chickens. Created and graded lab quizzes. Graded each student based upon participation and exam scores. Provided hands on opportunities for students to connect classroom concepts to real-world situations, such as the use of fistulas in cows. Currently present lectures for those that did not previously pass the course.

EXTRACURRICULAR ACTIVITIES

- Penn State Pre-Vet Club *8/14 – Present*
- Volé: The Penn State Ballet Club *1/15 – Present*