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Griffith, Sara, M. Identification and Characterization of Eimeria spp. in Western North American Bison Herds and Risk of Cross-Species Transmission, M.S., Department of Veterinary Sciences, August 2020.

Abstract

The American bison (*Bison bison*) is an iconic native wildlife species of the Great Plains of North America. Recently, farmed bison have also gained importance to the livestock industry across the United States and Canada. One of the plentiful diseases in young bison is coccidiosis, a diarrheal disease caused by protozoa that can result in significant morbidity. The goal of the present study was to investigate occurrence and identity of bison gastrointestinal parasites, specifically coccidia of the genus *Eimeria* in both farmed and free-ranging bison with focus on potential *Eimeria* species transmissibility between bison and cattle. Individual bison (up to one year of age) were sampled across Wyoming, Colorado, Nebraska, and South Dakota on six bison ranches (n=137) and in two free-range herds (n=70). Both helminth and *Eimeria* populations were assessed by fecal analyses. Morphological identification revealed presence of different helminth eggs and oocysts consistent with *Eimeria (E.) bovis, E. zuernii, E. ellipsoidalis, E. cylindrica, E. alabamensis, E. auburnensis, E. canadensis, E. pellita, E. subspherica,* and *E. bukidnonensis*, all of which are described in cattle. Additional *Eimeria* species specific ITS1 sequencing data along with generated phylogenetic maximum likelihood trees suggest that *Eimeria* species from cattle, namely *E. bovis, E. zuernii, E. alabamensis, E. cylindrica, E. brasiliensis, E. ellipsoidalis,* and *E. wyomingensis*, are genetically consistent with the detected bison coccidia. In conclusion, the study results indicate that bison harbor a variety of gastrointestinal parasites. Bison *Eimeria* species appear to be transmissible between different bovine species such as bison and cattle.

Identification and Characterization of *Eimeria* spp. in Western North American Bison Herds and Risk of Cross-Species Transmission

By

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A thesis submitted to the Department of Veterinary Sciences

and the University of Wyoming

in partial fulfillment of the requirements

for the degree of Master of Science

in

Animal and Veterinary Science

Laramie, Wyoming

August 2020

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DEDICATION PAGE

I would like to dedicate this first to my family, for always believing in me and encouraging me to pursue what I love and to never give up even if things seem hard. Also to Erin, who was always there for me during the hard times and never got tired of my rants. And to Zack, thank you for pushing me to be the best version of myself and always knowing how to make me laugh. Finally to Link, for reminding me the importance of a good walk.

ACKNOWLEDGMENTS

Throughout this project I received help and support from various people at the Wyoming State Veterinary Laboratory and from various ranches across 4 different states. I would first like to thank my advisor, Dr. Berit Bangoura, for trusting me to take on this amazing project. She has been the best advisor I could have asked for and none of this would have been possible without her. I would also like to thank my lab-mate Morgane Vandendoren for teaching me so much about various protocols and always being with me for collections, even when it took us to Jackson for a whole weekend. I would also like to thank my committee members Dr. Gigley and Dr. Fox for giving me great advice throughout this process. I also had much help from the generous people in the parasitology lab such as Katie Bardsley and Gunnar Malmstrom who helped me with samples and let me use their lab space.

Of course, this whole project would not have been possible without the generous people who let me come to their ranches and sample their bison. I had an amazing experience being around these amazing creatures, and I couldn't have done any of this without their cooperation and generous help. I also must give a huge thank you to Ben Wise who helped me collect samples up at the National Elk Refuge as well as Karl Held who helped me with collection at the Soapstone Prairie Natural Area.

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Bison Population Dynamics

History of American Bison

The known history of the North American Bison (*Bison bison*) is an in depth one, one that dates back as far as the 1500's. It is estimated that back in the 16th century there were as many as 30 to 60 million bison that occupied North America ("Timeline of the American bison"). However, as people began to occupy the land, especially moving further west, they brought changes that included habitat modification, disease introduction, and grazing competition. In the 1870's large numbers of bison were slaughtered and in 1884, their numbers had dwindled to around 325 animals total in the United States, including only 25 bison in Yellowstone National Park. However, it is said that the plains bison (*Bison bison bison*) was saved from extinction by five private herds that were established by ranchers, as well as a sixth herd that was established at the New York Zoological Park (Hedrick, 2009). In 1905, the American Bison Society was founded to begin to protect the remaining bison in the United States. This protection the bison received obviously worked, as there were 20-25,000 bison in public herds and 250,000 bison in private herds at the end of the 1990's ("Timeline of the American bison").

However, this huge population decline in the 19th century resulted in bottlenecking of the population and therefore a lack of genetic diversity in today's herds. Previous studies observed that genetically homogenous populations tend to suffer from harsher disease outbreaks than populations that are more genetically diverse (King & Lively, 2012). As of today, the bison's

susceptibility to diseases has to be studied further, because of their vast history that includes a large population reduction that caused genetic bottlenecking.

Along with the genetic bottlenecking of this species, Hedrick mentions that a number of conservation herds, and nearly all productions herds contain some cattle (*Bos Taurus*) ancestry. It is estimated that only 1.5% of plains bison don't have any cattle ancestry (Hedrick, 2009). This is important to consider when looking into cross transmissibility of diseases between cattle and bison, as many of them may share common ancestry and genetics.

Bison as a Wildlife Species

The American bison has been a wildlife staple in the North American ecosystem for hundreds of years. As of 2014, there were 9,855 bison in US Federal herds as reported by the National Bison Association ("Bison by the Numbers"). As of 2011, they also reported 9,008 bison located in state and other public herds, as well as an estimated 20,000 bison that are on tribal lands ("Bison by the Numbers"). The geographic distribution of plains bison conservation herds extends from northern Mexico, all the way up to Canada and parts of Alaska and as far west as California. Eighty seven percent of these plains bison conservation herds are located in the original range of plains bison, with the outliers being herds in California, British Columbia, and Alaska (Gates et al., 2010).

Bison are inherently social creatures that tend to move in large groups that generally contain cows, calves, and immature bulls, with large, mature bulls forming much smaller and separate groups for much of the year. It is observed that these groups move between locations based on forage quality and season. For example, bison that typically reside in Yellowstone National Park spend their summers up in higher elevations, and move to winter down in lower

elevations (Gates et al., 2010). It is also noted that bison can drastically shape the ecosystem and are known as a keystone species in many areas. Bison share their landscape with so many species of plants and animals; it is easy to understand how their various behaviors can influence the overall ecosystem. Bison wallows that fill with water not only support various wetland species of plants, but also provide a breeding habitat for a couple species of plains toads. Grazing by bison affects various vegetation species by seed dispersal, physical disturbance, as well as stimulating nutrient recycling. Their grazing can also help small rodent species such as prairie dogs by decreasing the height of their local grasses (Gates et al., 2010). These reasons, among many others, are why bison are essential in their ecosystem and provide vital resources and interactions as a wildlife species.

Bison demographics are still fluctuating ever since their huge population decline in the 1800's. Population numbers vary quite drastically depending on the location as well as the weather that year. Currently, population declines can be caused by various diseases, droughts, and even very harsh winters with deep snow. Wolves can also contribute to population decline, as they are the one of the only successful predators of bison, other than humans (Gates et al., 2010).

Bison as a Livestock Species

While it is obvious that bison have been an important wildlife species for hundreds of years in North America, they also emerged as significant livestock species in the recent years. According to the 2017 census, there were 183,780 bison in the United States residing on private ranches ("Bison by the Numbers"). Numbers rose very quickly as people began to realize the various advantageous characteristics of bison and their domestication potential. Bison overall

possess many traits that people believe make them preferable to cattle. They have a greater ability to digest low quality forage, an ability to defend themselves well against natural predators, an ability to survive harsh weather conditions, and a very low incidence of calving problems (Gates et al. 2010) While bison do have these advantageous qualities as a livestock species, one of their most problematic qualities is that they tend to respond poorly to handling. While cattle are fairly used to being handled and put into chutes frequently, bison tend to be more difficult and can actually be quite dangerous to the handler. As it states in the American Bison Status Survey and Conservation Guidelines of 2010, the key to handling bison is exploiting their natural instincts (Gates et al. 2010). Since bison are strongly motivated by food, the fear of predation, as well as their need to be in social groups, handlers tend to use these motivations to handle bison in the easiest way possible (Gates et al., 2010).

Bison ranchers are selecting bison for breeding by traits that are considered advantageous or ones that should be excluded. According to Gates (2010), many bison ranchers' primary goals are to increase calf production, feed-to-meat conversion, as well as the meat quality. The traits that these producers tend to select for to achieve these goals are ones like docility, easy handling, conformation, growth performance, and reduced agility (Gates et al., 2010).

With increasing use of bison as livestock species, the commercial bison herds might pose a potential threat to the conservation of the wild species. If these genetically selected commercial animals were to escape their ranches and mix with conservation bison herds, it could majorly impact the genetics of the conservation herds.

Diseases and Cross-Species Transmission

Bison diseases are a huge consideration for both free-ranging and ranched bison populations and as of today, there still is a great need for investigation. Bison in their different habitats and husbandry conditions have a lot of contact with other wildlife as well as livestock, such as cattle. Especially infectious diseases may be transmitted across species, i.e. from other – mainly ruminant - species to bison and vice versa. This section will focus on parasites, bacteria, and viruses commonly found in bison as well as various other ruminants.

Bacteria

While there are various relevant pathogenic bacteria, two bacteria are exceptionally important for both wildlife and livestock, with a high cross-transmission potential between cattle and bison. The most commonly known bacterial disease in bison is brucellosis, caused by *Brucella (B.) abortus*. Brucellosis has been a highly important disease for bison and cattle alike for many years. This disease was first observed in bison as a wildlife species in 1917 in Yellowstone National Park and has grown to be a large problem in that area for both free-ranging bison and elk populations. While there has been a large effort to control and eradicate the disease within private herds, there is a lot of debate around management of Yellowstone National Park and other free-range areas and how to confine the disease in those areas (Tessaro, 1989). *B. abortus* can cause abortions, retained placentas, infertility, and arthritis in ruminants, and as a zoonotic disease it can cause influenza-like symptoms in humans (Rhyan et al., 2013).

Another bacterial disease that has been found in Yellowstone bison is hemorrhagic septicemia caused by *Pasteurella (P.) multocida*. This disease was the very first contagious disease verified in bison when it killed a large number of them in Yellowstone National Park in 1911. This disease has since ran rampant through various free-ranging herds across the United States and

caused severe mortality among bison calves (Tessaro, 1989). Luckily, in 1966 the *P. multocida* P-1459 strain was isolated and utilized to generate an inactivated vaccine for bison calves (Heddleston & Wessman, 1973). This vaccine was effective, and this serotype of *P. multocida* that caused hemorrhagic septicemia is no longer a problem in the United States.

Viruses

There are important viral pathogens at the wildlife-livestock interface that are infectious for bison. Certainly, one of the most relevant is the malignant catarrhal fever virus (MCFV) that was first reported in captive bison between 1973 and 1976 with a case fatality rate of up to 100%. MCFV related disease in bison is similar to cattle and symptoms include ulcerative lesions in the alimentary tract (Tessaro, 1989). Domestic sheep are a source of infection, while sheep rarely develop symptoms. While many of the discussed infectious diseases feature cross-species transmission, none of them are as detrimental to wildlife species whereas going undetected in domestic host animals.

Parasites

Parasites found in ruminants are arguably the most extensive and broad group of disease-causing agents and are observed frequently in both wildlife and livestock. Since there is such a variety of ecto- and endoparasites in North American bison, this section will focus on the most relevant intestinal parasites only, comprising roundworms and various species of protozoa. While free-ranging bison are generally not treated with antiparasitic drugs, ranched bison may potentially be subject to antiparasitic treatment (Eljaki et al. 2016). Still, intestinal parasite control is not a common focus of commercial bison production (Eljaki et al. 2016).

Regarding roundworms, most common species such as *Trichostrongylus* and *Nematodirus* belong to the Trichostrongyloidea superfamily. Trichostrongylids are small, hair-like worms that

have a direct, and usually not migratory life cycle (Mike A Taylor et al., 2016). All species of trichostrongylids can cause great morbidity and mortality and thus need to be considered on all ranching practices as well as in free-ranging bison. The life cycle begins when the larvae are ingested where they develop to the third larval stage in about 7-10 days and are able to penetrate the mucosa of the small intestine where eggs are then laid and expelled in the feces (Mike A Taylor et al., 2016). *Nematodirus* species can be fairly variable in appearance, however infections are easily distinguishable from other trichostrongylid species based on their very large egg size seen by fecal analysis. Most trichostrongylid roundworm genera can do severe damage to the intestinal tract of young ruminants, including bison, and should always be considered in de-worming protocols in the livestock industry.

Toxocara (T.) vitulorum. is a very long, and large roundworm that is commonly found in ruminants such as cattle and water buffalo (Woodbury et al., 2014). Upon infection of calves, larvae migrate from the intestine into the lungs where they are then coughed up, swallowed, and then able to mature into adult worms within the small intestine. While this roundworm can be quite damaging to young calves, it doesn't usually cause patent infections in individuals older than 6 months. When infecting the older hosts, the larvae migrate to muscles and other organs such as liver and kidney where they enter a hypobiotic stage (Woodbury et al., 2014). Woodbury et al. (2014) showed a prevalence of 77.8% in affected bison herds and high worm burdens capable of inducing symptoms such as diarrhea, intestinal obstruction, weight loss, and even death.

Protozoa are another group of parasites that have a large impact on wild and domestic ruminants. Besides *Eimeria* species, which are reviewed with the related disease coccidiosis in detail below, the main two protozoa to mention are *Cryptosporidium* and *Giardia*. The first interesting thing to note about these two parasite genera is that they are both containing zoonotic

gastrointestinal parasite species, and because of their zoonotic implication and their wide distribution, they are considered highly important to both human and veterinary medicine. In wild ruminants the prevalence for *Cryptosporidium* ranges from 0% to 8.8% and ranges from 0% to 12.3% for *Giardia* (Geurden et a. 2009). The prevalence for "captive wild ruminants" can also vary considerably based on stocking density, management technique, as well as age and species (Geurden et al., 2009). Geurden et al. (2009) sampled a commercial bison farm in southern Belgium and found that of the 82 animals sampled, three bison calves were found to have *Cryptosporidium* infections and 19 calves were found to be positive for *Giardia*. They also determined age to be an important factor in these infections and attributed the low prevalence of *Cryptosporidium* to the fact that most of the bison calves sampled were older than one month of age, and thus less susceptible. For *Giardia*, all positive calves were actually older than one month.

Coccidiosis

Coccidiosis caused by the protozoan parasite genus *Eimeria* is an important disease in young ruminants and vastly understudied in bison. There is a lot of current information about this parasite in cattle however. From experiences with cross-transmission between bison and cattle in other infectious diseases, knowledge on cattle coccidiosis should be taken into account when researching bison coccidiosis. Most of the information on bovine coccidiosis refers to cattle and is compiled below.

Epidemiology, Pathogenesis, and Clinical Signs

Eimeria species infections are becoming increasingly important to the livestock industry as they impact calves' health and productivity, which is problematic especially under the currently

mounting economic pressure on livestock operations. While there are 12 valid species of *Eimeria* described in cattle (Bangoura and Daugschies 2019), only *Eimeria bovis* and *Eimeria zuernii* are responsible for severe disease, with *Eimeria alabamensis* being a less virulent pathogen (Daugschies & Najdrowski, 2005). The 12 valid species of *Eimeria* described in cattle include: *E. bovis, E. zuernii, E. alabamensis, E. auburnensis, E. bukidnonensis, E. brasiliensis, E. cylindrica, E. canadensis, E. ellipsoidalis, E. wyomingensis, E. pellita,* and *E. subspherica*. All cattle *Eimeria* species are considered to be strictly host specific and have a monoxenous lifecycle with both an internal and external phase. The internal or parasitic phase of this life cycle includes two different reproduction cycles within it, merogony (asexual reproduction) and gamogony (sexual reproduction). The external or environmental phase includes sporogony of the oocyst in the environment. Both internal merogonies and gamogony happen within the cells of intestinal mucosa, with the cell type differing for each species. Various species tend to develop in lacteal endothelial cells, lamina propria, the epithelial lining of the intestine, or in some cases the cytoplasm or nucleus of endothelial cells (Daugschies & Najdrowski, 2005). Each developmental step destroys many host intestinal cells, which is why this parasite is capable of causing serious damage.

Young calves that get infected with large numbers of oocysts from either *E. bovis* or *E. zuernii* can develop severe and sometimes hemorrhagic diarrhea, along with hyperthermia, anemia, dehydration, anorexia, and in severe enough cases, may die. Mortality for coccidiosis is quite variable and can be anywhere from 7-20% (Daugschies & Najdrowski, 2005). Even if mortality for this disease is comparatively low, many calves experience anemia and some may never recuperate to their full potential or they may be more susceptible to other diseases (Lassen and Ostergaard 2012). *Eimeria alabamensis* tends to have less of an effect on calves, with their

symptoms including watery diarrhea and reduced growth, however some of this may even be due to co-occurrence of other pathogenic *Eimeria* species or other diseases (Daugschies & Najdrowski, 2005).

Impacts

Coccidiosis has been proven to have severe effects on the livestock industry, both in terms of animal welfare and economy. With severe disease, animals have reduced growth and their sale weight might be decreased significantly. Additionally, the cost of coccidiosis treatment may amount to significant economic damage if many animals within the herd are infected. Lastly, there is the cost of mortality. According to Matjila and Penzhorn (2002), coccidiosis can cost cattle ranchers more than $400 million per year because of problems such as reduced weight gain, reduced feed efficiency, and susceptibility to other diseases. Lassen and Ostergaard found a similar pattern in their study on economic effects of *Eimeria* infections on dairy cattle. They discovered that the annual losses to the gross margin of the herd due to *Eimeria* ranged from 8-9% (Lassen & Østergaard, 2012), and factored subclinical effects as major part of the losses.

Control and Treatment

With coccidiosis causing such large problems within the livestock industry, it is important to take control measures to decrease the prevalence of this disease within a herd. Prevention is the best course of action for this disease, as many species of *Eimeria* can be very difficult to get rid of once they are found within the herd. Control measures that can be taken to prevent this parasite in the first place include good hygiene practices, lower animal density, as well as floor type or pasture type, depending on the operation (Daugschies and Najdrowski 2005). For animal housing,

commercial disinfectants can be used to decrease the number of oocysts in the area, as well as using slatted floors, so feces aren't able to accumulate in the pens. Since disinfection of a pasture is not possible, a rotating system should be implemented so that the animals aren't always on the same pasture, and they can be turned out to clean pastures every year. Wet areas of the pasture should also be fenced off and avoided, as this parasite accumulates in a moist environment (Daugschies & Najdrowski, 2005).

There are also prophylactic drugs used to assist control of this parasite. Most of these drugs have to affect the parasite at an early stage of its life cycle so that vast intestinal damage during internal development is prevented. Sulphonamides attack the asexual reproduction phase of the parasite but have also been shown to help with secondary infections, as they also can be effective against certain bacteria. Monensin is an ionophorous antibiotic that can also be effective against *Eimeria* if it is given in feed over an extended period of time. Other drugs that can be suitable prophylactic treatments for cattle include amprolium and decoquinate (Daugschies and Najdrowski 2005). While there are many options for prophylactic drug treatments, the very serious subject of anticoccidial resistance has to be considered. This type of drug resistance has been shown in poultry, and it is now a worry with cattle as well. This topic still needs to be investigated more, however these anticoccidials should be used with this resistance in mind and should not be used with high frequency and for extended periods of time (Daugschies & Najdrowski, 2005).

When preventive control measures are not sufficiently effective, treatment of the disease has to be considered. Once scouring calves are removed from the rest of the herd, they can be treated therapeutically with drugs like amprolium or sulfonamides that also target the gamont phase of the life cycle (Daugschies and Najdrowski 2005). They can also be given electrolytes, anti-diarrheal medication, and antibiotics if a secondary infection is present (Daugschies &

Najdrowski, 2005). In general, coccidiosis is a self-limiting disease, and once the internal life-cycle of *Eimeria* is concluded, the infection is cleared, unlike many other parasitic infections (Daugschies and Najdrowski 2005).

Diagnostics

The most common way to diagnose coccidia within an animal is through the examination of fecal samples by performing a fecal flotation. Oocysts can be found easily after the flotation through the use of a light microscope (Daugschies and Najdrowski 2005). It is recommended to examine the feces of multiple animals in the herd to get an estimate of the prevalence within the herd. Different *Eimeria* species are commonly distinguished by morphological characteristics, and for bison, currently the morphological keys for bovine *Eimeria* are utilized. Serological methods such as ELISA and Western blots have been developed for *E. bovis*, however it has major drawbacks such as having cross reactivity between various species (Daugschies & Najdrowski, 2005), and it is not commercially available. Furthermore, antibody tests may not distinguish between active and recent infections.

More recently, cattle *Eimeria* PCR tests have been developed, especially for research purposes. Today, it is fairly common to use PCR for exact *Eimeria* species diagnosis by targeting the internal transcribed spacer 1 (ITS1) region within the small subunit ribosomal DNA (SSU-rDNA) in these *Eimeria* species. This region lies between the 18S rRNA gene and the 5.8S rRNA gene and because of the heterogeneity of this region in different species, it is thought to be a suitable target for species specific primers (Kawahara et al., 2010). While this is a good region to design species specific primers, there are always some limitations such as the chance for cross-reaction between species. Kawahara et al. (2010) designed primer pairs specifically amplifying

ITS1 gene fragments of *E. bovis*, *E. cylindrica, E. ellipsoidalis, E. zuernii, E. alabamensis*, and *E. auburnensis*, respectively. They constructed phylogenetic trees for all of their isolates to describe the relatedness between the investigated species (Kawahara et al., 2010). Similarly, Pyziel et al. (2019) identified *Eimeria bovis* oocysts in European bison where they also used SSU-rDNA sequences for their alignment. With this PCR, they were able to discover that there was a high degree of homology found between *E. bovis* found in European bison and those found in cattle (Pyziel et al., 2019). Both of these studies show that PCR is on its way up to becoming the new way to detect *Eimeria* in livestock animals and will hopefully provide a future routine tool for reliable *Eimeria* species identification.

Chapter 2: Introduction

The American bison (*Bison bison*) has been roaming Western North America as a significant wildlife species for hundreds of years. In more recent years they have acquired importance as a livestock species as well. With bison continuing to gain attention, their interactions with other domestic ruminant species such as cattle is becoming more frequent and relevant. Both free-range and farmed bison have contact with species such as cattle, which can be extremely significant when it comes to spread of various infectious diseases. It is known that bison, much like other free-ranging wildlife species, can harbor different pathogens that can be transmissible to cattle. These pathogens can include bacteria, viruses, as well as various parasites; one of which includes the protozoa of the genus *Eimeria*. While there are 12 valid species of *Eimeria* described in cattle, and 8 more species that are uncertain in validity (Bangoura & Daugschies, 2019), there are only seven species that are currently reported in bison. *Eimeria (E.) bovis* and *E. bukidnonensis* were said to be the first species to be reported in 1930,

followed by *E. auburnensis*, *E. brasiliensis*, and *E. canadensis* found on a ranch outside of Gillette, Wyoming in 1975 (Ryff & Bergstrom, 1975). The findings of these species were then followed by *E. zuernii* and *E. ellipsoidalis* that were discovered and reported in Montana (Penzhorn et al., 1994). While *Eimeria* species are generally considered to be strictly host specific, all seven of these species described in American bison are consistent to those found in cattle and share the same morphological characteristics. Along with the species above, bovine species *E. alabamensis, E. cylindrica, E. pellita,* and *E. subspherica* have been reported in European bison (*Bison bonasus*) (Pyziel et al., 2014). While European bison are different from American bison geographically, they share the same genus. While the published morphological observations beg the question of cross-transmissibility of *Eimeria* species between different bovines, no evidence of this has been published so far.

Currently, the main identification method of *Eimeria* species in American bison is by morphology (Ryff & Bergstrom, 1975). However, this can be problematic due to morphologic features of these *Eimeria* species exhibiting high similarity, especially if it is not clearly proven that Eimeria from cattle are indeed consistent with bison *Eimeria* species at all. Without clear genetic data available for any bison *Eimeria* species, it can be difficult to definitively identify *Eimeria* down to a species level and decide if bison share cattle *Eimeria* species or harbor their own host specific variants. Sequence-based identification will be a useful tool to acquire a definitive diagnosis of *Eimeria* spp.

While not the most widely studied, bovine coccidia are highly important parasite species. Calves that are exposed to coccidia can suffer from a disease known as coccidiosis, which can cause severe diarrhea, weight loss, and in some cases mortality. These symptoms can cause huge economic losses in both the cattle and bison industries. Coccidiosis has been shown to cost cattle

ranchers more than $400 million dollars a year because of problems such as reduced weight gain, reduced feed efficiency, and enhanced susceptibility to other diseases (Matjila & Penzhorn, 2002). With these economic losses, prevention of coccidia infections in both commercial farming and potentially also wild populations is of the utmost importance.

Chapter 3: Research Goals

The overall aim of this study is to further investigate the coccidia fauna of bison. As there are only three reports of *Eimeria* in American bison (Penzhorn et al., 1994; Ryff & Bergstrom, 1975) with one of them being from an Italian zoo (Fagiolini et al., 2010), it is a novel subject that needs further research. The research goals of this study are to identify the gastrointestinal parasite spectrum in farmed and free-range American bison less than one year of age, identify *Eimeria* species variety and the number of *Eimeria* species present within American bison, and lastly to test the relatedness of American bison *Eimeria* species with cattle *Eimeria* species. With these research goals come three separate hypotheses. Hypothesis (1) being that there will be several *Eimeria* species present within both farmed and free-ranging American bison herds that will both morphologically and genetically resemble those found in cattle. Hypothesis (2) is that there will be a correlation between husbandry conditions and *Eimeria* spp. infections prevalence. Lastly, hypothesis (3) states that there will be a high prevalence of a variety of gastrointestinal nematodes as well as a high prevalence of *Eimeria* species in young American bison, with a higher prevalence to be expected in farmed bison compared to free-ranging due to higher transmission potential in confined husbandry. By studying prevalence of coccidia in bison, we can learn more about this parasite in regard to its impact on free-ranging bison populations and various bovine livestock species including bison and cattle.

Chapter 4: Materials and Methods

4.1 Study herd selection

Animals selected for sampling was done based on the compliance of either the rancher or the wildlife biologist in charge. With this convenience sampling, there were different herd numbers between farmed and free-range herds. There were six farmed bison ranches (n=137) sampled across Wyoming, Colorado, Nebraska, and South Dakoda with 10-50 individual samples taken from each herd. There were individual animals sampled (n=70) from two free-range herds sampled one being located on the National Elk Refuge near Jackson, Wyoming, and the other being located at the Soapstone Prairie Natural Area near Fort Collins, Colorado.

4.2 Questionnaire/ meta data collection

A questionnaire was filled out by an individual from each farmed herd. This questionnaire included questions on husbandry conditions such as number of bison on the operation, contact with cattle, disinfection measures, and past infection knowledge. The questionnaire can be viewed in its entirety in the appendix (Appendix A).

4.3 Fecal Collection

Sample collection varied slightly from farmed to free ranging herds, but the principle was generally the same. For farmed bison, a vehicle was taken out into the bison herd and younger bison (less than a year old) were observed. When the younger bison defecated, a large handful of the individual fecal sample was collected off the ground with an obstetrical (OB) glove and placed in a cooler (4°C-10°C). This process was done for all six bison ranches mentioned

above. For two of these ranches, this method was explained to the ranch owner and they performed it themselves and then sent samples directly to the lab for processing.

For free-range bison, the herd was located, and samples were collected off the ground in the area around the herd. Fecal samples were examined and judged based on consistency and color to be likely derived from a bison less than a year old then collected in an OB glove and placed in a cooler. This process was done for the two different free-range herds mentioned above.

4.4 Fecal Flotation

The feces collected were first judged based on fecal consistency following a well-established fecal scoring system as a parameter for clinical coccidiosis in cattle (Bangoura & Daugschies, 2007).

The feces were then examined by a semiquantitative fecal flotation method. Five grams of feces were placed in a 15mL conical tube. 10mL of water was added to the conical tube and the feces were mixed thoroughly with a wooden stir stick. The sample was spun down at 380 x g for 2.5 minutes. The supernatant was decanted, and water was added to bring the volume up to 15mL. It was spun down again at 380 x g for 2.5 minutes and the supernatant was decanted. 10mL of Benbrook's sugar solution (SG = 1.20) was added and mixed. The sample was spun down for 5 minutes at 380 x g. More sugar solution was added slowly until the fluid was slightly bulging over the top rim of the conical tube. A cover slip was placed on top of the conical tube and left for at least 10 minutes to allow flotation and attachment of parasite stages (oocysts, worm eggs) to the downside of the cover slip. The cover slip was then placed on a microscope slide and viewed under a microscope. Parasitic stages were identified (nematode eggs, tapeworm eggs, *Eimeria* oocysts). The different species of *Eimeria* found were identified and recorded

based on morphology described for cattle *Eimeria* species (M A Taylor & Catchpole, 1994). Other parasites were identified to a genus level (*Moniezia* species, *Trichuris* species, *Nematodirus* species, *Capillaria* species) or family level (trichostrongylid worms). The amount of parasite stages detected in each sample was scored semiquantitatively as "(+)" (rare stages), "+" (few stages), "++" (moderate amount of stages), or "+++" (numerous stages) for each individual parasite type detected.

4.5 Oocyst Purification and Storage

The fecal samples were placed in a two-liter beaker and then filled with water to cover the sample completely. Depending on the sample volume, the feces were then mixed either with a hand blender or a wooden tongue depressor until the mixture was completely homogenized. The homogenized feces were passed through 850 µm and 250 µm sieves (Gilson, Lewis Center, OH, USA) and rinsed with water. The mixture was then put back into the beaker and completely filled with water and left to sediment overnight.

The next day, the supernatant of the beakers was carefully decanted in one motion as to not dump out the bottom sediment layer. The sediment was mixed up in the remaining fluid and distributed evenly into four 500mL tubes. Water was added to balance the weight of the four tubes. They were then spun down at 1500 x g for 7 minutes at room temperature. The supernatant was discarded, and the process was repeated until the supernatant was light yellow in color. It could be washed again if needed to obtain a solution containing few fecal debris. Benbrook's sugar solution was added to the sediment layer and they were mixed thoroughly. Additional sugar solution was added to balance the weight of the tubes. There should be roughly an 8:1 ratio of sugar to sample. They were mixed further by inverting the tubes and

centrifuged again at 1500 x g for 7 minutes. The upper half of the supernatant was poured off into (a) large beaker(s). More of the supernatant was poured if initial oocyst contents was low (score (+) or low amount of initial fecal sample). The beaker was then filled with water in a 1:10 sample to water ratio. The beakers were set aside to sediment overnight again.

The next day the supernatant was poured off carefully and the bottom layer was mixed again with the remaining fluid. It was then poured into 500mL tubes once again, weighed, and centrifuged at 1500 x g for 7 minutes. The supernatant was poured off and the samples were washed again if they needed to be, based on the clarity of the solution.

If the samples had only *Eimeria* oocysts in them per semiquantitative flotation analysis performed beforehand, a 1:1 ratio of 4% potassium dichromate was poured into the bottles to obtain a final concentration of 2% potassium dichromate as antifungal and antibacterial preserving agent. Samples were then transferred to cell culture flasks and placed on a plate shaker.

If the samples had *Eimeria* oocysts as well as worm eggs; streptomycin and penicillin (1mL), and amphotericin B (1mL) were used per 50mL of sample. This was all added to a cell culture flask and placed on a plate shaker. The samples were then set aside to sporulate, being shook on the plate shaker once a day to allow oxygen to get into the flasks. Once sporulation occurred, the flasks were then stored at 4° C.

For the purification process, samples that had the same *Eimeria* species per the fecal flotation were pooled to increase oocyst number. Samples were only ever pooled within the same herd and there was no pooling of samples between different herds.

4.6 Excystation and DNA Extraction

For molecular analysis, *Eimeria* positive samples were subject to oocyst excystation (cracking) and subsequent DNA extraction. Excystation started by putting the sample from the flask into 50 mL conical tubes. These were then centrifuged down at 2061 x g for 6 minutes. The supernatant was discarded, and the sample was washed with non-sterile PBS. It was centrifuged once more, and the supernatant was again discarded. The pellet was suspended in 5-10 mL of PBS and placed into a 15 mL conical tube prepared with a central hole in the cap. The tube was then placed in an insulated ice bucket with ice packed around it. The probe of the ultrasonificator (Fisherbrand™ Model 120 Sonic Dismembrator) was then placed into the sample until it was completely submerged. The ultrasonificator was then ran in pulse mode for 6 minutes, 02 pulse on, 02 pulse off, at 60% amp. The sample was then centrifuged at 2465 x g for 10 minutes then the supernatant was discarded. The sample was subsequently stored at -80° C.

For DNA extraction from these samples, the protocol from the Macherey-Nagel Nucleospin soil kit was followed according to the manufacturer's recommendations (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany).

4.7 Amplification of ITS1 Region

A modified version of previously published PCR primer sequences were used, and targeted partial sequences of the ITS1 gene of various *Eimeria* species (Kawahara et al., 2010). Additional primers were created by using the Primer-BLAST tool on the NCBI website (Ye et al., 2012). Due to few sequences available in NCBI GenBank for most bovine *Eimeria* spp. along with relatively short fragment lengths reported, primer pairs spanned about 150 to 500 base pairs product length for the individual *Eimeria* sp. PCRs. Another factor contributing to the

short fragment length to be amplified was that the primers needed to be highly specific while similarities between different bovine *Eimeria* spp. were present for the target fragment. All primers that successfully amplified their species and were sequenced are indicated in Table 1.

Positive plasmid controls were made specifically for each *Eimeria* spp. tested for by inserting the cattle *Eimeria* spp. ITS1 partial sequences found from the NCBI GenBank database into a vector and cloning it. This was performed by Genewiz (South Plainfield, NJ) using the ValueGENE service.

These regions were amplified using 25 µM of each species-specific primer, 25 µM of each dNTP within a mix (Promega dNTP Mix), 1X PCR Buffer (Thermo Fisher DreamTaq 10X Buffer), and 0.625 U/reaction of polymerase (Thermo Fisher DreamTaq 5U/µL Polymerase) for a total volume of 50 µL. The PCR was ran on a Veriti thermocycler (Thermo Fisher Scientific) with an initial denaturation of 95° C for 6 min followed by 40 cycles of a denaturation of 95° C for 30 s, a variable annealing temperature depending on the primer used (Table 1) for 30 s, followed by an extension step of 72° C for 30 s then a final extension step at 72° C for 7 min.

4.8 Agarose Gel Excision and Amplicon Purification

A gel was run with 2% agarose and with a large comb inserted. At least 50µL of sample was loaded into the gel. The gel was run on a Thermo Fisher Scientific electrophoresis system anywhere from 120 to 180 volts depending on the size of the gel. It was then soaked in ethidium bromide for 30 minutes and imaged on a BioRad Gel Documentation system. Once imaged, the gel slice containing the DNA fragment was excised using a clean scalpel blade, cutting as close to the band as possible. Gel excision and purification was performed by

following the protocol of Thermoscientific GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA).

4.9 Sequencing and Data Analysis

Samples were sent in for Sanger sequencing following the protocol for a pre-mixed, purified PCR product from Genewiz (South Plainfield, NJ). The sequencing results were analyzed using the Nucleotide-BLAST tool on the NCBI website and poly peak parser (Hill et al., 2014). Sequencing data was then put into MEGAX 10.1 software (State College, PA) where it was aligned by muscle. Maximum likelihood phylogenetic trees were then created for each species isolate found using MEGAX 10.1 with 1,000 bootstrap replications.

Statistical analysis of the light microscopic findings and correlations with the questionnaire data was done using the PSPP statistical program (GNU PSPP 1.3.0). Kruskal-Wallis tests as well as bivariate correlations were used to determine if there were any statistically relevant correlations between findings within the samples and the husbandry conditions on the ranches. The data was separated out between farmed data, free-range data, and combined data that included both farmed and free-range.

Chapter 5: Results

5.1 Questionnaire Data

Through questionnaire data that was taken from each ranch, it was shown that the sampled herds featured variable herd sizes, with the average being about 1,300 bison per herd. Most of the operations had a rotating pasture system and their bison either had limited contact to

cattle through a fence, or none at all. Larger herds were usually separated into groups of some kind and water sources varied from well water to creeks, or various combinations. None of the ranches used any kind of routine disinfection measures during calf rearing and only two ranches were aware of past coccidal infections within the past five years. All ranches but one used some kind of not further defined antiparasitic drug on their operation, however only one ranch used any type of anticoccidial preventative treatment. Relevant questionnaire data can be seen summarized in Table 2.

5.2 Fecal consistency scoring

Fecal scores were assessed when the samples were examined, and for all samples there was an average fecal score of 1 (normal/pasty). There were 26 total samples on 3 ranches and both free-range herds that had fecal scores of 2 (liquid/semi-liquid) and there were no samples that had fecal scores of 3 (watery) or 4 (containing blood/tissue).

5.3 Parasite burden

Viewing the samples through a light microscope after the fecal flotation process revealed presence of different parasite stages. Various *Eimeria* species were found within the samples by using morphological descriptions available for cattle *Eimeria* species. Detected species included *E. bovis, E. zuernii, E. ellipsoidalis, E. alabamensis, E. auburnensis, E. cylindrica, E. canadensis, E. bukidnonensis, E. subspherica* and *E. pellita*. Various species of worms were also observed. These included *Moniezia, Nematodirus,* trichostrongylids*, Trichuris ovis, Capillaria,* and *Strongyloides*. *E. bovis* had the highest prevalence within all of the samples (n=207) with a percentage of 33.3%. This was followed by *E. ellipsodialis* with a prevalence of 32.8% and then

by *E. zuernii* at 13.5%. *E. alabamensis* had a lower prevalence of only 3.4%. While all of the average flotation scores for *Eimeria* species were (+), there were some that got as high as ++. Summarized data on all *Eimeria* species as well as all worm species found during the fecal flotation process can be seen in Table 3.

5.4 Impact of ranch management parameters on *Eimeria* burden

Statistical analysis on semiquantitative flotation results data alone as well as in conjunction with questionnaire data was performed. Presence of *E. zuernii* and *E. ellipsoidalis* oocysts in fecal samples were correlated both for combined data ($p < 0.001$) and farmed-only data ($p < 0.001$), indicating that *E. zuernii* and *E. ellipsoidalis* commonly appear simultaneously in the analyzed samples. No other species seemed to show this same correlation. Based on Kruskal-Wallis tests, presence and semiquantitative score for *Eimeria* spp. were negatively related to the use of anticoccidial drugs though no statistical significance was observed (*E. ellipsoidalis* $p < 0.001$, *E. bovis* $p < 0.079$, *E. zuernii* $p < 0.092$, *E. alabamensis* $p < 0.038$), i.e. ranches that didn't use any form of anticoccidial treatment were tentatively featuring a higher *Eimeria* burden in young bison. A similar trend was observed for the use of any unspecified antiparasitics, i.e. ranches using antiparasitics tend to have partially significantly lower *Eimeria* burdens within the herd offspring (*E. ellipsoidalis* $p < 0.001$, *E. bovis* $p < 0.009$, *E. zuernii* $p < 0.004$, *E. canadensis* $p < 0.001$).

5.5 Impact of parasite burden on clinical signs

There was no significant correlation between *Eimeria* burden for any given *Eimeria* sp. and the observed fecal consistency score. The same was true for the burden of any detected worm species/genus/family and fecal consistency score.

5.6 PCR and Sequence Analysis

All microscopically *Eimeria* positive pool samples were subject to conventional PCR. All described species-specific PCR reactions (see Table 1) were performed on every single sample, irrespective of the actual *Eimeria* spp. detected by light microscopy. Once pooled (by similar microscopic findings to enhance material volume due to commonly low *Eimeria* oocyst concentration observed; only pools within individual herds were created), there were a total of 90 samples analyzed with each primer pair. Fifty-one pool samples were shown to be positive for *E. bovis*, 21 were positive for *E. zuernii*, 26 came back positive for *E. ellipsoidalis*, 6 for *E. cylindrica*, 18 were positive for *E. brasiliensis*, 17 samples were positive for *E. wyomingensis*, and only one sample was positive for *E. alabamensis*. There were 38 pool samples that had two or more *Eimeria* species within them and 10 samples that had four or more *Eimeria* species. *E. alabamensis* and *E. wyomingensis* were the only two species not detected by PCR within free-ranging herds. There were many *Eimeria* species found during the fecal flotation process that matched with the PCR data, however there were some discrepancies. There were some samples that were positive from microscopy data that did not test positive by PCR, and some samples that were positive for a given Eimeria sp. via PCR, though this species was not detected by microscopy. The most important example of this is *E. brasiliensis* and *E. wyomingensis*. Both of these species were not seen under the microscope during the fecal flotation process at all;

however, many samples were positive when PCR was performed. Summarized PCR data can be found in Table 4.

Samples that were PCR positive for the respective *Eimeria* sp. were then amplified for gel excision and subsequent sequencing (Figures 1-7). Each species had one to nine successful sequences that were available to be matched with known cattle Eimeria spp. sequences in the NCBI GenBank database. All obtained *Eimeria* ITS1 partial sequences amplified with the named PCRs were aligned with published ITS1 gene sequences for cattle *Eimeria* spp. from NCBI GenBank to generate maximum likelihood phylogenetic trees (Figures 8-14). As result of our study, 35 individual bison *Eimeria* sp. isolates were uploaded into the NCBI GenBank as seen in Table 5.

For all isolated *Eimeria* sp. ITS gene sequences, a close relationship with known cattle *Eimeria* sp. sequences listed in the NCBI GenBank database could be shown (Figures 8-14).

Chapter 6: Discussion

The presented study was designed to answer three main hypotheses, the first being that there will be *Eimeria* species in farmed and free-ranging American bison herds that morphologically and genetically resemble those found in cattle. Our data suggests that American bison are susceptible to the same *Eimeria* spp. as cattle. This susceptibility to *Eimeria* that are present in cattle could be related to the breeding background of American bison. Not only have this species been through a genetic bottlenecking event, but it is estimated that only 1.5% of plains bison (especially in production herds) don't contain any cattle ancestry (Hedrick, 2009). It is essential that the past breeding and ancestry of American bison is taken into account when studying disease, especially disease that occurs in a common ancestor.

This data is corroborated both by morphological data and ITS1 based genetic data. All *Eimeria* ITS1 sequences found are similar to cattle *Eimeria* spp. data (see Figs. 9-14). Per our morphological and sequencing data, the following five *Eimeria* spp. are present in both free-ranging and ranched bison: *E. bovis, E. brasiliensis, E. cylindrica, E. ellipsoidalis,* and *E. zuernii*. *E. alabamensis* and *E. wyomingensis* were present only in ranched bison. All seven of the species found within bison are very similar genetically to those found within cattle and cluster specifically with the expected *Eimeria* sp. the respective PCR primer pairs were designed for.

In our fecal samples, generally more than one *Eimeria* spp. were present in each individual sample, which is why no overarching generic *Eimeria* PCR was performed based on several amplicons being expected in parallel for each sample. Since we only performed PCRs specific for the already known cattle *Eimeria* ITS1 sequences, potentially we may have missed *Eimeria* DNA other than the sequences we specifically looked for. Thus, it cannot be excluded that there may be additional *Eimeria* spp. present in bison apart from the known cattle *Eimeria* spp. The morphological identification did not reveal presence of unknown *Eimeria* species though, which is in line with previous descriptions by Penzhorn, Ryff, and Bergstrom. Hence, we assume that American bison generally shares *Eimeria* spp. with cattle.

Similarly, Pyziel et al. (2014) found cattle *Eimeria* spp. in European bison (*Bison bonasus*). The extreme bottlenecking of European bison has been researched extensively and it has been shown that the entire species are derived from 12 founder animals (Tokarska et al., 2011). However, none of these animals were shown to have cattle genetics, and while they can be fairly similar to the American bison, they don't seem to have a past breeding history with cattle. Importantly, *E. zuernii* and *E. bovis* have also been observed in water buffalo (*Bubalus*

bubalis) (Dubey, 2018). In conjunction with data from our study and from Pyziel et al. (2014), this may indicate that bovine *Eimeria* spp. are less host specific than initially assumed, and that a cross-transmission between different bovine host species may actually happen. According to the presented study, this includes the highly pathogenic species *E. bovis* and *E. zuernii* which may show practical relevance as disease agents to cattle and bison productions alike.

In the Western US, where free-ranging American bison potentially share pastures with both ranched bison and domestic cattle, this opens a broader need of preventative measures to ensure livestock health. Interestingly, neither free-ranging or ranched bison showed clinical signs associated with *Eimeria* spp., even with regard to pathogenic species like *E. bovis* and *E. zuernii*, nor did they show a high *Eimeria* oocyst excretion level. This might indicate that though bison are susceptible to cattle *Eimeria* spp. infections, these do not cause clinical coccidiosis and thus are not significant pathogens under the investigated conditions. This agrees with a study done at an Italian zoo that looked at gastrointestinal parasites of mammals (including American bison) where they found that there were no symptoms in any of the animals associated with parasite infection, including *Eimeria* (Fagiolini et al., 2010). This lack of clinical signs could be for a myriad of reasons, one of them being the immune system of bison calves that may react more quickly or successfully to this pathogen. It has been shown in cattle that CD4+ T cells as well as other lymphoid cells are important for a productive immunity against this pathogen and calves generally are supplied with these cells by colostrum uptake (Daugschies & Najdrowski, 2005). It would be important to see the difference in bison calves' immunity to this pathogen, and if perhaps some of that is because of immune cells received from their mother's colostrum. Daugschies and Najdrowski (2005) also mentioned that T cell response can impact the level and duration of oocyst excretion, which could perhaps be part of the reason a low number of oocysts

were observed within these herds. It is also important to note that possible resistance to coccidia and responsiveness to the pathogen is determined by the genetic background of the calf (Daugschies & Najdrowski, 2005). Because of different genetic backgrounds, bison may be somewhat more resistant to this disease or their immune system may be more responsive to it. There is still much to research in this area to understand American bison immunity and susceptibility to this pathogen.

For our second hypothesis we expected correlations between husbandry conditions and various *Eimeria* spp. present and their prevalence. The questionnaire data revealed interesting relationships between preventative drug use and *Eimeria* prevalence. Even though the sample size was probably not high enough to detect significant correlations (only bison from one farm were treated with anticoccidial drugs), some trends were visible from our data. One trend was when ranchers did not use anticoccidials on their ranch, there was a trend to have more coccidia found within the herd. This trend is fairly self-explanatory, as anticoccidials are meant to kill species of *Eimeria*, so if none are used, there will be more *Eimeria* found than in herds where anticoccidials are used. Another similar trend is that herds that used antiparasitics of any kind saw less *Eimeria* within their herd. This could be because of an increase in *Eimeria* co-infection when there are other parasites present that the antiparasitics would usually get rid of. In a study on diarrheic calves in Germany, it was shown that there was mixed infection with *Eimeria, Giardia*, and *Cryptosporidium* in young dairy calves (Gillhuber et al., 2014). Co-infections with *E. coli* F5 as well as Rotavirus and Coronavirus was also discovered (Gillhuber et al., 2014). These are all important gastrointestinal pathogens that can co-infect bison when various antiparasitics and other vaccines aren't used within the herd.

In regard to co-infections, *E. zuernii* and *E. ellipsoidalis* were shown to commonly occur together and appeared in many of the same samples. This is interesting, as it could show that infection with a more pathogenic species of *Eimeria* such as *E. zuernii*, can lead to co-infections with less pathogenic species like *E. ellipsoidalis* or the other way around. No other *Eimeria* species showed this type of correlation in this study.

Besides investigation of *Eimeria* prevalence and identity in the sampled herds, the collected fecal samples were also analyzed for gastrointestinal worm eggs to assess the spectrum and prevalence of these parasites to test our third hypothesis. In accordance with previous investigations in American bison (Zaugg et al., 1993) we found *Strongylus* spp., *Trichuris* spp., as well as the cestode species *Moniezia*, however with having a larger sample size than the previously mentioned study, our prevalence was shown to be higher.

Our study could not clarify if bison that graze on significantly larger pastures (ranched bison) or open spaces (free-ranging bison) than domestic cattle are actually subject to a lower infection pressure than cattle or if the multiplication of the parasites is less efficient in bison than in cattle due to genetic, immunity, or other differences. This point could be clarified e.g. by experimental infection studies, however, to our knowledge this type of study is lacking as of today.

This study was mainly focused on bison *Eimeria* infections; thus, we did not perform molecular investigations into the worm population. However, it would be interesting to utilize modern tools such as deep amplicon sequencing (Avramenko et al., 2015) to identify the trichostrongylid worms in Western American bison herds to a species level. This would enable a judgment to which extent they overlap with typical trichostrongylid pathogens in cattle and wildlife; and to evaluate the significance of the observed high prevalence to our livestock

operations and wildlife health. It seems likely that especially *Nematodirus* spp. and *Trichosgrongylus* spp. are commonly shared pathogens as also stated by Hoberg et al. (Hoberg et al., 2008).

We utilized two different *Eimeria* detection methods in this study, namely fecal flotation and conventional PCR. In our hands, it seems like both methods deliver reliable results, with each method featuring its benefits and downfalls. Important parameters for a decision on which method to use are time, money, and accuracy. For the latter, determining species by morphology alone can be difficult to the average diagnostician that is not specifically trained in *Eimeria* species differentiation. However, the fecal flotation method is fast and easy to perform, cheap, and allows for a certain level of quantification of the parasite burden. Additionally, it allows for detection of both protozoan and helminth pathogens at the same time. On the other hand, PCR can be easier for routine use in laboratories that are not well versed in microscopic *Eimeria* species identification and it can provide a definitive species diagnosis especially in less common hosts like bison. However, it tends to be more time consuming and costly.

In conclusion, this study could provide important genetic descriptions of multiple bison isolates of *Eimeria* species. It has also shown that these species of *Eimeria* found in bison are genetically similar to those found in cattle. This knowledge opens the door for other studies about *Eimeria* in bison, as there is much to still learn about this disease in this essential wildlife and livestock species. The next direction for this project along these lines could be an epidemiological study to continue looking into the cross-transmission of this parasite between bison and cattle in the field.

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Tables

Table 1: Primers used to detect various *Eimeria* species via PCR. Primers indicated with an asterisk were previously designed by Kawahara et. al. (2010); other primer pairs are self-designed using NCBI primer-BLAST (Ye et al., 2012) and ThermoFisher multiple primer analyzer (Thermo Fisher). All primer pairs specifically target the ITS1 gene region of the respective bovine *Eimeria* species.

Primers			
Name	Forward/ Reverse	Sequence	Annealing Temperature
E. ellipsoidalis fwd *	F	5'-CAA CGT TTT TCC TTT TCC TAT CA-3'	58°C
E. ellipsoidalis rev *	R	5'-ACT GCG ATG AGA GAG AGC G-3'	58°C
E. cylindrica fwd *	F	5'-GAC ATT TAA AAA ACC GAT TGG T-3'	58°C
E. cylindrica rev *	R	5'-GGC TGC AAT AAG ATA GAC ATA-3'	58°C
E. alabamensis fwd *	F	5'-CAT TCA CAC ATT GTT CTT TCA G-3'	56°C
E. alabamensis rev *	R	5'-GCT TCC AAA CTA ATG TTC TG-3'	56°C
E. bovis fwd *	F	5'-TCA TAA AAC ATC ACC TCC AA-3'	56°C
E. bovis rev *	R	5'-ATA ATT GCG ATA AGG GAG ACA-3'	56°C
E. zuernii fwd	F	5'-CCC ACT ACA TCC AAC CTC CTG-3'	62°C
E. zuernii fwd	R	5'-GCG TTC GGA AAT CTG ATG GT-3'	62°C
E. brasiliensis fwd	F	5'-CAT GGG TTC CCG TAT GTG GT-3'	62°C
E. brasiliensis rev	R	5'-TGC CTA ACC GGA ATG AAG GC-3'	62°C
E. wyomingensis fwd	F	5'-CGA TAC GCA TTG TGA ATT GC-3'	59°C
E. wyomingensis rev	R	5'-TAT TCA CCC CCT ACC ATT AAC C-3'	59°C

Table 2: Summary of relevant questionnaire data for all 6 ranches sampled.

Herd	Location	Herd Size	Husbandry Conditions	Contact to Cattle	Past Coccidial Infections	Anti-Parasitic Drugs	Anti-Coccidial Drugs
1	Wyoming	1500	Pasture only	Through fence	Yes	Yes	Yes
2	Wyoming	600	Rotating Pasture	Through fence	Yes	No	No
3	Wyoming	3000	Rotating Pasture	None	No	Yes	No
4	Colorado	700	Rotating Pasture	None	No	Yes	No
5	Nebraska	180	Rotating Pasture	None	No	Yes	No
6	South Dakoda	1,800	Pasture only	Through fence	No	Yes	No

Table 3: Parasite occurrence and prevalence in ranched and free-ranging bison herds from light microscopy investigation.

Parasite	% of ranched herds positive (n=6)	% positive all individual samples (n=207)	Presence in Free-Ranging herds (y/n)	Average flotation score for positive samples	Range of flotation scores for positive samples
E. bovis	100%	33.3%	Y	(+)	(+) to ++
E. zuernii	100%	13.5%	Y	(+)	(+) to ++
E. alabamensis	50%	3.4%	N	(+)	(+) to +
E. auburnensis	33.3%	2.9%	N	(+)	(+)
E. cylindrica	83%	3.9%	N	(+)	(+) to +
E. canadensis	83%	10.6%	Y	(+)	(+) to ++
E. ellipsoidalis	100%	32.8%	Y	(+)	(+) to ++
E. bukidnonensis	16.6%	1.0%	N	(+)	(+)
E. pellita	16.6%	1.4%	Y	(+)	(+) to +
E. wyomingensis	0%	0%	N	-	-
E. brasiliensis	0%	0%	N	-	-
E. subspherica	33.3%	1.0%	N	(+)	(+)
Trichostrongylid worms	100%	68.7%	Y	(+)	(+) to ++
Moniezia	100%	21.8%	Y	(+)	(+) to ++
Nematodirus	50%	25.1%	Y	++	+ to +++
Trichuris ovis	50%	5.3%	Y	+	+
Capillaria	0%	1.0%	Y	(+)	(+)
Strongyloides	33.3%	1.0%	N	(+)	(+)

Table 4: *Eimeria* spp. prevalence detected by conventional PCR.

Eimeria spp.	% of ranched herds positive (n=6)	Presence in Free-Ranging herds (y/n)
E. bovis	100%	Y
E. zuernii	83.3%	Y
E. alabamensis	16.6%	N
E. cylindrica	50%	Y
E. ellipsoidalis	100%	Y
E. brasiliensis	100%	Y
E. wyomingensis	100%	N

Table 5: Alignment of amplified bison *Eimeria* spp. ITS1 gene fragment sequences with cattle *Eimeria* spp. ITS1 gene sequences. (all obtained bison *Eimeria* sp. sequences of sufficient quality, n = 35)

American bison *Eimeria* sp. isolate	*Eimeria* sp.	NCBI Genbank database accession number for own submitted bison *Eimeria* sp. ITS1 sequence	NCBI Genbank database sequence (organism, accession number)	% Homology (Query cover %)
WY-1A	*E. alabamensis*	MT711497	E. alabamensis AB557607.1	100% (99%)
WY-1B	*E. bovis*	MT711488	E. bovis AB769588.1	100% (88%)
WY-2B	*E. bovis*	MT711489	E. bovis AB769575.1	97% (92%)
WY-3B	*E. bovis*	MT711490	E. bovis KU351711.1	96% (84%)
WY-4B	*E. bovis*	MT711491	E. bovis KU351711.1	98% (82%)
WY-5B	*E. bovis*	MT711492	E. bovis KU351709.1	95% (86%)
WY-6B	*E. bovis*	MT711493	E. bovis MK333255.1	96% (81%)
WY-7B	*E. bovis*	MT711494	E. bovis MK333255.1	95% (86%)
WY-8B	*E. bovis*	MT711495	E. bovis MN601274.1	96% (82%)
WY-9B	*E. bovis*	MT711496	E. bovis MK333255.1	96% (84%)
WY-1C	*E. brasiliensis*	MT711470	E. brasiliensis KU351740.1	94% (90%)
WY-2C	*E. brasiliensis*	MT711471	E. brasiliensis KU351740.1	95% (90%)
WY-3C	*E. brasiliensis*	MT711472	E. brasiliensis KU351740.1	92% (86%)
WY-4C	*E. brasiliensis*	MT711473	E. brasiliensis KU351740.1	91% (96%)
WY-5C	*E. brasiliensis*	MT711474	E. brasiliensis KU351739.1	96% (97%)
WY-1D	*E. cylindrica*	MT711485	E. cylindrica AB769615.1	94% (89%)

WY-2D	*E. cylindrica*	MT711486	E. cylindrica AB557616.1	93% (99%)
WY-3D	*E. cylindrica*	MT711487	E. cylindrica AB557616.1	95% (98%)
WY-1E	*E. ellipsoidalis*	MT711482	E. ellipsoidalis AB557621.1	98% (99%)
WY-2E	*E. elliposidalis*	MT711483	E. ellipsoidalis AB557620.1	98% (98%)
WY-3E	*E. ellipsoidalis*	MT711484	E. ellipsoidalis AB557619.1	97% (90%)
WY-1F	*E. wyomingensis*	MT711498	E. wyomingensis* AB769831.1	82% (100%)
WY-2F	*E. wyomingensis*	MT711499	E. wyomingensis* AB769831.1	82% (99%)
WY-3F	*E. wyomingensis*	MT711500	E. wyomingensis* AB769831.1	82% (100%)
WY-4F	*E. wyomingensis*	MT711501	E. wyomingensis* AB769831.1	92% (91%)
WY-5F	*E. wyomingensis*	MT711502	E. wyomingensis* AB769831.1	81% (97%)
WY-6F	*E. wyomingensis*	MT711503	E. wyomingensis AB769831.1	94% (92%)
WY-7F	*E. wyomingensis*	MT711504	E. wyomingensis* AB769821.1	90% (84%)
WY-1G	*E. zuernii*	MT711475	E. zuernii MH245200.1	99% (100%)
WY-2G	*E. zuernii*	MT711476	E. zuernii MH245201.1	99% (89%)
WY-3G	*E. zuernii*	MT711477	E. zuernii LC171338.1	98% (93%)
WY-4G	*E. zuernii*	MT711478	E. zuernii AB769663.1	99% (87%)
WY-5G	*E. zuernii*	MT711479	E. zuernii MH245201.1	97% (86%)
WY-6G	*E. zuernii*	MT711480	E. zuernii LC171339.1	97% (90%)
WY-7G	*E. zuernii*	MT711481	E. zuernii LC171337.1	98% (90%)

*For this bison *Eimeria* sp. sequence, besides homology with *E. wyomingensis* as listed, the NCBI GenBank database provided a close homology to one *E. cylindrica* sequence from cattle (AB769797.1). This *E. cylindrica* sequence seems somewhat random and does not match any other *E. cylindrica* sequences in the NCBI GenBank database, therefore, we disagree with this one single sequence hit.

Figures

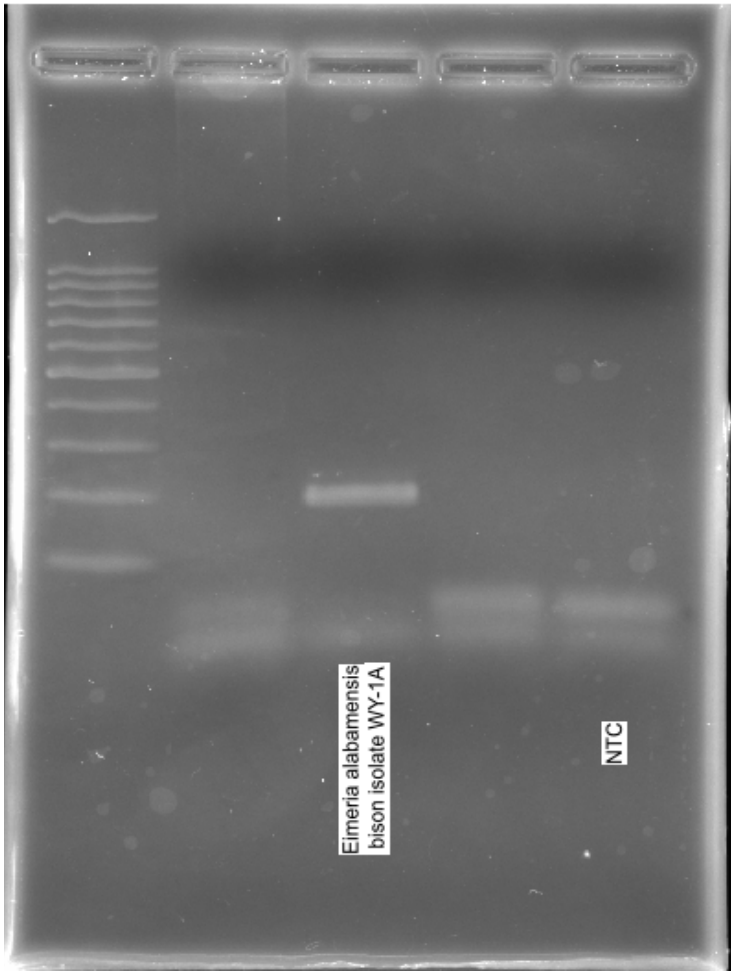

Figure 1: Agarose gel electrophoresis of the PCR products from American bison isolates of *E. alabamensis.*

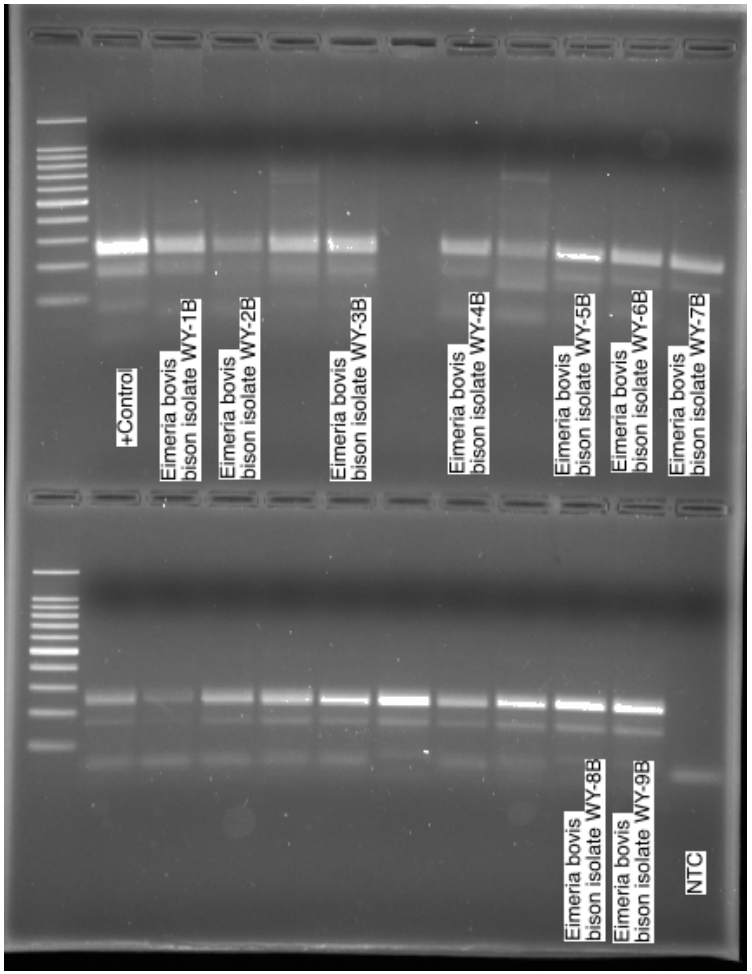

Figure 2: Agarose gel electrophoresis of the PCR products from an American bison isolates of *E. bovis.*

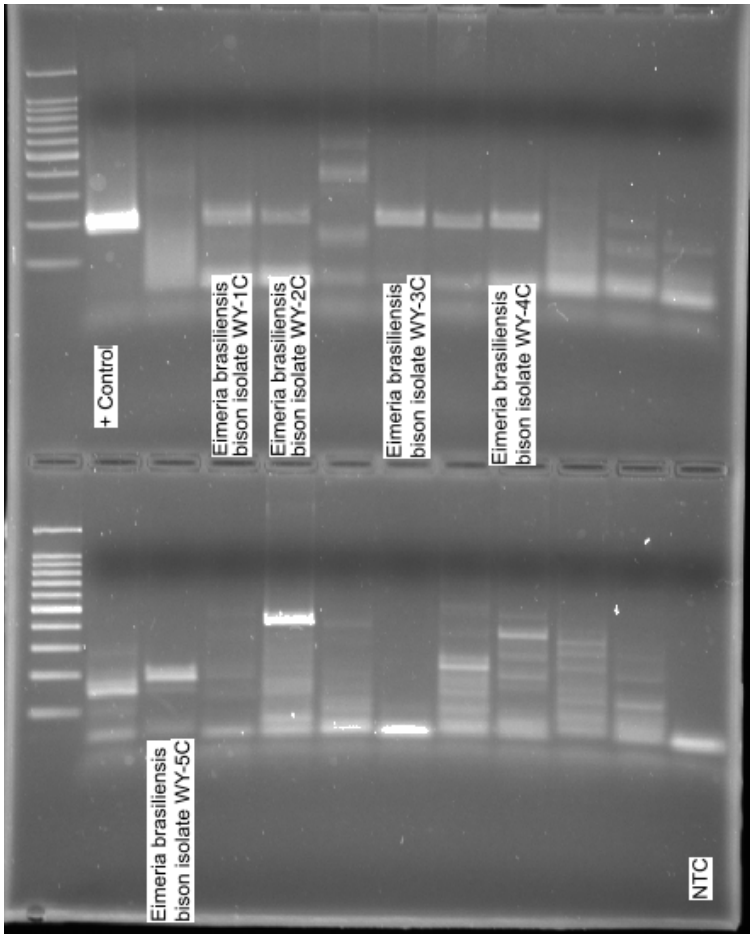

Figure 3: Agarose gel electrophoresis of the PCR products from American bison isolates of *E. brasiliensis.*

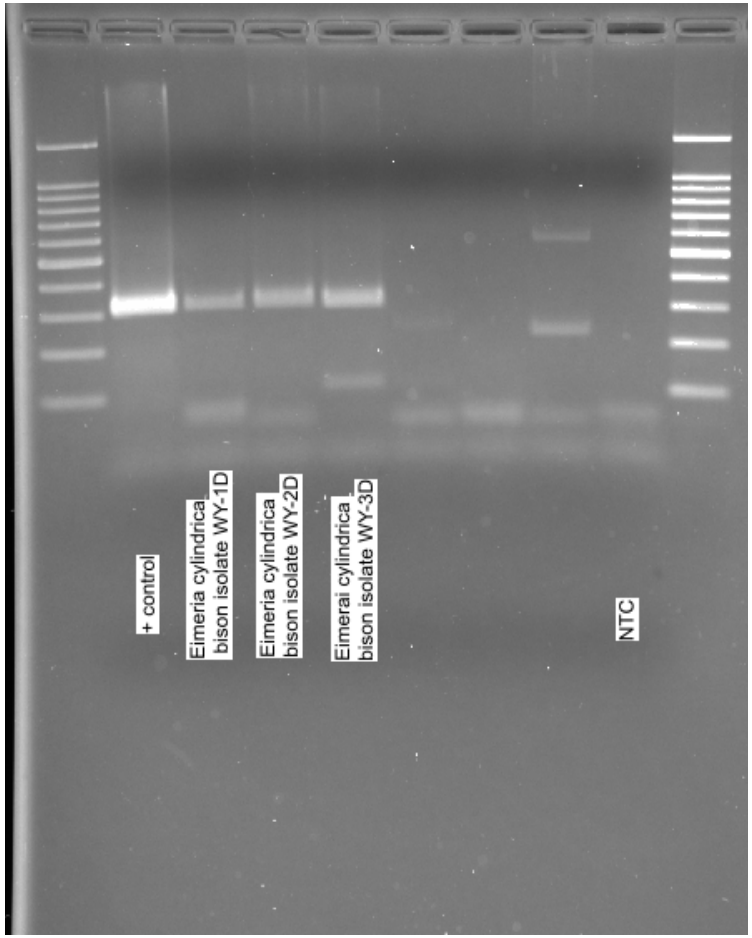

Figure 4: Agarose gel electrophoresis of the PCR products from American bison isolates of *E. cylindrica.*

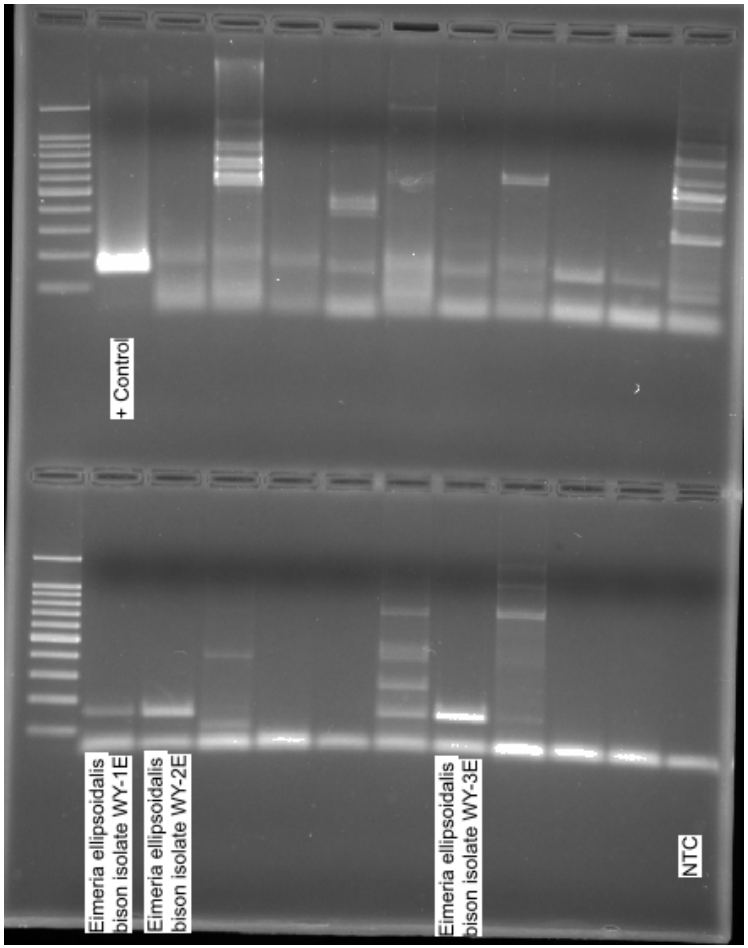

Figure 5: Agarose gel electrophoresis of the PCR products from American bison isolates of *E. ellipsoidalis*.

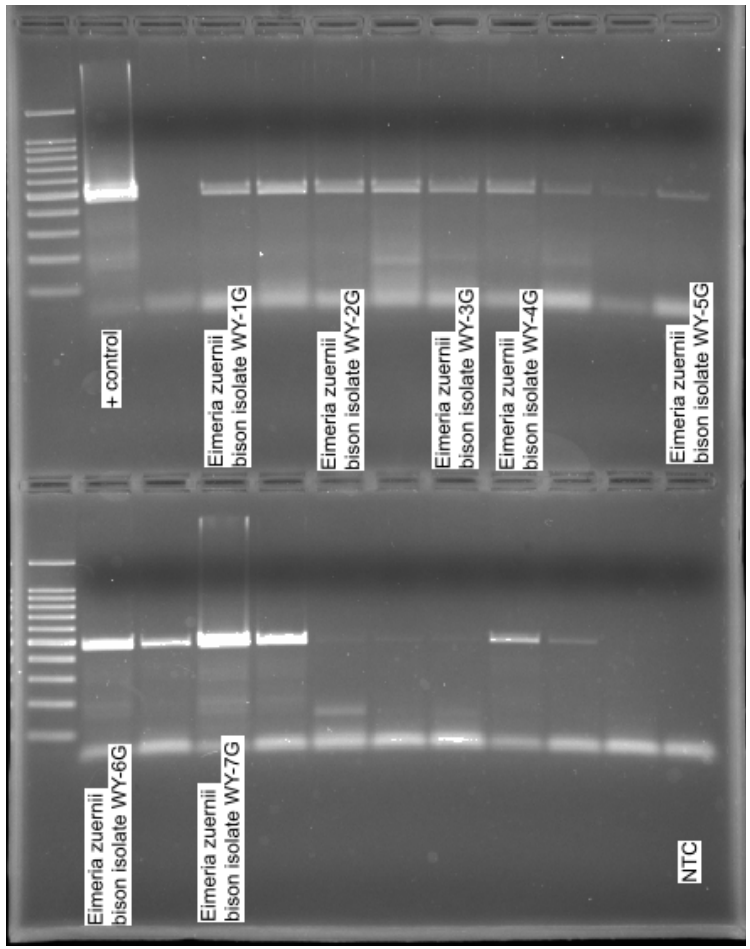

Figure 6: Agarose gel electrophoresis of the PCR products from American bison isolates of *E. zuernii.*

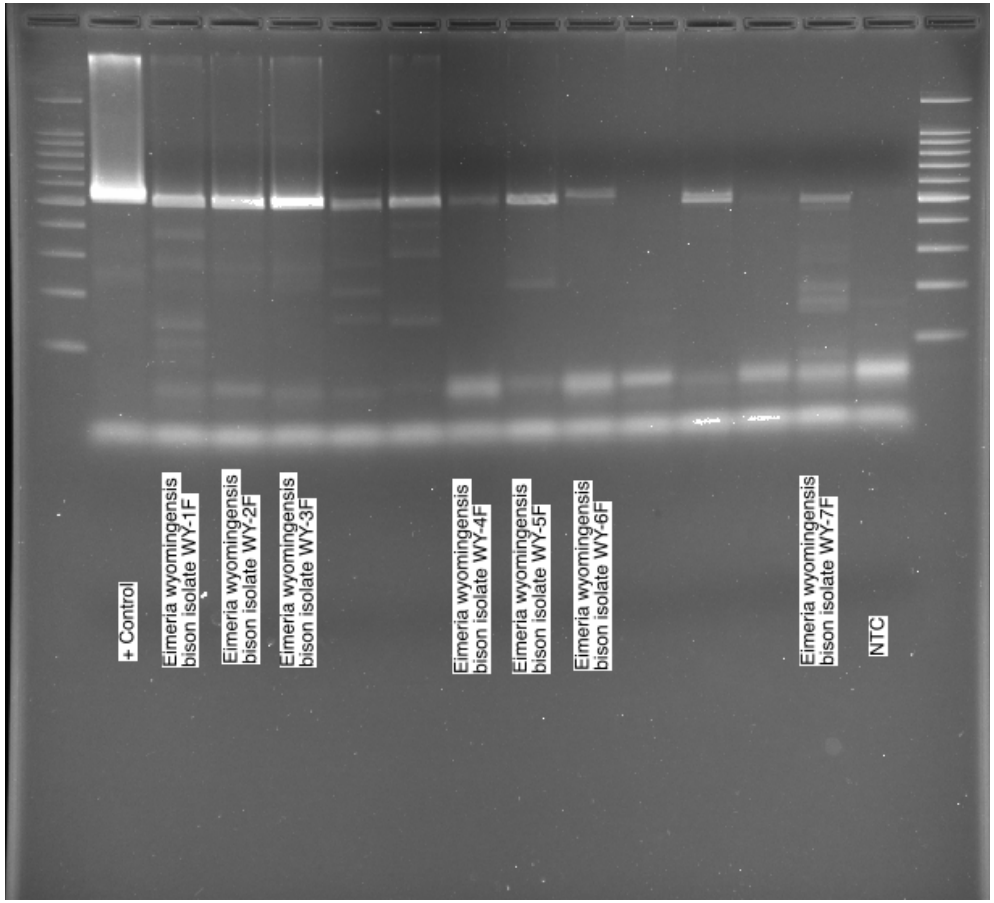

Figure 7: Agarose gel electrophoresis of the PCR products from American bison isolates of *E. wyomingensis*.

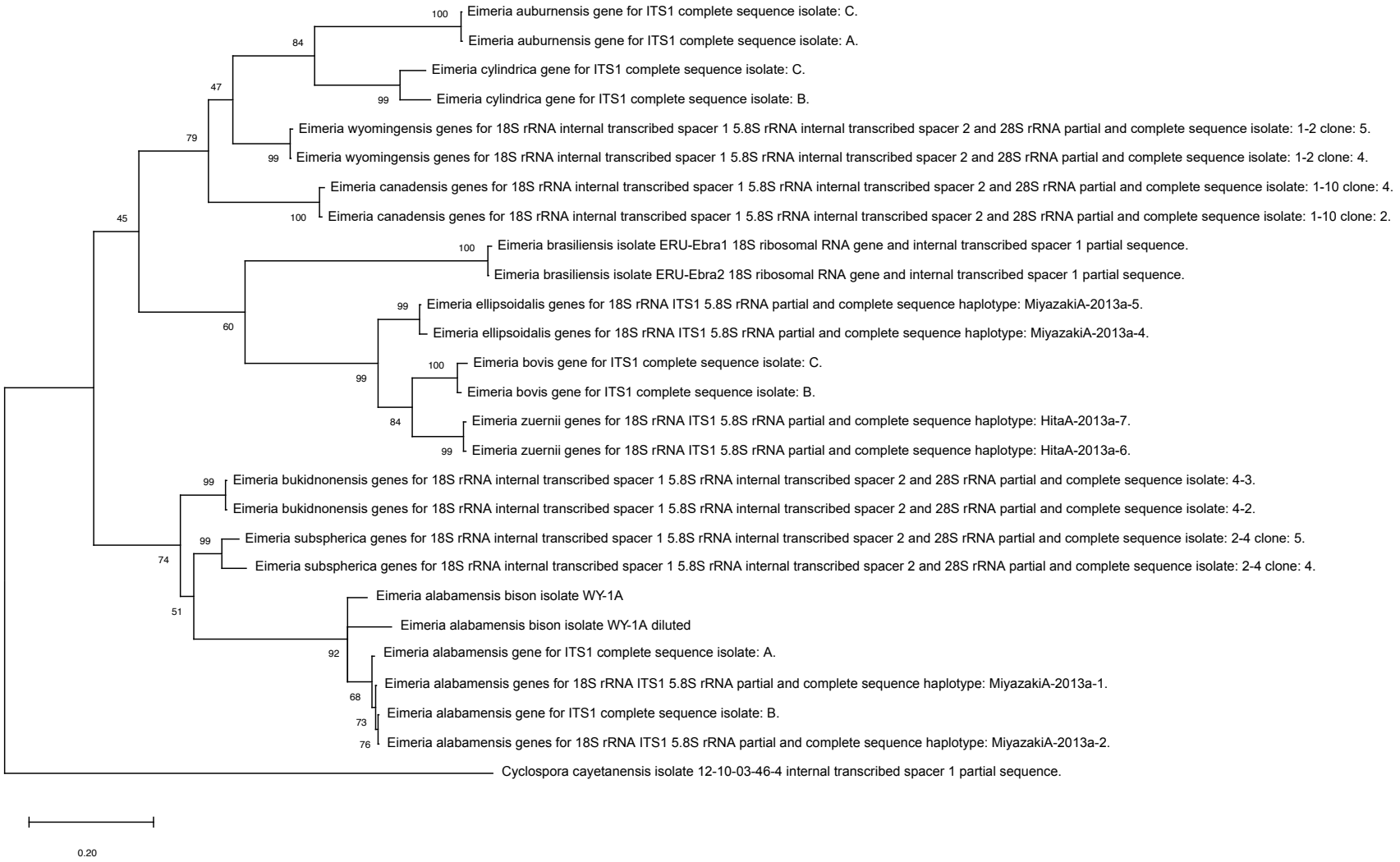

Figure 8: Maximum likelihood phylogenetic tree for *E. alabamensis* American bison isolates with 1,000 bootstrap replications.

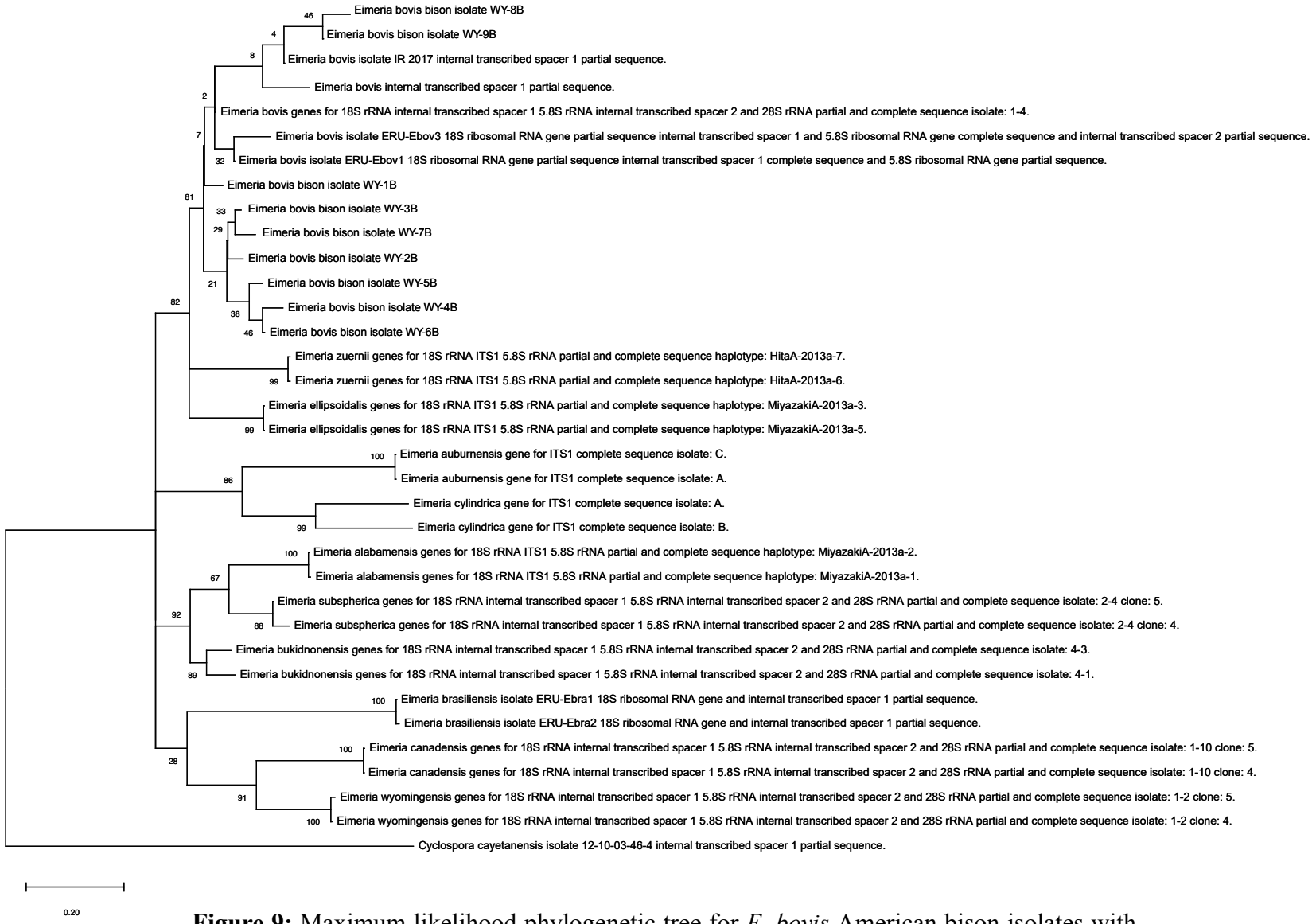

Figure 9: Maximum likelihood phylogenetic tree for *E. bovis* American bison isolates with 1,000 bootstrap replications.

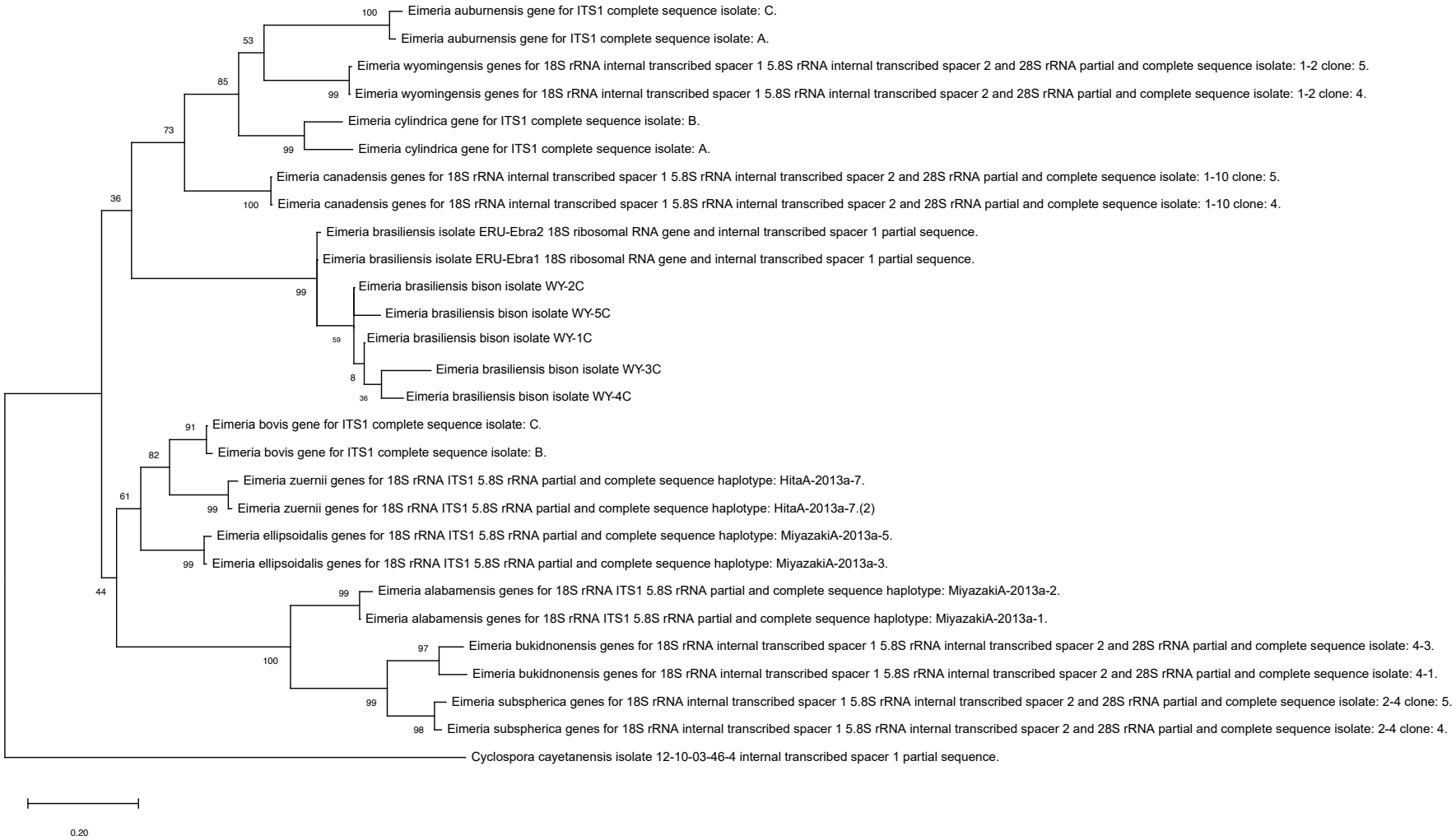

Figure 10: Maximum likelihood phylogenetic tree for *E. brasiliensis* American bison isolates with 1,000 bootstrap replications.

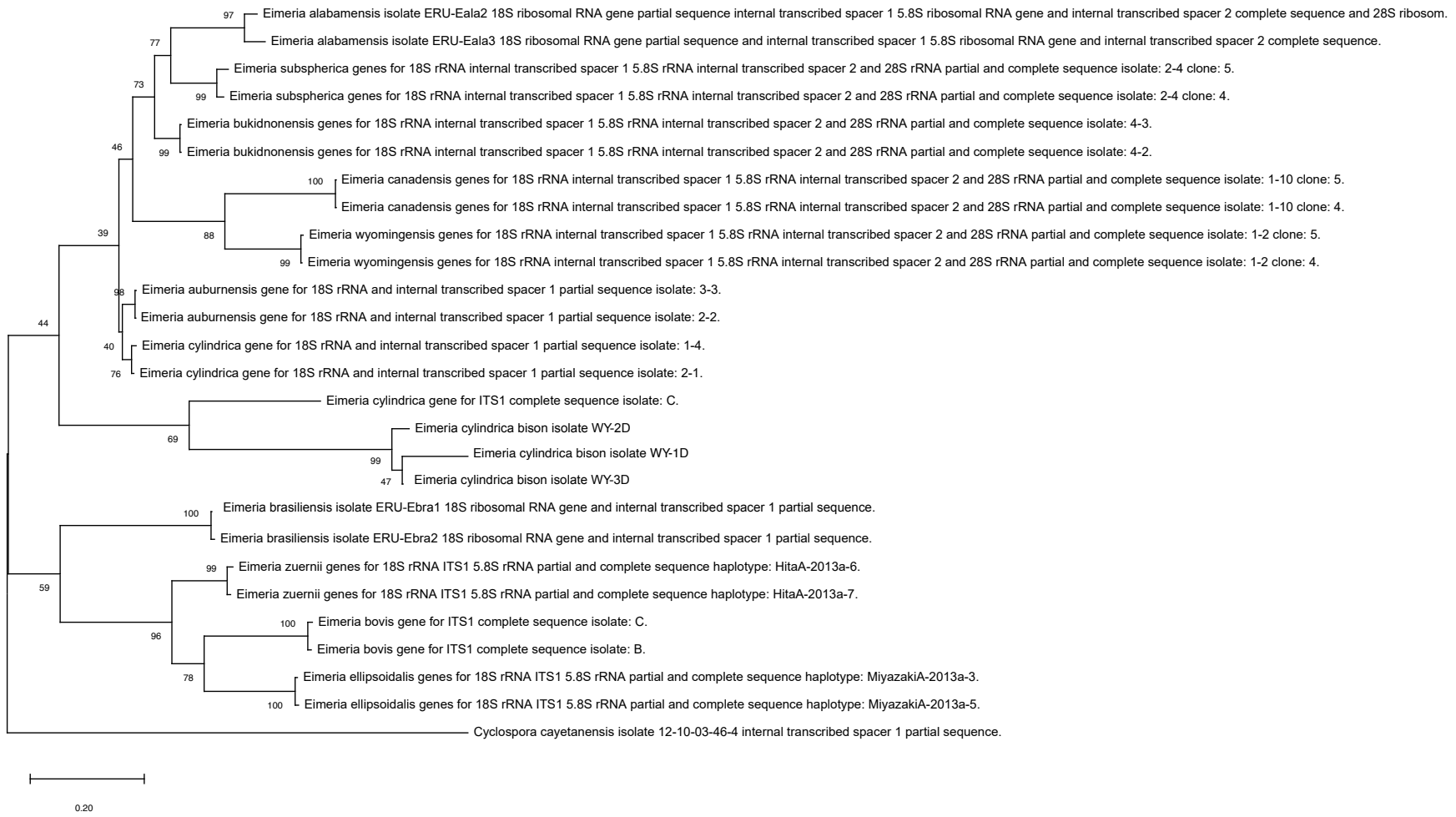

Figure 11: Maximum likelihood phylogenetic tree for *E. cylindrica* American bison isolates with 1,000 bootstrap replications.

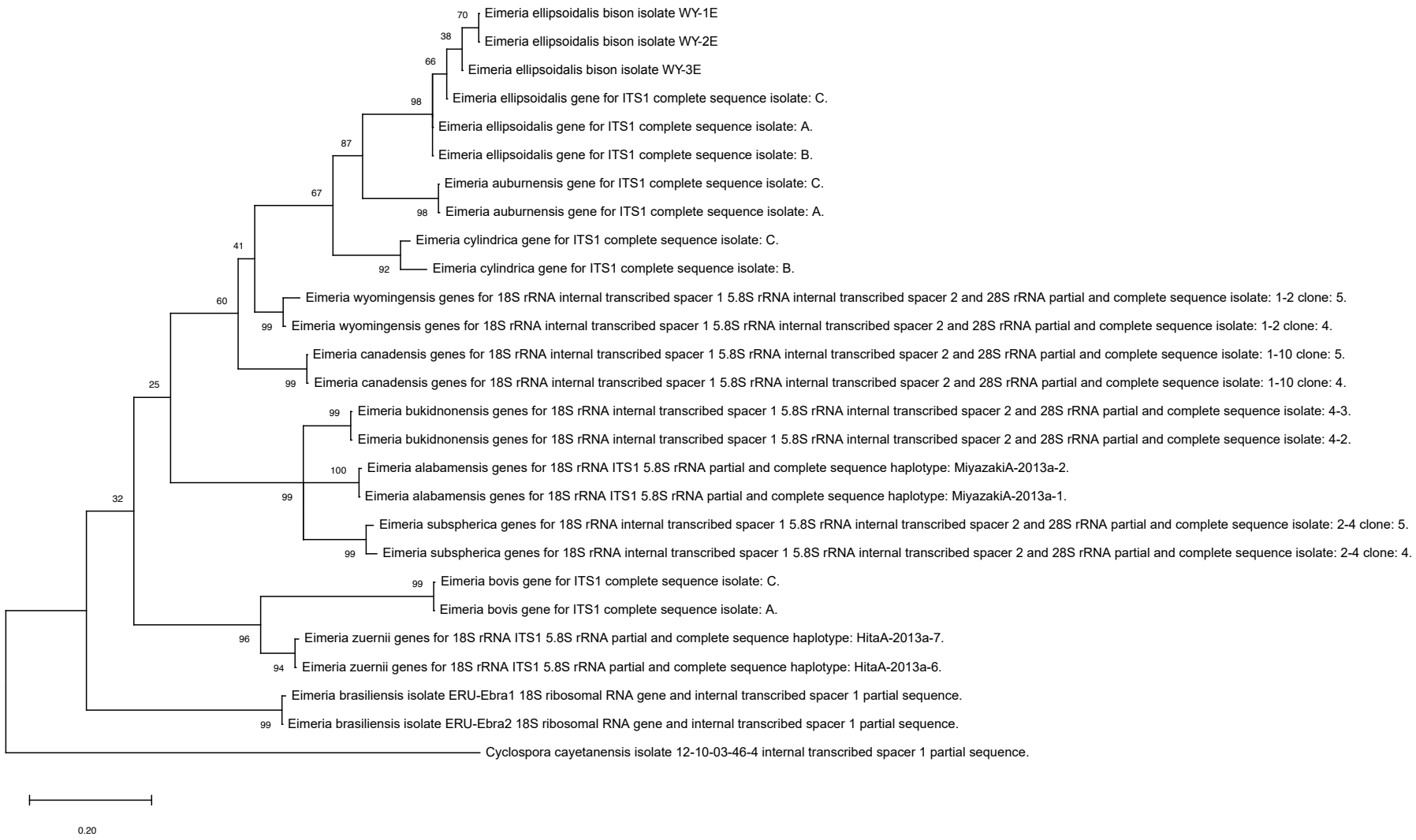

Figure 12: Maximum likelihood phylogenetic tree for *E. ellipsoidalis* American bison isolates with 1,000 bootstrap replications.

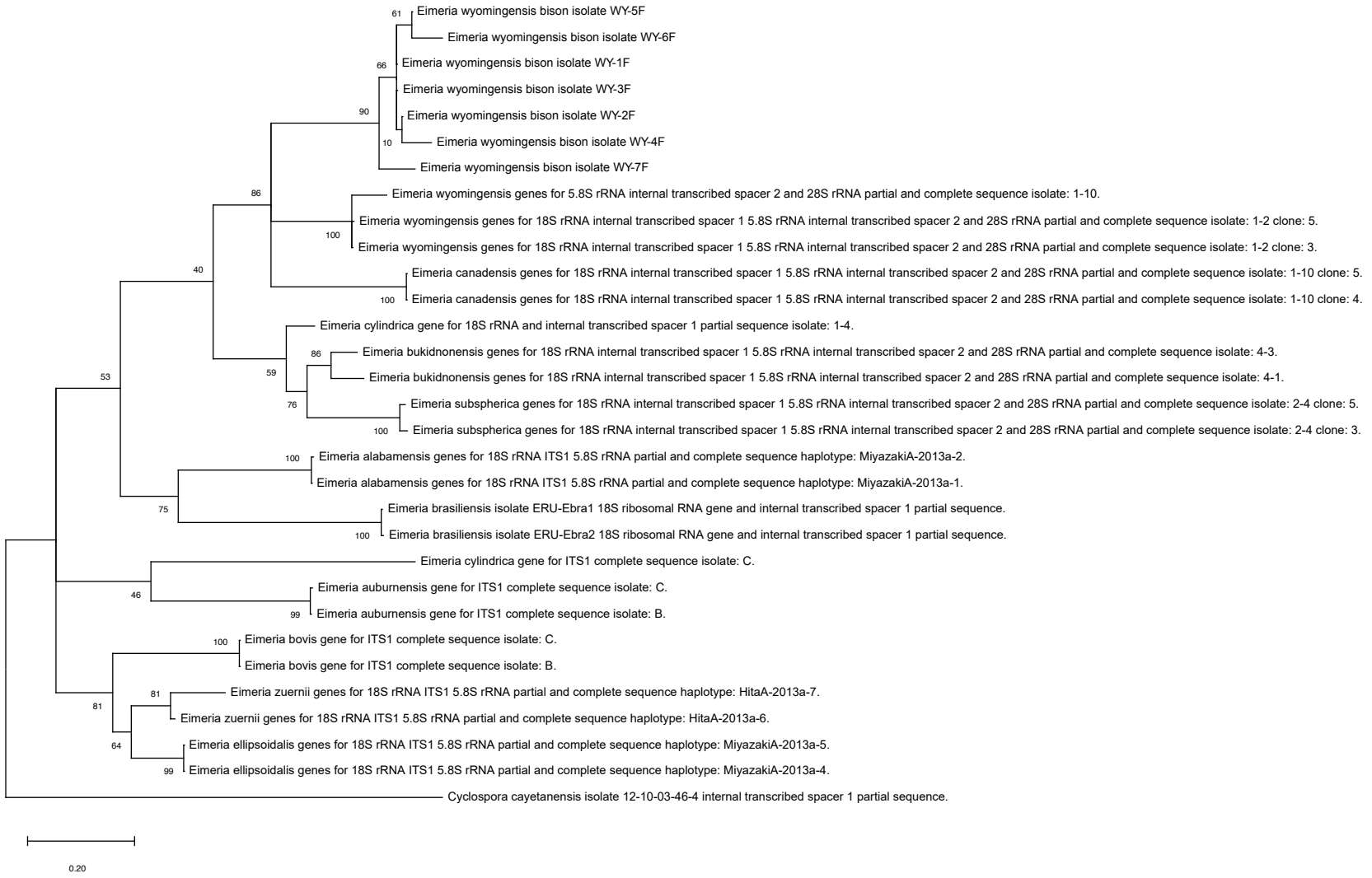

Figure 13: Maximum likelihood phylogenetic tree for *E. wyomingensis* American bison isolates with 1,000 bootstrap replications.

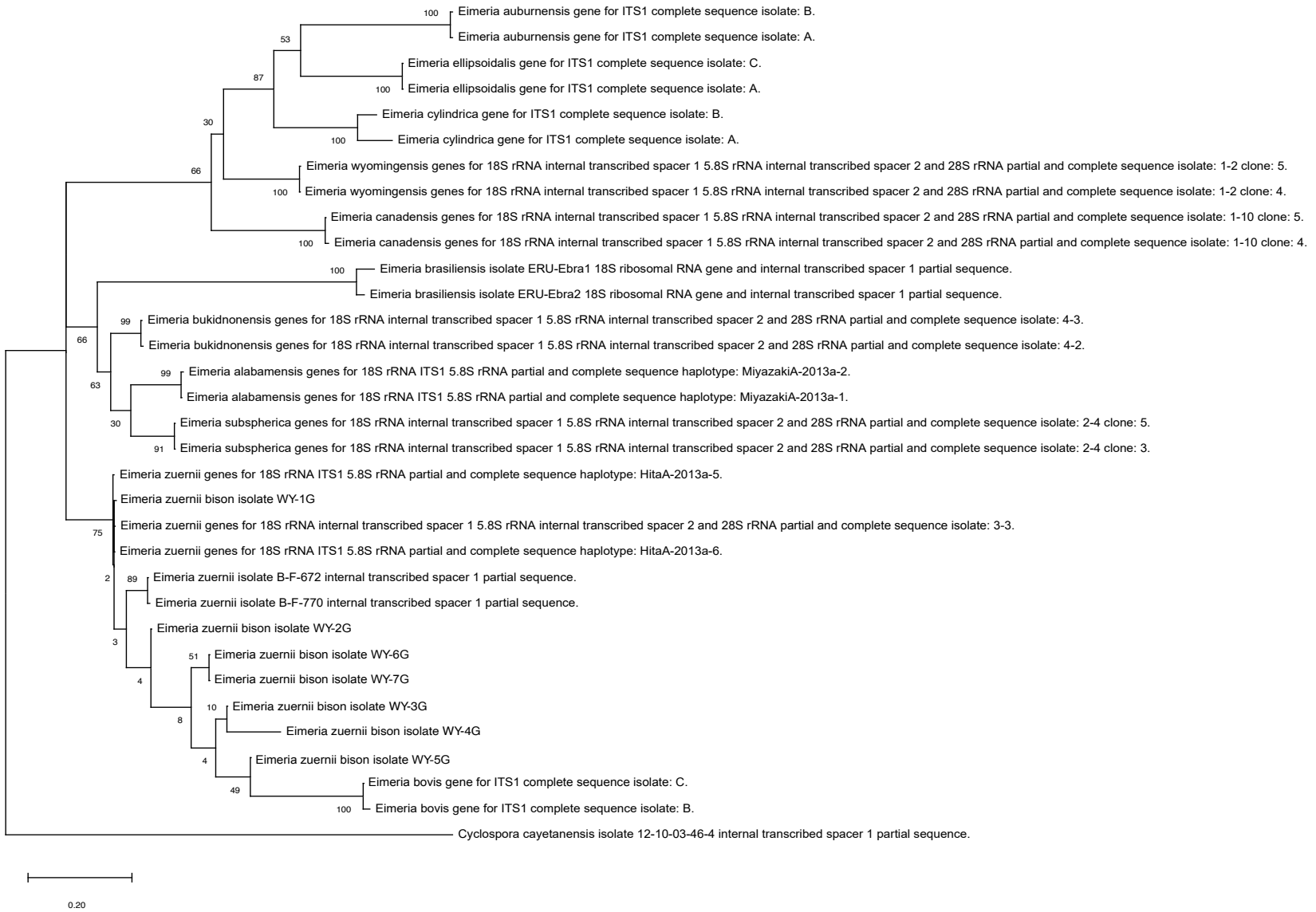

Figure 14: Maximum likelihood phylogenetic tree for *E. zuernii* American bison isolates with 1,000 bootstrap replications.

Appendix A

Questionnaire for Bison Operations

How many head of bison are on your operation?

What kind of operation is it? Meat/hobby?

How many calves do you usually have per year?

How many calves did you have this last calving season?

Which months of the year is calving season for your operation?

What are the husbandry conditions for your operation? Pasure only/rotating pasture/housing facilities (sheds)

What is the general contact to cattle? Are cattle kept on the same ranch?

How is water presented on your operation? Is there a watering hole or is the water raised up/in a trough? Water source? (pond/ ground water/ river)

Do you separate your herd into groups?	Yes	No
If yes, please describe:		
Are any routine disinfection measures established in calf rearing?	Yes	No
Are you aware of any calves in your operation currently presenting with scours/diarrhea?	Yes	No
Have you had a problem with calf scours over the last 5 years?	Yes	No
Are you aware of any calves in your operation that are currently infected with coccidian parasites?	Yes	No

Have coccidial infections been diagnosed on your property in the past 5 years?	Yes	No
Are antiparasitics administered to the bison on your operation?	Yes	No
Are coccidiostats (anti-coccidials) or anticoccidial feed additives administered to the calves on your operation?	Yes	No