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Incidence of Blackleg in Beef Cattle and Implications of Regional Differences

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INCIDENCE OF BLACKLEG IN BEEF CATTLE IN SOUTH DAKOTA AND IMPLICATIONS OF REGIONAL DIFFERENCES

by

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ABSTRACT

Incidence of Blackleg in Beef Cattle in South Dakota

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Blackleg is an acute, fatal disease that has been afflicting cattle since the late 1800's. It is caused by the anaerobic, spore-forming bacteria *Clostridium chauvoei.* This disease is characterized by swelling in areas like the legs, hips, chest, and back, and is caused by toxins released from *C. chauvoei* that turn the surrounding tissue black or red and give this disease its name. This study surveyed cattle veterinarians in South Dakota to determine the incidence of blackleg in South Dakota. The goal was to determine the incidence in South Dakota and discover any specific regions that were more susceptible to blackleg. After administering this survey to a number of veterinarians, we found the herd-level incidence in South Dakota over the past 45 years to be 18%, and the overall incidence to be 2.82 cases per 100,000 head. The counties in South Dakota that had the highest herd-level blackleg incidence rates were Lawrence, Douglas, and Davison counties. The low herd-level and overall incidence rates in South Dakota are consistent with common perceptions and indicate current preventative measures are effective. Regional differences may be explained by a few factors such as recent soil disturbance/excavation, rainfall patterns, and variation in vaccination practices through time.

KEYWORDS: Blackleg, Incidence, *Clostridium chauvoei*, Beef Cattle, South Dakota

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Chapter One: Introduction

Blackleg is among the oldest and most persistent diseases afflicting cattle today. Blackleg is also called black quarter, symptomatic anthrax, or emphysematous anthrax, and was first reported in 1870. It affects cattle and other ruminants worldwide (Mohler, 1923; Useh, Nok, & Esievo, 2003). Blackleg is most common in pastured cattle but can infect sheep and goats as well as fish, frogs, and whales; however, humans and other animals including birds are resistant (Pfeifer, 2018; Useh et al., 2003; Useh, Nok, & Esievo, 2006). Blackleg gets its name because often times the site of infection is a leg muscle, and the disease causes the muscle to become a darkened or black color (Ziech, Gressler, Frey, & Vargas, 2018). Currently there are no curative measures for a blackleg infection, but there are preventative measures (Ziech et al., 2018). The current and only preventative measure is a vaccine in use since 1930, but cattle that have been vaccinated still may suffer from blackleg (Drovers, 2013; Ziech et al., 2018). Blackleg is fatal and practicing proper preventative measures as well as developing curative measures is crucial for raising healthy cattle.

Blackleg is an infectious but non-contagious disease most often caused by the anaerobic bacterium *Clostridium chauvoei* (Robson, 2007b; Stampfli, 2020). A few cases of blackleg have been attributed to *Clostridium septicum* bacteria, but this is not common (Robson, 2007b; Stampfli, 2020). Blackleg can't be transmitted directly between animals because of the pathogenesis of this disease; instead, *C. chauvoei* is found in soil and ingested as dormant spores. Upon ingestion, *C. chauvoei* spores enter the digestive tract, then the bloodstream and muscles, where they remain dormant until germination conditions are present (Drovers, 2013; Robson, 2007b; Stampfli, 2020). Since *C.*

chauvoei is anaerobic, the conditions for germination include injury, bruising, excessive exercise, or other situations that result in reduced oxygen supply (Robson, 2007b). Once germinated, vegetative *C. chauvoei* cause blackleg onset and the release of toxins (Microbiology, 2016; Stampfli, 2020). These toxins cause muscle swelling, depression, and lameness (Tagesu, Tagesu, Hasen, Regea, & Tadesse, 2019). In the early stages swelling is small and hot but becomes larger and colder as the disease progresses (Stampfli, 2020). *C. chauvoei* toxins destroy surrounding tissue before entering the bloodstream and killing affected individuals within 12-48 hours (Drovers, 2013; Robson, 2007b; Stampfli, 2020). The vegetative bacteria produces gas, creating bubbles in the muscles leading to skin crackling and the release of odorous gas when the affected area is necropsied (Drovers, 2013; Stampfli, 2020). The toxins from *C. chauvoei* will turn the muscles dark red to black and cause them to become spongy and dry -- the most typical sign of blackleg (Drovers, 2013; Stampfli, 2020). The affected muscles are often the hind legs, but can be any muscle, with swelling often seen in the shoulder, hips, chest, and back (Tagesu et al., 2019). The onset of blackleg is sudden, and since the disease kills quickly, producers often fail to notice or don't notice in time for any curative intervention (Drovers, 2013; Robson, 2007b; Stampfli, 2020).

Although incidences of blackleg have been reported in cattle ranging in age from two months to adult, the most common age of incidence is from six months to two years (Robson, 2007b; Tagesu et al., 2019). One reason cattle this age are so susceptible is that during this time they are rapidly gaining weight and consuming large amounts of feed. Researchers have identified a positive correlation between high body condition and susceptibility to blackleg in cattle, which helps explain why younger cattle tend to suffer from blackleg more frequently (Robson, 2007b; Tagesu et al., 2019). Another reason younger cattle are more susceptible to blackleg is the relatively higher stress of castration, vaccination, dehorning, and weaning. These events provide a window of opportunity for clostridial diseases (Robson, 2007a).

Weather also appears to have an effect on the occurrence of blackleg. There are more reported blackleg losses during hot and humid weather (mostly in summer months), areas that experience high amounts of rainfall, and areas that have sudden cold periods (Pfeifer, 2018; Robson, 2007b; Tagesu et al., 2019). These associations may be attributed to soil displacement that brings spores to the surface after having been deeply buried before (Pfeifer, 2018; Tagesu et al., 2019). The correlation between specific weather patterns and blackleg occurrence was described in 2017 by Pfiefer and colleagues (2018). These researchers reported a higher number of blackleg outbreaks that could potentially be linked to the erratic weather patterns of the year and the increased moisture and soil disruption following Hurricane Harvey. This is an interesting correlation that warrants further study, because understanding when blackleg outbreaks are most likely to happen may allow for more timely prevention. However, vaccination seems to provide adequate protection against these spores and is the only practiced preventive for blackleg.

Vaccinations are usually given to cattle at around four months of age, and followed by a booster dose up to a month later (Drovers, 2013; Ziech et al., 2018). Since the colostrum received from the calf's mother within the first few days of birth is usually sufficient defense against the Clostridium family of bacteria, young calves up to four months old are protected from blackleg in most cases (Drovers, 2013). If there is a known risk for blackleg, some veterinarians may recommend newborn vaccination, however, subsequent vaccinations at four and five months are still required to provide sufficient immunity (Drovers, 2013; Robson, 2007a, 2007b). Veterinarians also recommend yearly booster shots, although not all producers follow this recommendation (Drovers, 2013; Robson, 2007b; Ziech et al., 2018).

There are three types of blackleg vaccines, and the type used depends on the area. The most common in the Midwest is the 7-way vaccine which contains six different killed organisms of the Clostridium family; *C. chauvoei, C. septicum, C. novyi, C. sordelli*, and *C. perfringens* types C and D (Drovers, 2013). Although blackleg is mainly caused by *C. chauvoei,* the bacteria *C. septicum, C. novyi,* and *C. sordelli* contribute strongly to the disease and have specific effects (Maas, 2002). *C. septicum* and *C. perfringens* types C and D cause malignant edema, while *C. novyi* causes black disease of the liver (Maas, 2002; Robson, 2007a). The other type of blackleg vaccine contains the additional organism *C. hemolyticum* and is therefore called the 8-way vaccine. It is used in areas with abundant liver flukes, as *C. hemolyticum* infects susceptible livers and is also a cause of Redwater, a disease similar to blackleg (Drovers, 2013; Maas, 2002). The third and final type of blackleg vaccine is the 9-way vaccine, containing *C. tetani* (Daly, 2019). This vaccine gives protection against tetanus and is used in specific locations where tetanus is rampant or in castrated bull calves (Daly, 2019).

Both 7 and 8-way vaccines are generally effective but there is some debate on this matter (Drovers, 2013; Useh, Nok, et al., 2006; Ziech et al., 2018). Recent studies have found 7- and 8- way vaccines are not as effective as some think. Recent pathogenesis and toxin research has provided a much deeper understanding of blackleg and found the current vaccines only provide moderate to poor protection (Useh, Nok, et al., 2006; Ziech et al., 2018). However, since the 7 and 8-way vaccines are the only preventive measure available, they are widely used (Drovers, 2013; Maas, 2002; Stampfli, 2020). Proper vaccination practice is crucial, and it could be argued that many reported blackleg outbreaks were a result of improper vaccination techniques or improper carcass disposal (Drovers, 2013; Robson, 2007b). Both 7 and 8-way vaccines are meant to be delivered subcutaneously in the neck, as intramuscular delivery can cause damage to the muscles, e.g. lumps at the injection site (Drovers, 2013; Maas, 2002). Another mistake that can account for occurrence of blackleg, despite vaccination, is improper storage and use of the vaccine. Both 7 and 8-way blackleg vaccinations are most effective up to 24 hours after opening (Maas, 2002; Robson, 2007b). Additionally, containers not used shortly after opening or are stored at incorrect temperatures are subject to contamination that may cause the vaccine to become ineffective (Robson, 2007b). Furthermore, many producers do not continue to vaccinate their animals annually. Many producers assume the disease is not a problem in their herd, but since the spores can lay dormant in the soil for extended periods of time, blackleg will always be a potential threat (Drovers, 2013; Robson, 2007b; Stampfli, 2020). Lastly, if a blackleg outbreak occurs, proper carcass disposal procedures are crucial to prevent further infection in the herd. Proper disposal procedure is to bury or burn the carcass where it lies, because movement of the carcass may cause *C. chauvoei* spores to spread (Robson, 2007b). Spores can also be released by opening a carcass (Robson, 2007b). Minimizing vaccination and carcass disposal risks are important for producers to control blackleg, because there are no thoroughly effective curative measures (Robson, 2007b; Tagesu et al., 2019).

Since blackleg kills so quickly, it is frequently not diagnosed in time for any curative measures, which is one reason preventative vaccines are so crucial. If an animal is diagnosed with blackleg, the only curative interventions are large doses of penicillin and/or surgical removal of the affected area(s) (Tagesu et al., 2019). However and most frequently, this is not enough to reverse the damage *C. chauvoei* has caused, and survival rates are extremely low (Tagesu et al., 2019; Ziech et al., 2018). If the damage can be reversed, the surviving animal is usually lame and its market value decreases, which is detrimental to producers (Drovers, 2013; Tagesu et al., 2019).

Vaccination continues to be an important measure for controlling blackleg outbreaks, but there is recent research into specific aspects of this disease that may help develop more effective means of control (Ziech et al., 2018). Recent interest in unexplained aspects of blackleg has been renewed to explain why this disease has persisted for over 150 years without a cure. One prominent area of new research is the pathogenesis of blackleg (Tagesu et al., 2019; Ziech et al., 2018). Blackleg pathogenesis isn't fully understood, possibly due to many difficulties with studying *C. chauvoei* in the lab, but there is a hypothesis that may hold keys to developing an effective cure (Tagesu et al., 2019; Ziech et al., 2018). This method describes how spores are typically ingested, enter the muscles, and remain dormant until trauma or conditions that favor germination of spores into the vegetative state are met (Tagesu et al., 2019). Then, the vegetative bacteria release toxins causing necrosis before entering the bloodstream and bringing rapid death (Tagesu et al., 2019). However, the types of toxins and their exact function in mortality aren't fully understood or characterized (Useh, Nok, et al., 2006; Ziech et al., 2018). Further research into these areas may help develop another vaccine that targets these steps of pathogenesis and can more effectively prevent blackleg.

Some toxins have been identified in the pathogenesis of blackleg and include hyaluronidases, hemolysins, and deoxyribonucleases (Useh et al., 2003; Ziech et al., 2018). Five specific toxins have been characterized, including hemolysin D, DNase, CctA, neuraminidase NanA, and hyaluronidase Nag (Ziech et al., 2018). Hemolysin D, also called hemolysin III, has still not been definitively identified in *C. chauvoei* but is similar to the toxin produced by *C. perfringens;* the scope of its involvement is unknown (Ziech et al., 2018).

CctA, or *C. chauvoei* toxin A contributes to the virulence of blackleg (Frey, Johansson, Bürki, Vilei, & Redhead, 2012). This toxin is a hemolysin that belongs to the β barrel pore forming leukocidin family (Frey & Falquet, 2015; Frey et al., 2012; Rychener et al., 2017). CctA accounts for the majority of hemolytic and cytotoxic activity of *C. chauvoei* and is particularly detrimental to calf nasal epithelial cells, which may explain the common occurrence of blackleg in animals under two years of age (Frey et al., 2012; Ziech et al., 2018). CctA strains isolated worldwide from the past 50 years were shown to be evolutionarily similar, indicating this specific toxin is highly conserved (Frey et al., 2012; Rychener et al., 2017). Because of this, development of vaccines to neutralize this toxin might prove effective. Recent studies involving guinea pigs have revealed that CctA prepared with recombinant gene technology was effective in prevention of infection (Frey & Falquet, 2015; Frey et al., 2012). Further development and tests of this proposed vaccine are needed, but this line of research is promising.

NanA is an important neuraminidase/sialidase involved in cleavage of sialic acids that facilitates the spread of blackleg in an organism (Useh, Nok, Ambali, & Esievo, 2004; Useh et al., 2003). Neuraminidase activity is directly related to the pH and temperature of the surrounding tissues, and these optimal conditions coincide with the known anaerobic conditions under which *C. chauvoei* operates most efficiently (Useh et al., 2004; Useh et al., 2003). The NanA gene coding for neuraminidase was found to be conserved worldwide throughout the past 60 years of analysis on *C. chauvoei* strains, indicating that it's evolution has paused (Ziech et al., 2018). Since neuraminidase is an important factor in the pathogenesis of blackleg, research aimed at using neuraminidase inhibitors as treatment for blackleg is underway and should continue (Useh et al., 2004; Ziech et al., 2018). A few neuraminidase inhibitors (Paranitrophenyl oxamic acid, salicyl oxamic acid, diethylpyrocarbonate, N-ethylmaleimide) have been found to effectively stop *C. chauvoei's* neuraminidase activity in vitro (Useh et al., 2004). Some in vivo treatments using medicinal plants known to contain neuraminidase inhibitors have also been successful in the prevention of blackleg in rural Nigeria (Useh et al., 2004). There has been significant progress concerning neuraminidase and its role in pathogenesis and treatment of blackleg, and hopefully these findings will be incorporated into a commercially available vaccine in the near future.

Hyaluronidase NagH, a γ-toxin, has recently been sequenced and identified as an important component in pathogenesis of blackleg (Dangi, Yadav, Tiwari, & Nagaleekar, 2017). The gene *nagH* (hyaluronoglucosaminidase) in *C. chauvoei* that codes for the NagH toxin acts as a virulence factor and breaks down hyaluronic acid (Dangi et al., 2017; Ziech et al., 2018). This enzymatic activity makes it easier for *C. chauvoei* to spread in connective tissues and leads to muscle destruction (Ziech et al., 2018). No research concerning methods to stop *nagH* from spreading *C. chauvoei* has been reported, since characterization of this gene is so recent (Ziech et al., 2018). It has been hypothesized that the disaccharides remaining after hyaluronic acid breakdown provide nutrients for *C. chauvoei*, which further increases the spread of blackleg in the surrounding tissues (Ziech et al., 2018). These hypotheses indicate the importance of further research into the *nagH* gene and activity of hyaluronidases in blackleg.

DNase (deoxyribonuclease) is a β-toxin that aids in myonecrosis (Ziech et al., 2018). In *C. chauvoei* there are variations in toxin production among different strains, however the genes for DNase (specifically, genes encoding exo-deoxyribonuclease VII) were conserved in all strains studied (Rychener et al., 2017; Ziech et al., 2018). Unlike the other toxins, since DNase production varies among strains in expression, it might be more beneficial to further characterize the genes responsible for these differences before researching methods to counteract its enzymatic activity.

Cellular antigens are another area of research that could be expanded (Ziech et al., 2018). Flagellar antigens (specifically flagellin) protect against *C. chauvoei* in mice; this has implications for the composition of future vaccines (Frey & Falquet, 2015; Ziech et al., 2018). The importance of these antigens is highlighted in the increased genetic similarity of various *C. chauvoei* strains, because the expression of cellular antigens is nearly identical (Frey & Falquet, 2015; Ziech et al., 2018). This indicates if a vaccine could be developed that increased immunity to *C. chauvoei*, that vaccine would be effective for almost all known strains of the bacteria (Scott, 1926; Ziech et al., 2018).

Extended research into surface-associated antigens, as well as somatic antigens, could further efforts for a more effective vaccine (Ziech et al., 2018).

In sum, *C. chauvoei* is a very dangerous bacterium, and there are a number of different strains (Scott, 1926). However, these strains are almost genetically identical to one another and act like a single strain (Frey & Falquet, 2015). They were identical in virulence genes for CctA, NanA, and NagH, which indicates evolution of *C. chauvoei* has paused (Frey & Falquet, 2015). This information is beneficial because any vaccines or alternative therapies concerning these virulence factors would be effective on all strains of blackleg.

Much research is being done into the pathogenesis of blackleg; however, there are still important deficits in knowledge about this disease. Because vaccines have been in use for many years, most producers assume blackleg is no longer a threat to their herds. However, recent research has found these existing vaccines provide only poor to moderate protection against blackleg (Uzal, 2012). In order to conduct further research into pathogenesis, toxins, and more effective vaccines, more knowledge must be gathered about cases of blackleg. Most producers don't report known cases of blackleg, and many others just bury their dead cattle without ever knowing the cause of death, because blackleg kills so quickly. The present study aims to address some of these deficiencies by gathering information from veterinarians regarding their experience with blackleg. I asked questions about the current preventative measures (namely, vaccination), as well as the history of blackleg throughout South Dakota. Determining the incidence of blackleg allows for expansion of research on curative and preventative measures. Additionally, more information available to farmers and veterinarians about advances in understanding of blackleg are important tools that may help manage blackleg (Uzal, 2012).

Chapter Two: Methods

To study the incidence of blackleg in South Dakota, a short ten-question survey was administered to 334 beef cattle veterinarians registered with the SD Veterinary Medical Association. They were asked the following questions with a comment box at the end of the survey;

1. In what county/counties do you practice?
2. How many years have you spent in beef cattle veterinary practice?
3. How many beef clients do you have? If you practice with more than one beef cattle veterinarian, enter “your” share of the clients.
4. What is the average herd size of your beef cow-calf clients?
5. To what proportion of your cow-calf clients do you recommend blackleg vaccination? (0-24,25-49,50-74,75-99,100%)
6. What proportion of your cow-calf clients vaccinate calves for blackleg? (0-24,25-49,50-74,75-99,100%)
7. Approximately how many beef calves in your practice area have you determined or highly suspected to have died from blackleg throughout your career?
8. Of the cases above, how many different farms were affected?

9. Were the blackleg cases above from herds that were vaccinated for blackleg? (all, most, few, none)
10. Do you perceive any recent changes in the effectiveness of the blackleg vaccination over time? (Works better, no difference, works worse)

These questions were composed with consideration for animals most at risk for blackleg: beef calf exposed to pasture. Therefore, feedlot and dairy cattle were not included in this survey and only data from beef cow-calf operations was used. For practices with more than one veterinarian, respondents were asked to only enter “their share” of the beef clients to ensure there was no overlap. There were 38 respondents total. Though some of these respondents listed counties in Iowa and Minnesota in addition to their South Dakota areas, this survey was sent to members of the South Dakota Veterinary Medical Association exclusively. Therefore, these veterinarians claim to practice in South Dakota to at least some degree, and their South Dakota entries were treated the same as the rest of the data. Data from Iowa and Minnesota counties was excluded. Out of the total respondents, 86% practice exclusively in SD. The other 14% of respondents still practice in some South Dakota counties, but also work in Iowa and Minnesota.

After administering the survey and collecting the data, it was entered in SAS (Carey, NC, 2012) to calculate the mean, standard deviation, and sum of the responses to each open-ended question (questions 1-4, 7-8). Frequencies were calculated from the questions with multiple-choice answers or percentages (questions 5 ,6, 9, 10). Frequencies were also calculated from open-ended questions using Excel. Lists were compiled comparing the answers from the multiple-choice questions and the MEANS procedures from the other questions. After analyzing data as a whole (treated as a single area), data was separated by county to create a representative map from available information.

Herd-level incidence was calculated by taking the number of herds affected divided by the total number of herds. This was done with the data as a whole and separated by county. To find out the within-herd incidence of blackleg per farm, the number of reported blackleg cases was divided by the number of farms affected. Overall incidence was calculated by taking the number of reported blackleg cases divided by the total population at risk. The population at risk was defined as the total number of animals serviced, calculated by multiplying the average herd size by the number of farms. All of these calculations were done for both the entire career of each veterinarian, which spanned 45 years, and on a yearly basis.

Formulas:

a. Herd-level incidence= number of farms affected/ total number of clients serviced
b. Within-herd incidence= number of blackleg cases throughout career/ number of farms affected
c. Overall incidence= number of reported blackleg cases throughout career/ total population at risk
    i. Total population at risk= (average herd size*number of farms)
d. Average yearly calculations= incidence (herd-level or overall)/ 45 years

Chapter Three: Results

Herd-level incidence was 18%. This means that throughout the 45 years these veterinarians have practiced medicine, they found blackleg affected 18% of the herds they have serviced. On a yearly basis, 0.4% of herds serviced by South Dakota veterinarians were affected.

Within-herd incidence was 1.4. This data shows that within a herd that has been affected by blackleg, 1.4 animals out of that herd will experience the disease.

Overall incidence of blackleg in South Dakota in the past 45 years was 2.8 cattle per 100,000 head. On a yearly basis, overall incidence was .06 cases per 100,000 head.

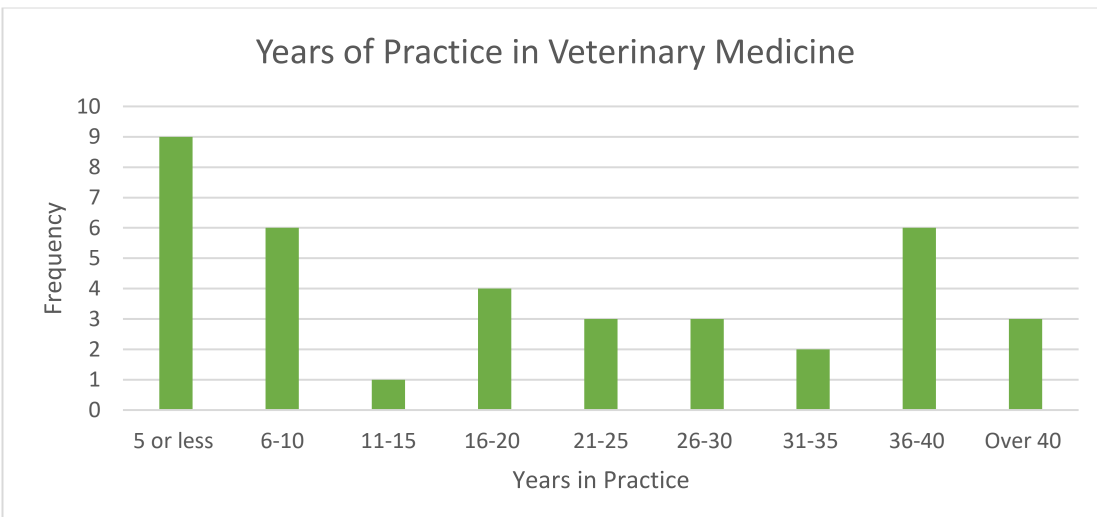

Figure 1: Years of practice in veterinary medicine. The most common response was 5 or less years of practice. The least common was 11-15 years. However, there is a lot of variation. There was a maximum of 45 years of experience and minimum of one year of experience included in this survey.

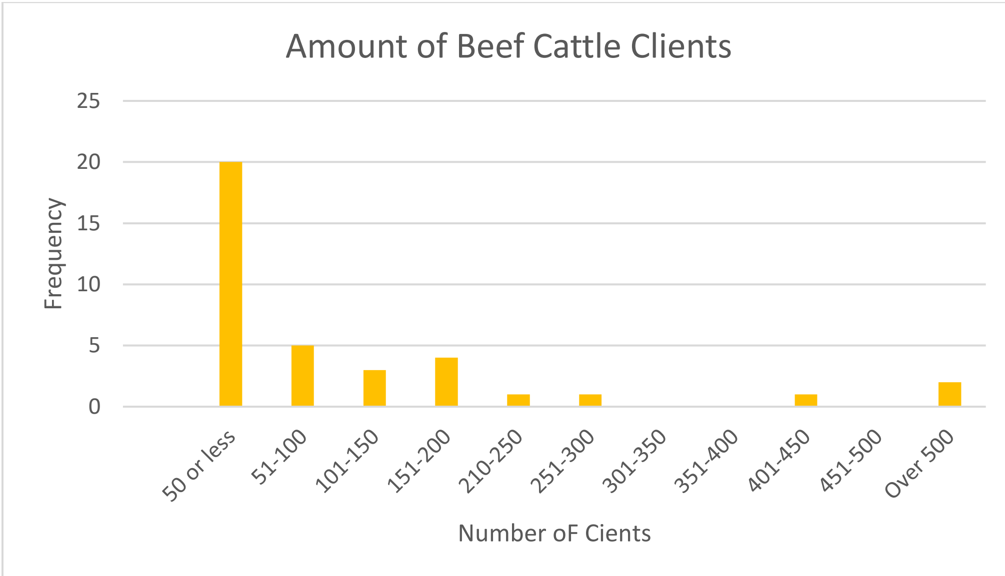

Figure 2: Amount of beef cattle clients that each South Dakota veterinarian services. Over half of the responding veterinarians service 50 or less beef cattle clients. As the number of clients per individual veterinarian increases, the veterinarians that service those clients drops drastically. By servicing a smaller number of clients, a stronger patient-provider relationship can be formed. The number of clients serviced is also dependent on the region of practice, as one region may have more or less clients that have beef cattle.

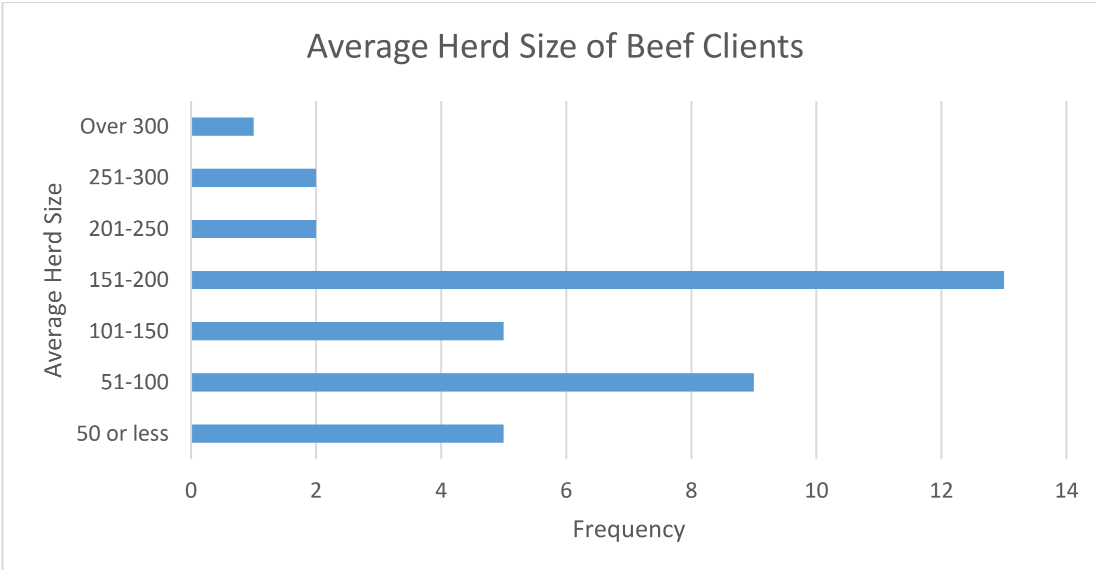

Figure 3: Average herd size of beef cattle clients in South Dakota. Most of the herds are between 151-200 head of cattle. The majority of veterinarians service herds 200 head or less, with 86% of veterinarians in this category. There are very few vets who deal with clients with average herd size over 200 head. However, this is an average; many vets deal with clients that have a lot of variation. Again, regional differences have to be taken into account; some areas are able to support large numbers of beef cattle, while others aren't as well-equipped.

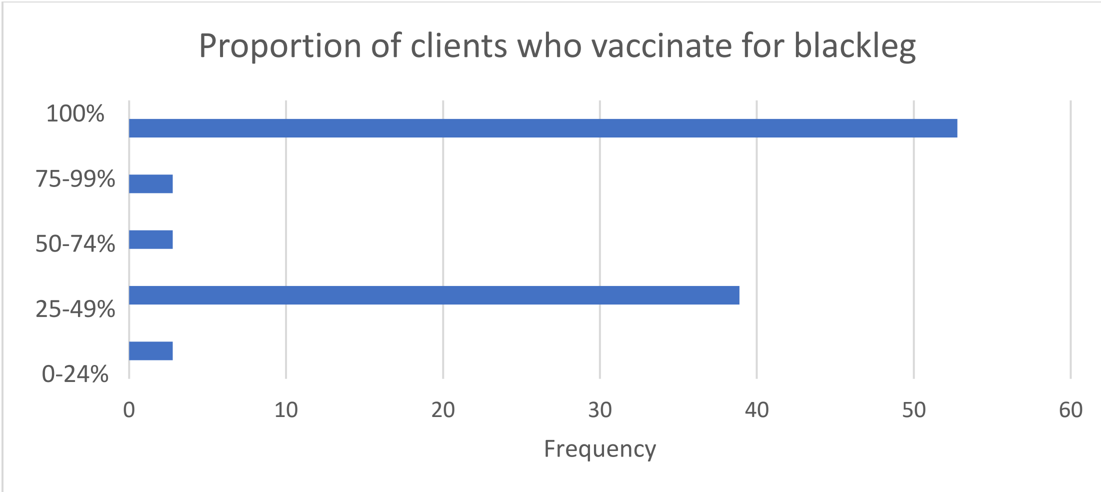

Figure 4: Proportion of clients who vaccinate for blackleg. The most modal response was that all of their clients (100%), vaccinate. The second most frequent response was just under half, with 25-49% of clients vaccinating their animals.

Although the vaccination practices of clients may vary, the recommendation for vaccination practices by veterinarians does not vary widely. 97% of South Dakota large animal veterinarians recommend the blackleg vaccination to all of their clients, while only 3% do not recommend the vaccination to any client.

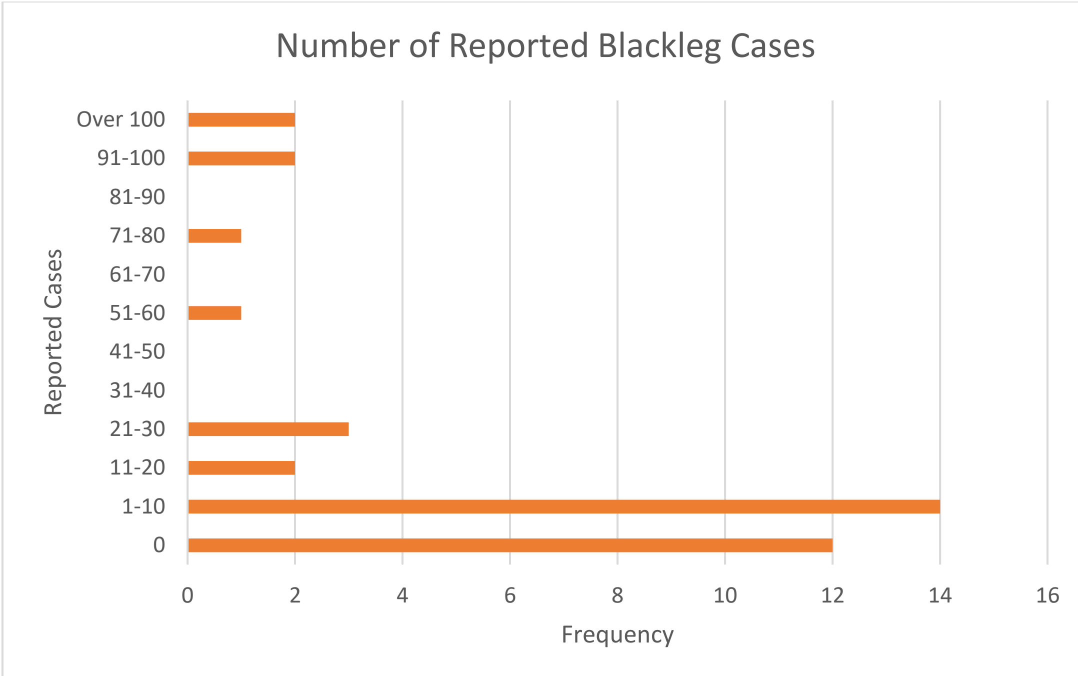

Figure 5: Number of reported blackleg cases by South Dakota veterinarians. The data is largely skewed, with over 84% of respondents reporting less than 30 cases in their career. The most common response was 1-10 cases, while the least common responses were 51-60 and 71-80 cases. The minimum was 0 and the maximum was 140 reported cases.

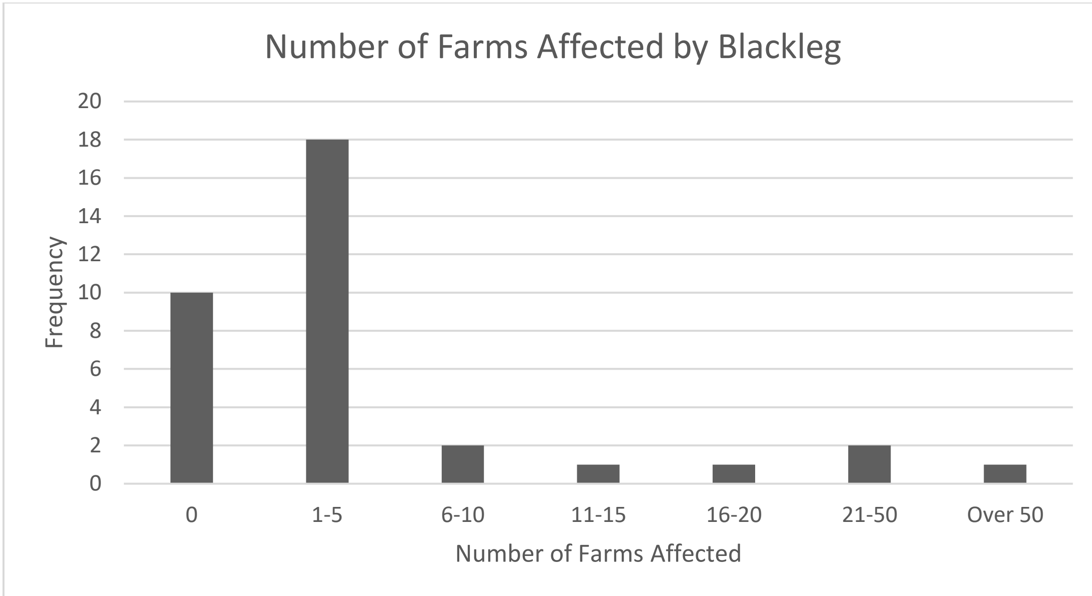

Figure 6: Number of farms affected by blackleg. There was a large gap in the middle of the data, with only two farms in the large interval of 21-50 cases. The majority of veterinarians reported low numbers of farms that experienced blackleg, with 80% of veterinarians reporting 10 or less individual farms in their career. This tracks the data from number of blackleg cases reported, as the most common number of cases was less than 30.

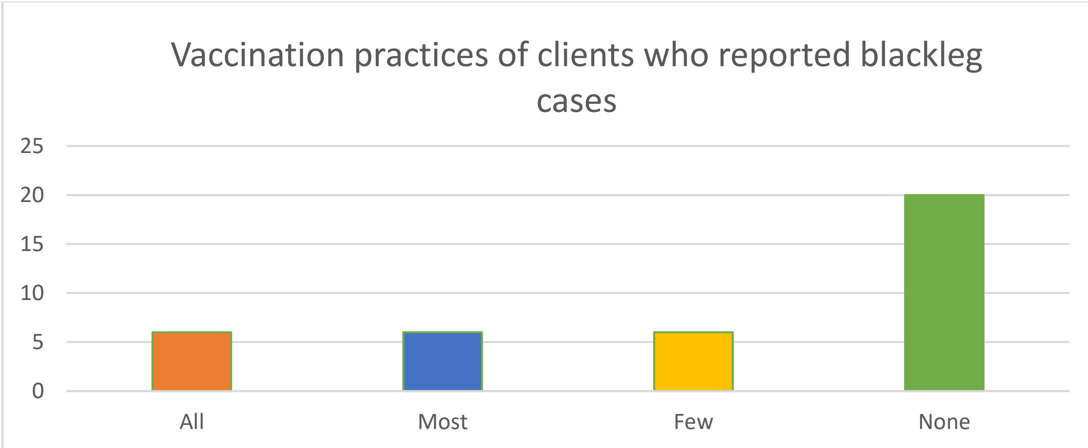

Figure 7: Vaccination practices for clients who reported blackleg cases. The majority of reported cases were from producers that did not vaccinate their cattle for blackleg. There was no difference between producers who vaccinated all their animals, most of their animals, or few of their animals. This indicates that the presence of vaccination practices correlates to reduced incidences of blackleg.

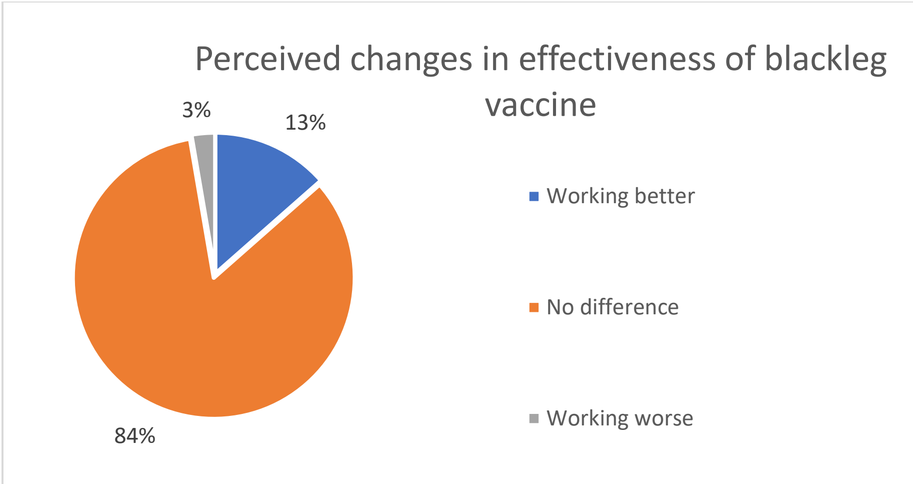

Figure 8: Perceived changes in blackleg vaccination effectiveness over time, with the dominant view being no difference in effectiveness over time (84%). Only a small fraction of respondents claimed the vaccine was working better (13%), with an even smaller fraction claiming the vaccine was working worse (3%). This suggests that management of blackleg can be maintained solely with the 7-way vaccine currently used, as the effectiveness has not varied through time.

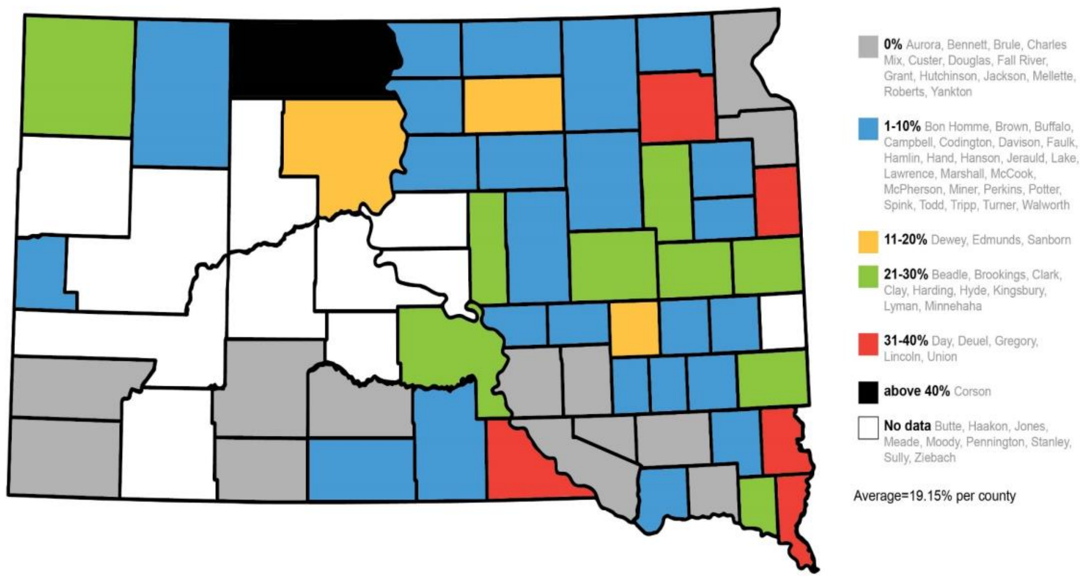

Figure 9: Map of blackleg herd-level incidence split by counties in South Dakota. There were 66 total counties, 56 of which had reported data. The average was 11.3% incidence per county. The majority of the counties had an incidence level of under 10%. The least common level was above 40%, with only one county in each of this categories.

County Herd-Level Incidence		
Incidence	**Number of Counties**	**Percentage**
0%	13	19.70%
1-10%	24	36.36%
11-20%	6	9.09%
21-30%	3	4.55%
31-40%	9	13.64%
Above 40%	1	1.52%
No data	10	15.15%

Table 1: Breakdown of county blackleg herd-level incidence. As shown in Figure 4 above, the majority (36.36%) of counties had an incidence level of below 10%. 0% incidence was the second most common, at 19.7% of counties in this category. 15.15% of counties in South Dakota did not have any data available in this study.

Chapter Four: Discussion

Because blackleg has been around for such a long time, it continues to be an important area of research in the veterinary field. Many producers and veterinarians believe the incidence of blackleg is low because of the vaccinations available, and this study reports both a low overall and herd-level occurrence in South Dakota that is consistent with these views (Abreu et al., 2017; Robson, 2007a). In a span of 45 years, the overall incidence was 2.82 cattle per 100,000 head, and 18% of herds in the state were affected. Additionally, 1.4 animals within the affected herds presented with the disease. This data indicates current vaccination practices are effective in managing blackleg.

However, there is definite regional variation in the occurrence of blackleg. Upon breaking down the data by county, there are regions that have much higher incidences than their neighbors. There are many factors that may account for these regional differences. One of these factors is soil disturbance. In one study, three cases of soil excavation in Montana, Minnesota, and the Lake Superior area were linked to incidences of blackleg shortly following the excavation (Barnes, Bergeland, & Higbee, 1975). This excavation can either turn up soil containing previously buried spores, or provide the correct conditions for spore germination (Disasa, Balcha, & Negewo, 2020). When farmers disturb the soil in pastures or grazing area, they are increasing the risk of a blackleg infection. Informing producers about the dangers of soil disturbance without subsequent vaccination practices may be an important step to help reduce blackleg incidence.

A few other factors that influence blackleg incidence are rainfall and drought (Useh, Ibrahim, Nok, & Esievo, 2006). Higher rainfall has found to be correlated with higher blackleg occurrences (Pfeifer, 2018; Robson, 2007b). Rainfall influences blackleg rate as it can cause soil disturbance and runoff, which brings infectious spores to the surface where they are then ingested and cause disease in cattle. These spores would have been previously buried and not as easily accessible. Drought also increases blackleg infection rate as it results in shorter vegetation and soil that is easily moved by wind, both of which bring infectious spores closer to cattle and easier for them to ingest (Eilerts, 2020).

The county in this study with the highest blackleg incidence was Lawrence county, with a 50% herd-level incidence. The second-highest incidence rate was in Douglas and Davison counties, with 39.9% herd-level incidence. Davison and Douglas counties are neighbors in southeast South Dakota, while Lawrence county is in western

SD. More research into weather patterns in these counties would be needed to establish concrete links between recent rainfall or drought and blackleg, but it is a factor that cannot be discounted.

The regional variation in herd-level incidence and the overall incidence could be affected by variance between vaccination practices in different clinics; however, this factor was accounted for by analyzing data from survey questions 5-9. The veterinarians were asked about their vaccination recommendations, efficacy of the vaccine, and whether their clients follow their recommendations. The majority of South Dakota veterinarians in this study recommend the blackleg vaccination to their clients, and most of those clients vaccinate. According to the majority of South Dakota veterinarians surveyed, the blackleg vaccine they use has not varied in efficacy through time. As for the vaccination itself, there are very few types of vaccinations used to fight blackleg, so any variation would be minute. There are three types; 7-way, 8-way, and 9-way. The difference between the 7-and 8- way vaccines is the addition of one more antigen, *C. hemolyticum*, but the 7-way vaccine is most commonly used in the Midwest (Drovers, 2013; Robson, 2007a). The 8-way and 9-way are used in specific areas and under specific conditions that usually aren’t common in South Dakota (Daly, 2019). Just like with any other vaccine, multiple companies make these products, but the type used in an area is based on veterinarian and producer preference.

Although the vaccines themselves may not vary by much, no data was collected on how often the vaccinations were administered, and this could be a cause for the high incidence rate of blackleg. For cows, vaccinations are recommended yearly or immediately following an outbreak on a farm (Drovers, 2013; Robson, 2007b; Ziech et al., 2018). Calves are vaccinated at around four months and a booster is recommended a month later (Drovers, 2013; Robson, 2007b; Ziech et al., 2018). If these practices are not followed, vaccinations from previous years may not be effective against blackleg. The infectious spores that cause blackleg can remain dormant in the soil for extended periods of time, which requires yearly management (Stampfli, 2020). Failure to vaccinate cows yearly, or failure to administer a booster in calves, can allow blackleg to infect and kill animals. Almost all 19 veterinarians surveyed in this study that provided comments specified the cases they saw were either from herds that don't vaccinate at all, or don't vaccinate according to procedure. Not vaccinating according to procedure means the affected animals either weren't given a booster (as calves) or vaccinated yearly (as cows), which could have contributed to their deaths. Many veterinarians surveyed also championed the effectiveness of the 7-way vaccine, and noted its cost and efficiency make it a popular product that many insist their clients utilize. Ultimately, veterinarians cannot force their clients to do anything, and the decision to follow recommended vaccination practices is in the hands of each producer.

The long range of this study also impacts the incidence calculations. Survey participants were asked how many cases they had seen throughout their career. The longest career response was 45 years, while the shortest was one year. Variation in these numbers, advances in research and technology, and new information about blackleg throughout this timespan are all influential.

First, vaccination practices of certain veterinarians or producers has changed in the last 45 years to be more effective in managing blackleg. For example, some used to only vaccinate cows once when they were calves, but now recommend yearly vaccination. Some now recommend the booster to calves when they previously hadn't. Another variation seen through time was that some veterinarians surveyed in this study noted the occurrence of additional organisms in the environment that can facilitate blackleg influence their vaccination practice; specifically, *C. sordelli* and *C. perfringens* Types A and C. Others noticed that the age of onset in blackleg has increased and have changed their vaccination recommendations to yearly according to this difference, in addition to stressing the vaccination of cows and bulls rather than just calves. Some note they have relied on multiple companies' vaccines to combat this disease. As research continues to advance concerning blackleg, vaccination recommendations and veterinary practices change to facilitate lower and lower rates of blackleg.

Survey Limitations

One possible confound of this study was the potential underreporting of blackleg. Because of the short kill time of this disease, affected cattle may be buried with cause of death unknown and without a veterinarian present. Often times, no symptoms of blackleg are even noticed (Daly, Miskimins, Good, & Stenberg, 2009; Stampfli, 2020). Cattle affected with this disease may also not be discovered for an extended period of time, complicating diagnoses of blackleg (Daly et al., 2009). One vet who responded to the survey in this study stressed this problem and detailed their experience with the difficulty in diagnoses. This issue is hard to rectify since many producers do not have resources to either a call to a vet or bring the deceased animal to a lab. However, if more information was available to producers about the importance of reporting diseases like blackleg, this issue may be lessened. Information sent out to producers about nearby necropsy labs (specifically, colleges) would also be beneficial. In South Dakota, the South Dakota State University (SDSU) Animal Disease Research and Diagnostic Laboratory (ADRDL) is the main resource for producers to bring their deceased animals in for testing (Daly et al., 2009). Many states have at least one diagnostic lab, either at a college or sometimes a wildlife and natural resources establishment (USDA, 2021). However, these places may not be well known, and the more information available about these institutions, the more well-prepared farmers are to continually manage blackleg.

Blackleg can also be hard to diagnose for a few reasons. Typically, the affected animal presents with muscle swelling that gets larger as the disease progresses (Disasa et al., 2020; Stampfli, 2020). The swelling fills up with gas, and the muscles turn dark red or black (Disasa et al., 2020; Stampfli, 2020). The affected muscles are often the hind legs, shoulder, hips, chest, and back (Tagesu et al., 2019). Unfortunately, factors such as quick decomposition, high temperatures, quick death rate, and differing clinical presentations all influence diagnoses (Daly et al., 2009). Culturing *C. chauvoei* from the deceased animal is also difficult due to its anaerobic nature and other bacterial growth around the affected site (Daly et al., 2009; Stampfli, 2020). Often other clostridial organisms like *C. sordelli*, *C. novyi*, and *C. septicum* may be present in a culture from the affected animal or cause symptoms that resemble those of blackleg, which complicates diagnoses (Daly et al., 2009; Disasa et al., 2020; Stampfli, 2020).

Another factor complicating diagnoses and contributing to the potential underreporting of blackleg is the age of onset of this disease. Some veterinarians in this survey noted they have been seeing more cases in cows and bulls, rather than young and quick growing calves that is often typical for blackleg (Disasa et al., 2020). This onset in older animals may be caused by environmental factors discussed previously, or failure to vaccinate yearly. Although it is rare, blackleg has been known to cause a fetal infection as well (Abreu et al., 2017). This is a potential problem when researching incidence because lots of producers will not send in a fetus for testing. However, fetal infections are scarce and because of this are not as big of a factor as the increased incidence in adult cattle.

Conversely, some blackleg cases that were reported may not have actually been caused by blackleg. The clinical presentations may vary and this could cause a misdiagnosis (Abreu et al., 2017; Daly et al., 2009). The most common way blackleg is identified is by tissue necrosis, but sometimes this way of diagnoses isn't conclusive (Stampfli, 2020) There are numerous other infections caused by the *Clostridium* family of bacteria, such as tetanus, overeating or enterotoxaemia, clostridial hepatitis, black disease, and malignant edema (Robson, 2007b). Clostridial bacteria release toxins that act upon the animal and in most cases of disease are fatal (Robson, 2007b). This mode of action is consistent throughout different clostridial species and may complicate blackleg diagnoses. Specifically, malignant edema presents very similarly to blackleg. It is characterized by swelling, tissue necrosis, and weakness, which are all signs of blackleg (Randolph, 2021; Robson, 2007b). Misdiagnoses of malignant edema or other clostridial diseases may cause some error in this study.

This was a small study with a sample size of 38, with a response rate of 11.38%. A larger sample size and stronger response rate would be a better representation of the entire state, and more data provides more accurate results. Because of this small size and the question design, there was some error introduced. We organized information by county, but it is impossible to know what proportion of the county was represented and to what extent. Many vets also cross county lines which introduces more error. These errors are hard to avoid but with the available data a representative map was created.

Additionally, this survey may be biased, as only veterinarians interested in blackleg might have participated. The only way to avoid this bias would be to have a response rate of 100%, which is almost impossible to achieve. Since the response rate was so low, bias may be a bigger factor that usual, but it is still unavoidable.

Finally, the overall and herd-level incidences calculated is subject to error because some of the beef cattle producers in South Dakota don’t use veterinarians. Therefore, the blackleg rate may be higher or lower depending on the incidence in those herds and how the producers manage blackleg. There is no way in this study to account for producers that don’t use veterinarians, as this survey was only administered to veterinarians and they are usually the ones to diagnose an animal with blackleg.

Summary

Although the herd-level incidence varied by region, both the overall incidence rate and total herd-level incidence were very low. Considering all the animals at risk in the population, a very small amount have been affected in the past 45 years. This is indicative of effective preventative measures in South Dakota. Most of the veterinarians in this survey reported they recommend the vaccination to all of their clients, and most of their clients follow those recommendations. They report the efficacy of this vaccine has not varied through time. Blackleg is deadly and requires constant management, but data from this survey shows management of blackleg in South Dakota is effective.

Overall, blackleg is a deadly disease that affects beef cattle and is important in South Dakota. Locating regions that are more prone to blackleg can influence veterinary/vaccination practices and recommendations, which can lead to healthier animals and happier livestock producers. Furthermore, making more information available to producers and veterinarians about blackleg as research progresses is a necessary step toward successful blackleg management.

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