A herd health and cost savings analysis for the hyperimmunization of sheep in polyclonal antibody production

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ABSTRACT

The purpose of this research project was to determine the outcome of decreasing the use of adjuvant while immunizing sheep for polyclonal antibody. To justify the change in protocol, a herd health analysis was conducted to track site reactions and a financial analysis based on Net Present Value to determine future financial benefits. For this study 199 cross bred ewe lambs were utilized for titer test analysis after 6 months of hyperimmunization with Universal Barbiturate antigen, saline, and adjuvant. The population was broken down into four protocols based on the injection ratio. These protocols, with their respective injection ratios were Current Protocol #1 (1/1/1), Current Protocol #2 (0.5/1/1), Test Protocol #1 (1/1/0.5), and Test Protocol #2 (1/1/0). In addition to the titer test, 128 sheep were randomly selected for site reaction data collection. Based on a graded scale, animals were graded based on the physical reaction sites over their lumbar regions where the injections took place. The results from both data collection methods were then compared to determine the best methods to decrease sight reactions and increase immune response rate. The results from this data compared the costs associated with adjuvant use and developed a cost savings analysis for each protocol used and helped to determine the best method for the company moving forward. The results concluded that the newly proposed method of injection offered a significant cost savings initiative, decreased site reactions, and still produced high immune response to maintain the expected rate of production.

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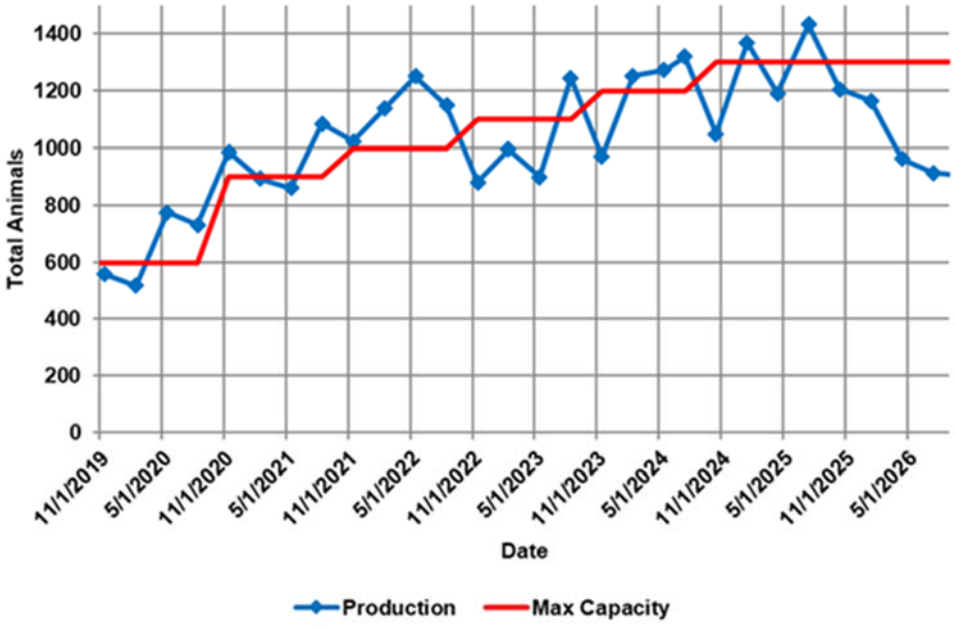
CHAPTER I: INTRODUCTION

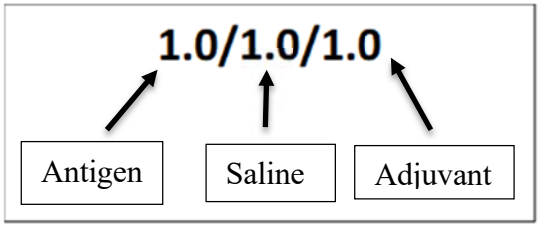
Siemens Healthcare Diagnostics has been a major player in the polyclonal antibody production industry for several years. As the industry progresses, keeping up with a growing demand and increased costs of materials are two of the biggest challenges the company faces. Additionally, all raw product is derived from livestock, primarily sheep. These small ruminants are key to producing adequate volumes of serum in a reasonable response period to keep up with projected demand. As shown in Figure 1.1, the projected demand for production sheep producing antibody is growing immensely causing many aspects of the process to be analyzed for cost savings potential, animal health, and efficiency. Maintaining herd health is a primary focus of the company. Working hand in hand with the USDA, maintaining a healthy environment for the sheep and meeting demand expectations are major factors when making decisions for the company.

Figure 1.1: Projected Growth of Animal Demand



1.1 Background

Antibody production has been used for decades to provide the raw material needed for healthcare companies to develop test kits for hospitals and doctors' offices. These test kits are used for a variety of reasons, but the polyclonal department of Siemens Healthcare focuses on primarily addictive and therapeutics drugs. Sheep, the most commonly used animal for antibody production for the company begin the process in the "test phase." Sheep usually arrive in flocks of 150-200 head and after a health status quarantine phase, are hyperimmunization for a minimum of 6 months prior to the first titer test. The six month time period has been determined as an average time frame for an immune response to have developed a high enough value to be recognized and the selection process for production animals can begin. Additional testing may take place at 9 and 12 months depending on the response rates thus far and the demand for inventory of the antigen of choice. Animals who qualify for production will continue to be immunized until they are no longer used for antibody collection. As you can see, the immunization of these animals is critical and is a huge factor in their potential success for production use. The potential for increasing productivity, decreasing cost, and maintaining herd health are all drivers for evaluating a herd health and cost savings analysis for the immunization protocol. Currently the most discussed step in the procedure of immunization, is the use of adjuvant and the combination of the adjuvant with different antigens. An immunization consists of three parts and is explained in a ratio format.



Protocols are antigen specific and animals are assigned to only one. On average, a flock of two hundred animals are dedicated to a program where they are further broken up into groups where each one received a certain ratio of antigen to saline to adjuvant combination. Overtime, some animals have been found to develop site reactions to these immunizations. The majority are small lesions, commonly found to be sterile when swabs have been taken and tested. It is a well-known fact that many adjuvants have been found to cause these types of site reactions which is why the company specifically uses Freud's Incomplete Adjuvant for all injections with the exception of the initial injection which contains Freud's Complete Adjuvant. Adjuvant is an additive with an oil appearance used to boost immune response in combination with an antigen. While using the incomplete Freuds adjuvant, site reactions have been more controlled but are most commonly administered at a 1:1 ratio of antigen/saline mixture. This raises the question that if the adjuvant ratio was decreased would the animals still have a similar immune response than they would have with an equal ratio? Would this open up for a potential decrease in future site reactions? If this is the case, does the ratio of antigen to adjuvant play a major role in both immune response and physical reactions? Both antigen and adjuvant are expensive additives to this process and since the antigen variable changes so frequently depending on the program being produced, would cutting down on adjuvant increase cost savings across the board for all programs? Further analysis into different adjuvants and their individual success rates could also provide a foundation for future studies.

1.2 Cost Implications

Incomplete adjuvant costs $1.15 per injection of each animal. There is 1.38 mL adjuvant used per animal injection including a calculated overage to ensure proper dosage

is met. The facility is expecting major growth in not only production animals but in capacity, allowing for more test animals to be injected at one time. Decreasing adjuvant use by 50%, also shown as a 0.5 ratio, would result in a decrease in cost per injection to $0.57. Overtime, this decrease in cost of adjuvant could have an impact on financial budgets. Narrowing down success rate potential based on adjuvant ratio could decrease the head count of animals needed to start each program, immune response will be narrowed down and subject to meeting titer expectations between 6 and 9 months post injections. All of these costs are constants within the process and will impact the overall costs associated with the immunization directly.

1.3 Animal Health Implications

In theory, by decreasing adjuvant use there is potential for a decrease in site reactions observed. As demand steadily grows, just as Figure 1.1 shows, there will be double the animals injected within the next 2 to 3 years. With a huge increase of immunized animals, the susceptibility of more animals presenting with a site reaction also increases drastically. Site reactions are a concern with respect to animal welfare and management and are closely monitored as animal quality of life and health are a top priority. It is on a very rare occasion that an animal has a reaction that is chronic and therefore regardless of immune response, that animal is not allowed to continue as a test or production animal. Some animals are naturally more sensitive to immunizations and protocols are in place to monitor the animals more likely to react. The trial conducted is primarily focused on decreasing the physical reaction rate of the herd overall but especially in the animals who are more susceptible.

1.4 Potential Outcomes

More animals are being injected and therefore costs associated with incomplete adjuvant are growing in addition to antigen. Having a better understanding of what ratio combinations animals will respond to will also help cut down on lead times and increase success rate accuracy. Increasing success rates will increase accuracy of selecting animals who will respond best and utilizing less adjuvant will decrease site reactions. Overall, the goal of conducting this research is to determine the potential for determining which ratios increase response rate to antibody production, leading to more animals added to the production line, and less animal turnover as production will be more efficient. The more accurate the initial test bleed is in relation to the immunization protocol, the quicker animals can be deemed fit for production.

CHAPTER II: LITERATURE REVIEW

Siemens Healthcare has acquired several years of experience as well as dedication to R&D programs and prior studies. Hyperimmunization has been a common method to boost animals used for antibody production. This procedure is based around outside research and has been tailored to fit the working scenario at the facility. The research and method of inoculation has primarily focused on changing antigen ratios only to modify success rates. Hyperimmunization includes mixing an injectable solution using lyophilized antigen, saline, and Freud's adjuvant into a solution that is easily transferable into an animal. This method coincides with previous research supporting this approach.

2.1 The Use of Adjuvant

Research pertaining to the use of adjuvant focuses on determining the best type or combination of adjuvant to be used, and the potential for skin sensitivity responses, and success rates (Aucoturier 2001). Different combinations of adjuvants have been researched to determine the best fit for response rates and to discover the direct impact on animals depending on what immunogen combination is being used. Research has shown that using a water to oil emulsion mix is the best solution overall but is entirely dependent on the end goal of the company (Aucoturier 2001). This mixture adopted by Siemens Healthcare, has been found to have a high response rate and extensive longevity perfect for animals on production for several years based on the antigen projects executed. The downside to a water and oil mixture are the potential for site reactions (Aucoturier 2001). On the contrary, an oil to water emulsion was found to execute shorter term response rates that were less reactive than the previously discussed combination (Aucoturier 2001). Overall, prior research agreed that a water to oil emulsion worked the most efficiently and was adopted

by Siemens Healthcare utilizing saline as the "water" component and incomplete Freud's adjuvant for the oil mixing component. Despite success, the animals receiving the injections are still prone to local site reactions. These reactions could be in response to either the antigen or the adjuvant being used for injections or the combination there of. Several different forms of adjuvant have been studied for traits such as rate of success based on immune response rates and the potential for physical reactions to develop (Bomford 1980). This research has helped scientists determine which adjuvant would fit their situation best (Bomford 1980). Overall, the Freud's Incomplete Adjuvant (FIA) was discovered to be the best adjuvant for research/ production use for Siemens Healthcare at the Hollister Facility and was highly recommended from research based articles (Bomford 1980). FIA was found to increase productivity of Immunoglobulin 1 and 2 production in the immune system, decrease site reaction rate and helped maintain a longer immune response compared to other adjuvants (Bomford 1980). The increase in immune response is what the company is focused on looking for when evaluating test bleeds to determine which animals will work on production best (Bomford 1980). Research based on company statistics show using Incomplete Adjuvant above the other adjuvant continues to produce a consistent immune response regardless of what antigen type it has been paired with while minimizing physical reactions. Some researched protocols have found success using complete adjuvant for the initial boost to heighten the initial immune response and then the incomplete form is utilized for the remainder of the injections in the study (Bomford 1980).

2.1.1 Complete adjuvant

Complete adjuvant has a quicker immune response but has been found to increase lesion development at injection sites (Lisa C. Halliday, et al. 2004). In support of using complete adjuvant as part of the protocol, evidence from histological data of New Zealand

rabbits whom were injected with complete adjuvant did not suffer from any long term internal or health related side effects when injected consistently with an immunogen and complete adjuvant mixture (Lisa C. Halliday, et al. 2004). Using complete adjuvant as an additive for immune response in polyclonal antibody production based on this data, is a positive trend, but one should be aware of the potential side effects even if they are limited or less than others (Lisa C. Halliday, et al. 2004). Overall, recognizing research findings, Siemens' immunization protocol only uses complete adjuvant in the first injection and/or in smaller loading doses that make up the same volume as one initial injection and then continues with incomplete thereafter. The remaining injections are administered with Freud's Incomplete adjuvant.

2.2 The Use of Antigen

As discussed in the *Vaccine* journal, utilizing a large protein for the antigen to bond to and react with the adjuvant is important to ensure the antigen is absorbed by lymph nodes and is responded to by the immune system (Aucoturier 2001). In addition to using a large protein, common methods to increase antigen susceptibility include varying methods of adjuvant, what type of species is receiving the injection, the ideal immune response timeline and longevity, and the location of injection on a specific animal (Aucoturier 2001).

2.3 Methods of Injection

Other studies have focused on varying sites of injection. The lumbar region is commonly used for sheep per protocol for Siemens Healthcare, but previous research has used foot pads in rodents, subcutaneous locations over the back, shoulder/neck, and armpit, and intradermal locations close to lymph nodes depending on the species and their specific anatomy (BEH and Lascelles 1985). Sheep immunization has been found to be most

efficient with a large molecule bound antigen that assists with carrying the antigen to a node in the lymphatic system. This increases the potential of the antigen to reach and react within the immune system to increase antibody production (BEH and Lascelles 1985). The antigen may be carried by a larger protein molecule, where it will be carried through the lymphatic system and expose itself to reactive cells such as B and T cells and macrophages (BEH and Lascelles 1985). The reactions that now occur may heighten the immune response, and without this assistance, the antigen may have a poorer success rate traveling from the point of inoculation to the immune system (BEH and Lascelles 1985). The lyophilized antigen used by the company has already been bound to a larger molecule prior to development of the injectable solution, and therefore may be more receptive to the animals and can be dependent on the type of antigen being used. Once bound to a larger molecule, the antigen is lyophilized and housed in a freezer until rehydrated with a 0.9% Sodium Chloride injectable solution and then mixed directly with incomplete adjuvant. This injectable solution is administered across the lumbar region easily susceptible to lymph nodes.

2.4 Variation of Species Used in Antibody Production

The major short comings of existing studies are due to primary dedication to rodents and other small laboratory species. Rodents have been found to have a quicker response rate but much smaller blood volume produced for testing and production. Sheep have become ideal production animals for Siemens Healthcare as they are easy to house together, have a relatively good response rate (6-9 months), and are a manageable size to handle and restrain. Site reactions and severity differ based on the study, antigen/adjuvant in use. The use of saline to adjuvant type solution with an antigen bound protein has been

determined by research to be successful in producing an immune response but increasing success and efficiency leaves some unknowns (Loewi, Holborow and Temple 1966). Despite extensive research, no studies have been found that focus on the ratio of adjuvant in comparison to various site reactions depending on volume and the effects of response rate and incomplete adjuvant ratio decreases. The following research trial helps bridge that gap.

CHAPTER III: MATERIALS AND METHODS

3.1 Materials

A total of 199 animals were used for test bleed collected and 128 of those were observed for site reactions. The population of animals tested were long yearling, cross bred ewes. The study was conducted at the Siemens Healthcare facility in Hollister, California. Materials used to manage animals on a daily basis were a specialized ewe maintenance ration, wheat straw, white salt blocks, and automatic waterers. Immunization protocols require the use of a chute-based operating system, 21-gauge needles, 3cc syringes, and a clipper set with attached 40 blade. The materials needed in preparation of immunogens include lyophilized Universal Barbiturate antigen, ICU Medical 0.9% Saline solution, Freud's BD Complete and Incomplete adjuvant, 60 mL snap cap containers, 60 mL and 30 mL clear plastic bottles, rubber stoppers, 14 gauge needles, syringe adaptors, 20 mL syringes, and the use of a sonicator and scale.

3.2 Trial Animal Preparation

All ewes arrived from supplier a based in central California to the Hollister facility where they were held in quarantine for 3 weeks prior to the start date of the trial. They were vaccinated against Caseous Lymphadenitis (Case-Bac Vaccine), and Enterotoxemia from clostridium perfingens C & D (CD/T Vaccine). They also received a pour on and oral drench for the treatment/prevention of liver flukes, additional internal parasites, biting/sucking lice, and face/horn flies. Once the trial sheep completed the quarantine period they were given two forms of identification: ear tags in both ears and a corresponding RFID button. They were then sorted and moved to their permanent housing

environment for the duration of the study. They were housed in groups of 20 in open air pens on expanded metal. They received a specialized ration once a day based on weight and water ad lib. Appropriate enrichment opportunities were provided. They began the trial in September 2019 and the ewes were injected with varying ratios of Universal Barbiturate antigen, ICU Medical 0.9% Injectable Saline solution, and BD Freud's adjuvant ranging from (0.5/1/1, 1/1/1, 1/1/0.5, 1/1/0). All animals for this research trial were dedicated to the Universal Barbiturate antigen program.

3.3 Study Design

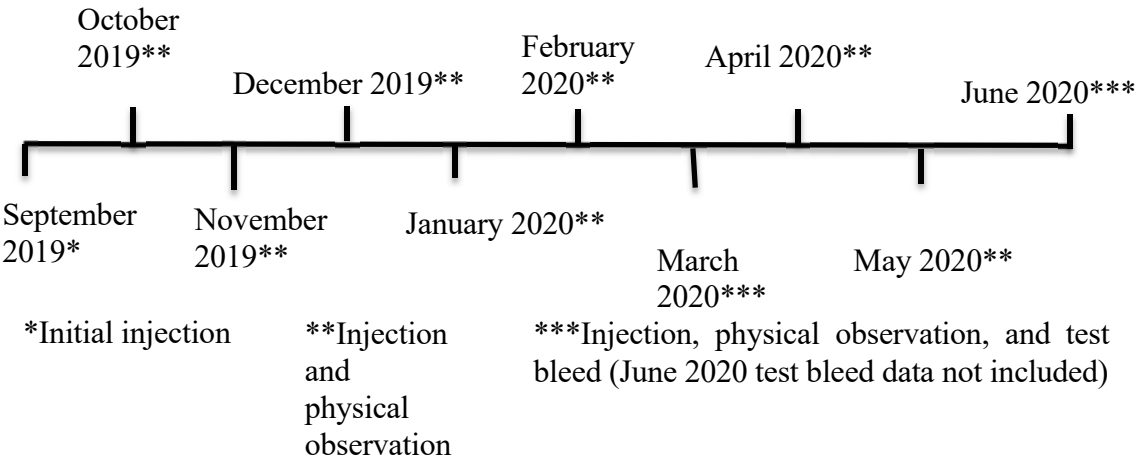
The research trial was broken into two parts, the physical site reaction data observations and the immune response based on separation test analysis. All the animals used were treated with an antigen that has a familiar chemical makeup and protein carrier molecule that is similar to many others used by the company. Ideally to see this study from all aspects, incorporating methods already used by the company were used to set a current baseline. Also, two new methods were used to estimate the response rate with 50% less adjuvant and 100% less adjuvant to justify the usage of adjuvant at all. This study was set up to utilize 199 cross bred ewes broken down into 4 protocols. These protocols are described as Current Protocol #1, Current Protocol #2, Test Protocol #1, and Test Protocol #2. For analysis purposes, the trial animals were broken down into varying ratios to test theories and are referred to as Current Protocol #1 (1/1/1), Current Protocol #2 (.5/1/1), Test Protocol #1 (1/1/0.5), and Test Protocol #2 (1/1/0). Each protocol has a varying number of animals assigned as these animals also are being utilized by the company for production purposes, and although the research data is important, the potential risk for decreasing antibody was still a possibility so more animals were dedicated to the most common ratio of injection (1/1/1) with some also assigned to the 0.5/1/1 ratio. If the trial

was run without priority to production, each protocol would have an even number of sheep dedicated to it. The immune response reviewed 100 head dedicated to Current Protocol #2 and 50 head assigned to Current Protocol #1. 39 head were treated with the Test Protocol #1, the same as in the site reaction observations and 10 sheep were dedicated to Test Protocol #2 as seem in Table 3.1. Due to the company having to maintain product development while investigating this research trial, animal head count dedicated to each protocol varied. The largest head count was dedicated to protocols that were either proven or had an increased potential to maintain an immune response. The site reaction trial was designed to have 40 animals dedicated to Current Protocol #1 and 39 animals dedicated to Current Protocol #2 each. Also, the Test Protocol #1 was assigned 39 sheep while Test Protocol #2 was assigned 10 sheep for site reaction observations also observed in Table 3.1. Data was collected for both research trials and utilized for analysis of the impact of adjuvant on immune response and physical reactions.

3.4 Method of Data Collection

To obtain the proper forms of data needed to analyze both the financial analysis and the herd health potential based on USDA guidelines, two different methods of data collection were utilized. For the duration of the trial, both blood samples and physical observations were made to develop a conclusive picture of the benefits and downsides to the potential of adopting the changes made in the protocol. The trial lasted for nine months from initial injection in September 2019 through the final site reaction data collection in Jun 2020 as seen in the timeline diagram below.

Figure 3.1 Study Timeline



3.4.1 Method of Data Collection for Immune Response

The method of data collection for titer analysis produces serum used to study the amount of antibodies produced and overall immune response. This method included drawing a 15 mL blood sample, or a "test bleed" with a serum separating tube. Prior to drawing blood, a surgical clip is applied to the jugular vein on either side of the neck to free the area of long wool and debris. A 21-gauge blood collection needle is used with a vacutainer holder and vacutainer blood collection tube. Once the test bleed is collected, the blood tubes are left to rest on their side for 30 minutes and then are put in the centrifuge for 30 minutes and the serum separated of the top was sent to the lab for evaluation. The serum was used to test for varying values of success rates. The separation value determined by the difference found by subtracting the "Neg" value from the "Cal 5" value. The "Neg" value is similar to plain urine and used as a reference point with no barbiturates present. The "Cal 5" has some barbiturates in it, therefore it helps to determine the quality of antibody collected from each animal. The total separation value was ideal in the 200 range. The values found for animals in the single digits or lower double digits were deemed inadequate

for production purposes and also did not show potential for future responses. Values greater than 500 were resulting from “noise” from the instrument used to read the sample. This means no antibody response from those samples and they also cannot be utilized for production. Analyzing the response rate to inoculation determines whether the animal has a superior/moderate/or minimal response rate. The animals with a superior response rate were put directly into production. The moderate animals were retested three months post the initial injection and animals with minimal response were culled from the herd.

3.4.2 Method of Data Collection for Physical Site Reactions

Animals observed for physical site reactions were observed one day post injections on a monthly basis for nine months, from September 2019 to June 2020. Physical observations were based on a numeric scale. Sheep were randomly selected for physical observations and utilized less sheep due to the time restrictions physical observations took in addition the daily productivity procedures required on a daily basis for the company. Once a month, one day post inoculation, the lumbar region of each sheep was evaluated for indication of reaction from the injections. When the animals are injected, they are inoculated over 6 sites subcutaneously across their lumbar, with three injections on either side of the spine. Animals were ranked on a graded scale from 1-4 from no reaction to several open, draining abscesses requiring medical attention. The scale used to evaluate reactions sites are described in Table 3.2.

3.5 Financial Analysis Methodology

Taking into consideration the titer test and physical observations, a financial analysis can be determined based off the findings and used to determine the best strategy for the company moving forward. Using the trial as a starting point when all dedicated animals are inoculated with the Universal Barbiturate antigen and adjuvant. The results will

justify which ratio of adjuvant will produce the best titer response, and with hopeful support from the site reaction observations, will also aid with cost savings. The cost-based analysis utilizes head count and the overall cost difference between methods to determine the total cost savings per year for the use of adjuvant as seen in Table 3.3. After the data has been collected and combined, the results will be used to determine the cost of adjuvant for the duration of the trial per protocol and for all the animals at the full extent of the trial. Additionally, the cost savings potential and NPV will show the impact the decrease of adjuvant use can have over the next 6 years. Overall, the production demand is climbing and therefore the animal head count increasing provides a huge opportunity to save materials cost associated with adjuvant. By comparing the costs of proven protocols with the researched ones within this trial will present the savings per year if the method is adopted into the entirety of the immunization approach for all animals subject for inoculation regardless of program they are dedicated to.

Table 3.1: Study Design Outline

	Ratio	# of Animals-Immune Response	# of Animals-Site Reaction
Current Protocol #1	1.0/1.0/1.0	50	40
Current Protocol #2	0.5/1.0/1.0	100	39
Test Protocol #1	1.0/1.0/0.5	39	39
Test Protocol #2	1.0/1.0/0.0	10	10

Table 3.2: Site Reaction Grade Scale for Data Collection

GRADE	DESCRIPTION
1	No reaction (no balding, raising up of skin, present abscesses, or other signs of irritation)
2	Presence of small/ isolated reaction abscesses or a larger single site reaction (not open, usually cause a balding of the area to distinguish where the abscess may occur)
3	Presence of a larger abscess or many small reaction sites, open and draining
4	Several open abscesses or a larger one that is not healing within a reasonable amount of time. Also, if wool follicle death starts occurring indicated by black wool growth. Site veterinarian will be made aware of animal's condition and plan to move forward will be made.

Table 3.3: Cost per Sheep per Protocol

Protocol	Ratio	Price of Adjuvant per Sheep	
Current Protocol #1	1.0/1.0/1.0	$	1.15
Current Protocol #2	0.5/1.0/1.0	$	1.15
Test Protocol #1	1.0/1.0/0.5	$	0.57
Test Protocol #2	1.0/1.0/0.0	$	-

CHAPTER IV: RESULTS

4.1 Physical Site Reaction Results

Of the 128 animals observed for physical reactions, 35% were found to have no reaction, 52% had mild reactions, 8.5% were reactive, and 4.6% were determined to be very reactive. No reaction inferred there was no lesion or abscess apparent over the lumbar region of the sheep for the entirety of the research trial. Mild reactions were based on 1-5 times an animal was ranked to have a grade 1 reaction. A grade 1 reaction consists of small or isolated abscesses or a single larger site reaction. These are not open or draining, may be a reaction in the healing process, or showing a balding area were the reaction is developing. The reactive sites were found to have 6-9 grade 1 reactions over the 9 month testing period. Lastly, animals found to be very reactive presented 1-3 grade 2 reaction sites. A grade 2 included several small or one larger abscess actively open and draining. These reaction sites generally took longer to heal but healed well and healthy skin and wool was present. The trial did include a stage 3, including several open and draining abscesses of various sizes were open, actively draining, and taking a lengthy about of time to heal. Generally, if healed, black wool follicle replaced what once were pink follicles and white wool, and the site veterinarian would be utilized to discuss the animal's further career as an antibody producer. For the duration of this trial, there were no grade 3 animals determined while collecting data.

4.1.1 Results of Site Reactions per Protocol

The sheep dedicated to Current Protocol #1 showed animals responding in several different ways. The majority of animals presented with minimal reactions, and a few did not have any signs of site reactions for the duration of the trial as shown in Figure 4.1. Additionally, animals also presented with reactive and very reactive lesions on their

lumbar region. Although, not nearly severe enough to affect the animal in a negative manner, taking note of this is imperative as this protocol is currently the most common approach to immunize. The Current Protocol #2, as shown in Figure 4.2 showed less animals with no reaction but had more animals who presented with minimal reactions. The Current Protocol #2 also had fewer reactive and very reactive site reactions than the primary protocol. The Test Protocol #1 had a significantly higher head count of sheep who did not have any site reactions for the duration of the trial in comparison to any of the other protocols associated with the research. Also, there were many animals with minimal reactions but had no reactive sites and only had one very reactive sheep. Figure 4.3 shows the drastic increase in decreasing site reactions as shown in the higher values of the no reaction and minimal reaction categories. Lastly, Figure 4.4, the baseline for this trial depicts all animals receiving the Test Protocol #2 all presented with no reactions. The lack of reactions is most likely associated with the lack of adjuvant present in the immunizations associated with the protocol. Figure 4.5 shows all of the protocols in comparison to one another. The protocols with the most ideal outcome were found between Control Protocol #2 and Test Protocol #1. On average as seen in Figure 4.6, the highest average value is represented by Current Protocol #2 while Test Protocol #2 has the lowest site reaction average count. Ideally, the average would be low as the least amount of site reaction is preferred. Test Protocol #2 has a huge decrease in site reaction compared to the other protocols due to a lack of adjuvant therefore not providing an agent to cause a site reaction.

4.2 Immune Response Results Analysis

After 199 titer tests were evaluated for the Universal Barbiturate antigen, there was a 5% success rate for animals injected with Current Protocol #2 and a 3% success rate with

Test Protocol #1. Making up the combined 3% success of the animals tested resulted in six animals going directly onto production as the superior animals. 102 of the remaining animals will continue to be boosted with the antigen and retested after 9 months post initial injection known as moderate responders. The 91 animals shown as minimal responders will not being utilized for production or retesting had a significantly low immune reaction and were culled from the herd. Of the six animals showing high response rates, three were observed throughout in the injection process for site reactions. Two of them were found to have minimal reaction and one had no physical reaction.

4.2.1 Results of Immune Response per Protocol

In Figure 4.7 the Current Protocol #1 resulted in the majority of animals falling into the moderate and minimal titer reaction categories. This resulted in a larger portion of this protocol being retested at 9 months post initial immunization and the population that showed minimal reaction were culled from the trial. Current Protocol #2 showed a different trend compared to the previous. As seen in Figure 4.8, Current Protocol #2 had some animals fall into the superior category and were put directly onto production. The majority of animals were seen in the moderate column where they will be re-tested and some were still culled from the minimal sector. Similar to this protocol, the Test Protocol #1 as shown in Figure 4.9, had a very similar outcome with an animal presented with superior data while a larger majority showed a moderate response rate. Lastly, Test Protocol #2 had no superior or moderate responses as seen in Figure 4.10. All of the animals dedicated to the Test Protocol #2 resulted in minimal immune response and were all culled from the program before it progresses to the next test phase. A side by side comparison of all four protocols can be seen in Figure 4.11 where it is clear the Current Protocol #2 dominated the other three with respect to the titer test. Also, the Test Protocol

#1 shows a positive trend, similar to the Current Protocol #2, and increased potential for further utilization of both of these methods in future programs. Lastly, the average immune response per protocol was calculated as seen in Figure 4.12. Figure 4.12 shows how Test Protocol #1 has the higher of the immune response values while Test Protocol #2 has the lowest. Similar to the average results found in Figure 4.6, for Site Reaction Average Analysis, Test Protocol #2 has the lowest immune response due to the lack of adjuvant included. Ideally, the higher the immune response value, the more product the animal can produce due to a high antibody productivity rate.

4.3 Statistical Analysis of Protocol Results

In addition to the general results found from the raw data collected for both the site reaction and immune response trials, further investigation was conducted into the statistical significance of the data found. Table 4.1 and Table 4.2 show the average and standard deviation of the raw data collected for immune response and site reactions. These tables show Test Protocol #1 with the highest average immune response rate and the second lowest site reaction average, only behind Test Protocol #2. Test Protocol #2 had an unusually low site reaction average due to the lack of adjuvant used also resulting in a very limited immune response. With respect to the standard deviation calculations, Current Protocol #2 had the largest standard deviation for immune response and Current Protocol #1 and Test Protocol #1 had the larger standard deviations compared to the other two protocols for site reactions. Based on the average calculations, prior to the t-statistic

analysis, the null hypothesis assumes that based on similar results, the values are either equal or very close.

4.3.1 t-Test Analysis per Protocol for Immune Response Data

To further investigate the statistical significance of the data found by the immune response data, t-test analysis was performed. As seen in Table 4.3, Current Protocol #1 and Current Protocol #2 are not found to be statistically significant and the null is not rejected. The findings for Current Protocol #1 compared to Test Protocol #1 and Current Protocol #2 compared to Test Protocol #1 also show similar findings shown in Table 4.4 and 4.5 where they are all not statistically significant as shown in the tables. Table 4.6 on the other hand shows a very significant and high t statistic where Test Protocol #2 is not similar to Current Protocol #2 and therefore we reject the null.

4.3.2 t-Test Analysis per Protocol for Site Reaction Data

After conducting t-test analysis on the site reaction data as well, very similar results were produced. The mean difference between Current Protocol #1 and Current Protocol #2 were also concluded to be statistically not significant due to the similarity of t values as shown in Table 4.7. Also, Current Protocol #1 and Test Protocol #1 have a test statistic less than that of the critical value displayed in Table 4.8. Both Tables 4.9 and 4.10 resulted in t statistic values that were greater than the critical t values and therefore statistically significant and resulted in the null being rejected. These t test analysis between Current Protocol #2 and Test Protocol #1 and Current Protocol #2 and Test

Protocol #2 resulted in t values of 2.5 and 11.67, both of which are much greater than 1.99, the critical value.

4.4 Financial Analysis Results

The financial results of the research trial demonstrated potential for the company to increase cost savings if Test Protocol #1 was adapted into the current protocols. As shown in Table 4.1, the current cost of adjuvant per sheep is $1.15 per injection. The price of adjuvant has remained constant over the past several years and is expected to remain consistent. When utilizing the Test Protocol #1 the adjuvant cost per injection decreases to $0.57. For the duration of the trial, the most expensive protocols were Current Protocol #1 and #2, but Protocol #1 shows a higher cost as there are more animals dedicated to that method. Furthermore, Table 4.2 shows the projected cost savings over the next 6 years of the company's production potential while utilizing Test Protocol #1. Cost savings per animal is $0.58 and peaks during years 2022-2023 at $8,526. All six years show a positive savings trend ranging from $5,394 to $8,526 per year. Overall, the Net Present Value (NPV) determined for this projection utilizing a 2% inflation rate was found to be $47,554.41. This positive difference in cash flows means that this financial decision would have a positive impact on fiscal budgets moving forward.

Figure 4.1: Current Protocol #1 Site Reaction Results

Figure 4.2: Current Protocol #2 Site Reaction Results

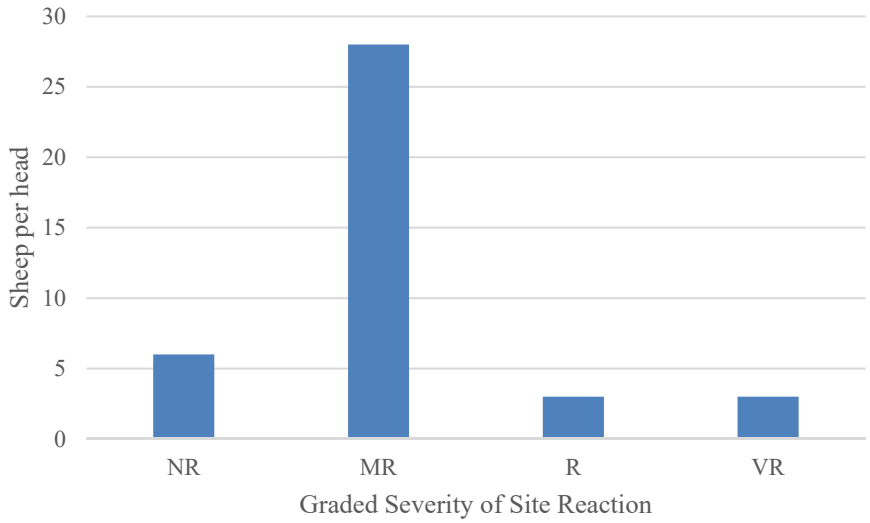


Figure 4.3: Test Protocol #1 Site Reaction Results

Figure 4.4: Test Protocol #2 Site Reaction Results

Figure 4.5: Comparison of Site Reaction Results per Protocol

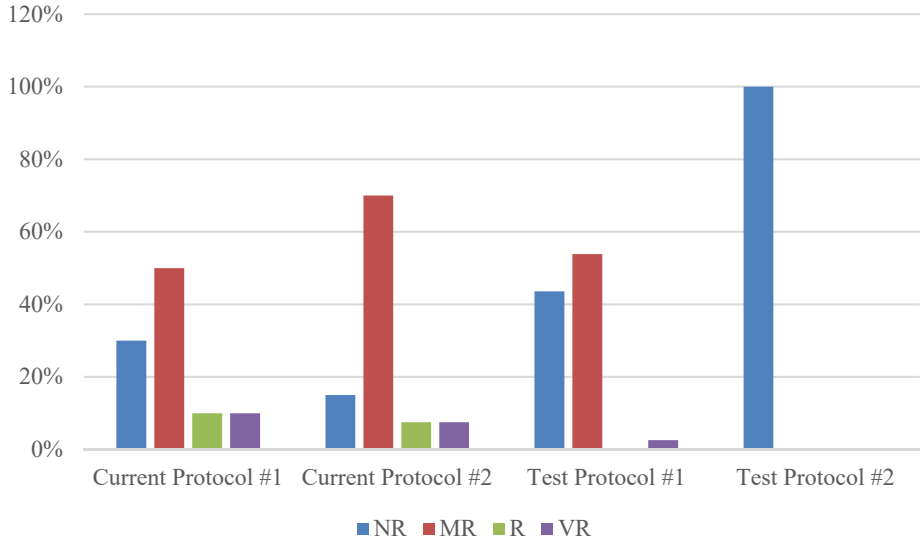


Figure 4.6: Average Site Reaction Results per Protocol

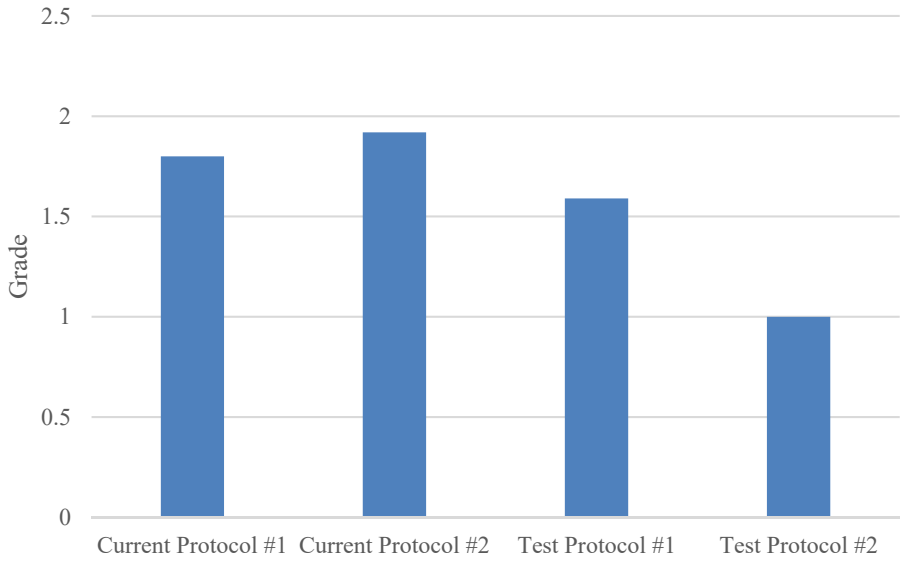


Figure 4.7: Current Protocol #1 Immune Response Results

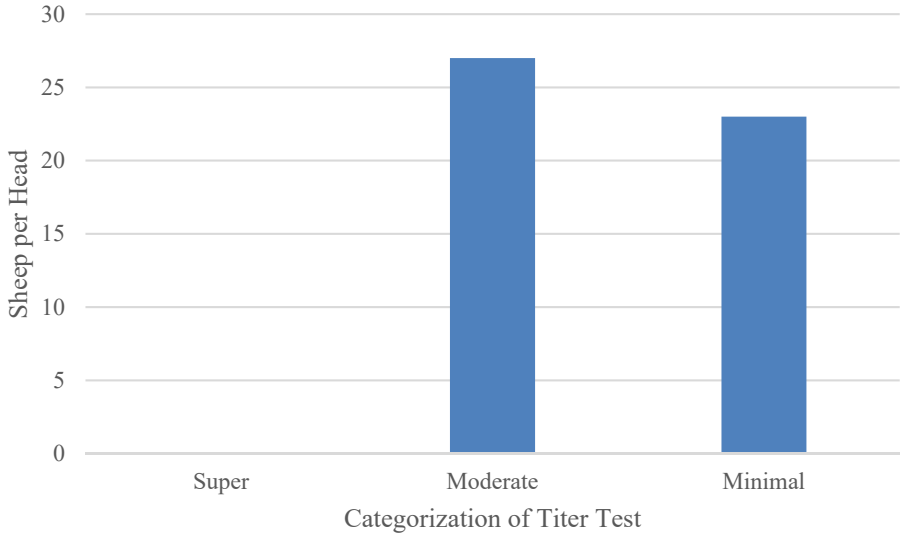


Figure 4.8: Current Protocol #2 Immune Response Results

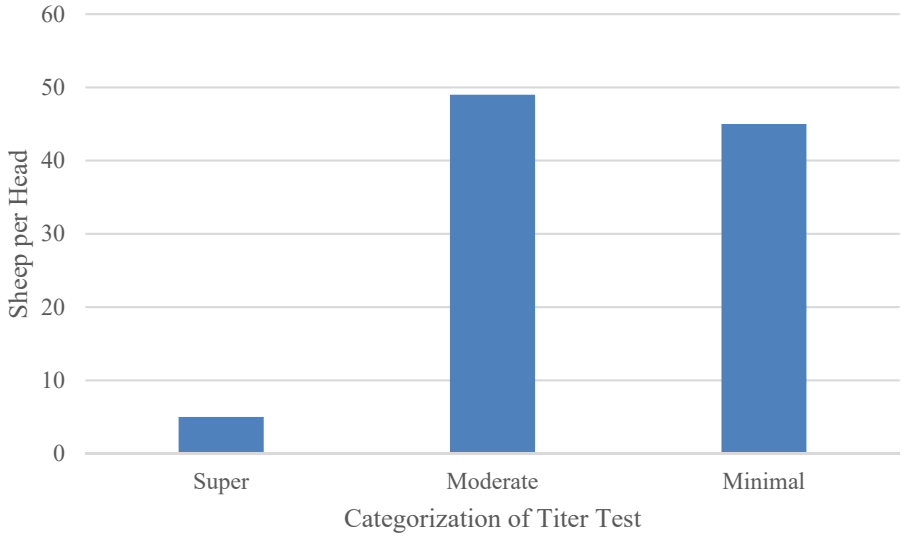


Figure 4.9: Test Protocol #1 Immune Response Results

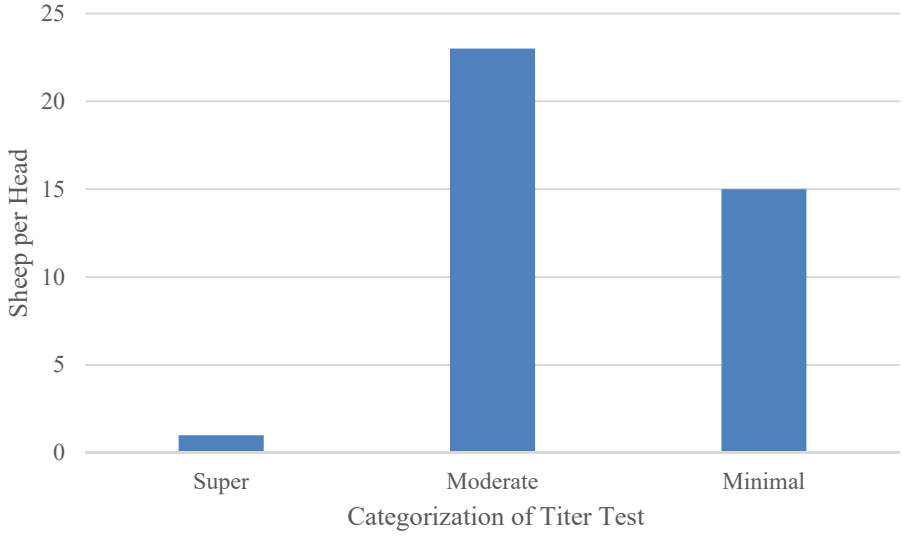


Figure 4.10: Test Protocol #2 Immune Response Results

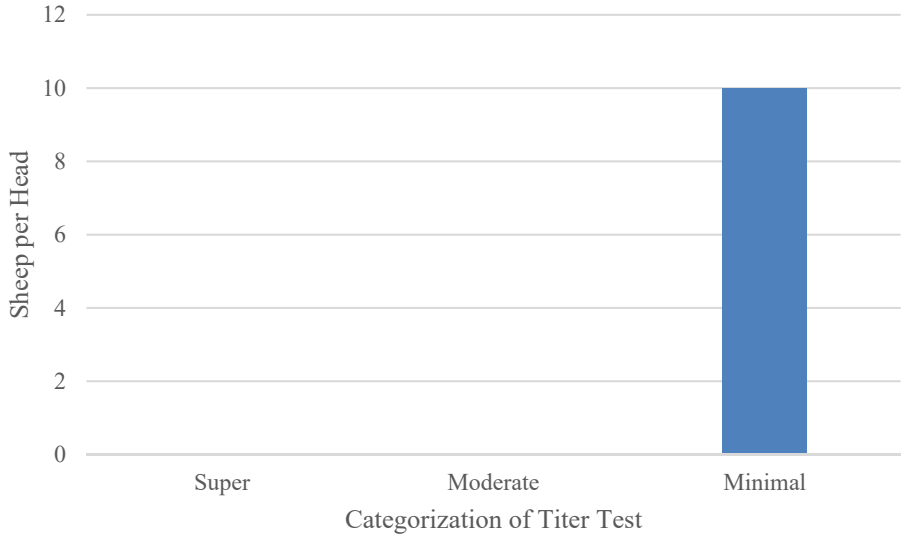


Figure 4.11: Comparison of Immune Response Results

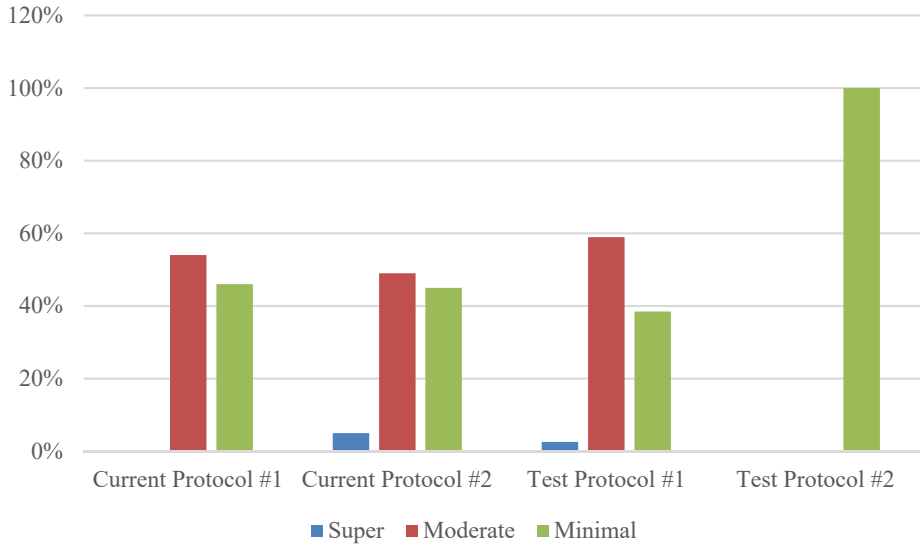


Figure 4.12: Immune Response Average Measured by Separation Values

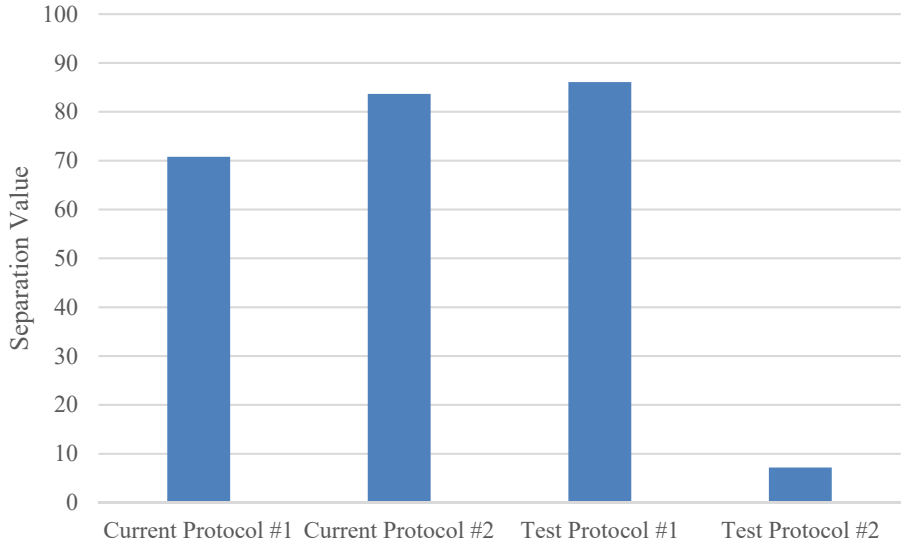


Table 4.1: Average Calculation for Trial Statistics

	Ratio	# of Animals-Immune Response	Average	# of Animals-Site Reaction	Average
Current Protocol #1	1.0/1.0/1.0	50	70.8	40	1.95
Current Protocol #2	0.5/1.0/1.0	100	83.67	39	1.92
Test Protocol #1	1.0/1.0/0.5	39	86.1	39	1.59
Test Protocol #2	1.0/1.0/0.0	10	7.2	10	1

Table 4.2: Standard Deviation Calculations for Trial Statistics

	Ratio	Standard Deviation-Immune Response	Standard Deviation-Site Reaction
Current Protocol #1	1.0/1.0/1.0	50	40
Current Protocol #2	0.5/1.0/1.0	100	39
Test Protocol #1	1.0/1.0/0.5	39	39
Test Protocol #2	1.0/1.0/0.0	10	10

Table 4.3: Current Protocol #1 v Current Protocol #2 – Immune Response t-Test Analysis

	CP#1	*CP#2*
Mean	71.73469388	84.15151515
Variance	1860.157313	3625.374768
Observations	49	99
Hypothesized Mean Difference	0	
df	127	
t Stat	-1.437779952	
P(T<=t) one-tail	0.076477984	
t Critical one-tail	1.656940344	
P(T<=t) two-tail	0.152955968	
t Critical two-tail	1.978819535	

Table 4.4: Current Protocol #1 v Test Protocol #1 – Immune Response t-Test Analysis

	CP#1	*TP#1*
Mean	71.73469388	85.57894737
Variance	1860.157313	2488.466572
Observations	49	38
Hypothesized Mean Difference	0	
df	73	
t Stat	-1.361155508	
P(T<=t) one-tail	0.088825789	
t Critical one-tail	1.665996224	
P(T<=t) two-tail	0.177651578	
t Critical two-tail	1.992997126	

Table 4.5: Current Protocol #2 v Test Protocol #1 –Immune Response t-Test Analysis

	CP#2	*TP#1*
Mean	84.15151515	85.57894737
Variance	3625.374768	2488.466572
Observations	99	38
Hypothesized Mean Difference	0	
df	80	
t Stat	-0.14126353	
P(T<=t) one-tail	0.444008437	
t Critical one-tail	1.664124579	
P(T<=t) two-tail	0.888016874	
t Critical two-tail	1.990063421	

Table 4.6: Current Protocol #2 v Test Protocol #2 –Immune Response t-Test Analysis

	CP#2	*TP#2*
Mean	84.15151515	7.222222222
Variance	3625.374768	0.944444444
Observations	99	9
Hypothesized Mean Difference	0	
df	99	
t Stat	12.69438063	
P(T<=t) one-tail	8.58966E-23	
t Critical one-tail	1.660391156	
P(T<=t) two-tail	1.71793E-22	
t Critical two-tail	1.984216952	

Table 4.7: Current Protocol #1 v Current Protocol #2 – Site Reaction t-Test Analysis

	CP#1	*CP#2*
Mean	1.794871795	1.921052632
Variance	0.325236167	0.236842105
Observations	39	38
Hypothesized Mean I	0	
df	74	
t Stat	-1.04528012	
P(T<=t) one-tail	0.149648013	
t Critical one-tail	1.665706893	
P(T<=t) two-tail	0.299296026	
t Critical two-tail	1.992543495	

Table 4.8: Current Protocol #1 v Test Protocol #1 – Site Reaction t-Test Analysis

	CP#1	*TP#1*
Mean	1.794871795	1.605263158
Variance	0.325236167	0.353485064
Observations	39	38
Hypothesized Mean I	0	
df	75	
t Stat	1.427541667	
P(T<=t) one-tail	0.078787199	
t Critical one-tail	1.665425373	
P(T<=t) two-tail	0.157574398	
t Critical two-tail	1.992102154	

Table 4.9: Current Protocol #2 v Test Protocol #1 – Site Reaction t-Test Analysis

	CP#2	*TP#1*
Mean	1.921052632	1.605263158
Variance	0.236842105	0.353485064
Observations	38	38
Hypothesized Mean I	0	
df	71	
t Stat	2.533629237	
P(T<=t) one-tail	0.006748933	
t Critical one-tail	1.666599658	
P(T<=t) two-tail	0.013497866	
t Critical two-tail	1.993943368	

Table 4.10: Current Protocol #2 v Test Protocol #2 – Site Reaction t-Test Analysis

	CP#2	*TP#2*
Mean	1.921052632	1
Variance	0.236842105	0
Observations	38	9
Hypothesized Mean I	0	
df	37	
t Stat	11.66666667	
P(T<=t) one-tail	2.92302E-14	
t Critical one-tail	1.68709362	
P(T<=t) two-tail	5.84605E-14	
t Critical two-tail	2.026192463	

Table 4.11: Summary of Financial Analysis for Trial (9 months)

	Sheep (per head)	Cost of Adjuvant per Sheep	Total for duration of trial
Current Protocol #1	99	$ 1.15	$ 113.85
Current Protocol #2	50	$ 1.15	$ 57.50
Test Protocol #1	39	$ 0.57	$ 22.23
Test Protocol #2	10	$ -	$ -

Table 4.12: Summary of Financial Analysis and NPV

Years	2020	2021	2022	2023	2024	2025	2026	Total
Number of Projected Animals	775	1050	1225	1225	1025	1200	900	7400
Cost of Current Protocol #1	$ 1.15	$ 1.15	$ 1.15	$ 1.15	$ 1.15	$ 1.15	$ 1.15	$ 1.15
Cost of Test Protocol #1	$ 0.57	$ 0.57	$ 0.57	$ 0.57	$ 0.57	$ 0.57	$ 0.57	$ 0.57
Cost Difference	$ 0.58	$ 0.58	$ 0.58	$ 0.58	$ 0.58	$ 0.58	$ 0.58	$ 0.58
Total Cost Savings Per Year	$ 5,394.00	$ 7,308.00	$ 8,526.00	$8,526.00	$7,134.00	$8,352.00	$6,264.00	$ 51,504.00
NPV	$47,554.41							

CHAPTER V: DISCUSSION

The research trial conducted had many components to determine not only the financial potential but the animal health impact as well. The healthcare industry is constantly being driven by increasing demand for test kits and analysis tools for not only drugs of abuse but therapeutic drugs, proteins, and hormones. Making financial decisions in this industry not only incorporates justifying the positive impacts on projected budgets but has to also ensure animal well-being and limitation of impact on the quality of product outcome. Taking all of these factors into consideration, this is why the research trial incorporated two phases of research data collection.

The site reaction study justified the changes in protocol used to immunize and ensure that there were no further negative health connotations beyond what were already associated risks with injecting a foreign material subcutaneously in a small ruminant. The titer test provided insight into the immune response rate for each animal based on the protocol they were assigned to. The immune response rate determined whether an animal held potential to work as a production animal for product development. Taking into consideration the results of these two tests, a financial analysis proved that adjusting the adjuvant levels in immunizations, specifically in Universal Barbiturate injections, the reaction response was stilled maintained at a sufficient level and the cost potential was cut in half. Looking further into the next 6 years of company projected demand, and calculating the potential NPV, the company would greatly benefit from this change if adopted into all protocols for all various antigens.

Based on the site reaction results, both the least common regular protocol “Current Protocol #2” and the newly proposed “Test Protocol #1” proved to have a positive response rate that could maintain the current production expectations.

Given the results from the titer test, the 3% success rate is a positive outcome. After the secondary test bleeds occurring after 9 months this percentage will grow to 8-9% projected. The overall goal of 5-6% will be used to maintain inventory development over the length of time the animals are on production. The results also coincide with the physical site reaction findings where the majority of animals with no reaction or minimal reactions also had moderate to super response rates. Just as seen previously both the “Current Protocol #2” and “Test Protocol #1” resulted in the most non-reactive and minimally reactive animals but whom still showed an immune response. When observing the physical results of the “Test Protocol #2,” there are no site reactions what so ever which is very appealing but this method also produced the most non-responsive animals according to the titer test with values so high there was no potential for antibody production what so ever. This being recognized means that in order for a sheep to have a triggered immune response to the antigen for antibody production, there needs to be some adjuvant utilized. Furthermore, based on the results stemmed from the trial the only method to produce animals within the first 6 months which is generally the bare minimum amount of time allowed before test bleeds are initiated for the first time, the “Current Protocol #2’ and “Test Protocol #1” were the only methods to have animals ready for production. Based on the titer test results the “Current Protocol #1” does have some immune response but the values were generally low enough that there was some immune activity but that they would either never increase enough to be an adequate candidate for production or who showed a

peak to the initial hyper immunization and then plateaued off and eventually will have a decreased titer value. As the “Current Protocol #2” and “Test Protocol #1” both produced relatively similar outcomes, with the current protocol showing slight dominance in titer test results versus the test protocol producing fewer reactive and very reactive site reactions. With this similarity, it seems an easy decision to lean towards further execution of the test protocol as the decreased adjuvant use portrays a massive financial influence. The current protocol does show a decrease in antigen compared to the most common “Current Protocol #1,” but the cost per antigen type greatly varies. Utilizing the decrease in adjuvant would impact all programs across the board as it is a constant factor not a variable. The increased efficiency of animal response occurring at 6 months, decreases the lead time for production to begin, therefore the animal is producing antibody available for product in a quicker manner. This decrease of lead time, and inventory support opens up even more cost savings potential as more programs could be started per year, therefore production can continue to grow, and the company will have more available product ready for customers.

CHAPTER VI: CONCLUSION

6.1 Overview of Key Results

Overall, the importance of this research trial was to establish the potential for a new baseline immunization protocol that would not only provide a fiscal benefit to the laboratory diagnostics sector of Siemens Healthcare but also have a key focus on the animal health and management. With so many variables taking place in this animal-based production system, making changes are not just paper and protocol changes. They must be justified on all fronts. After the 9 month trial of collecting monthly site reaction data and further analysis of titer tests taken at 6 months post initial immunization, it was proven that the traditional current protocol could be improved upon. Both a lesser used protocol and a new approach garnered proactive approaches to not only allow for production potential to be improved upon while taking into consideration the animals health and wellness but also solidifies a huge cost savings potential. The newly adapted protocol allowed for the greatest cost savings potential which was strengthened by the drastic projected growth the company is facing with a yearly $5,000-$8,500 savings that maintains well over the next 6 years with potential to continue to climb.

Major contributions to this change were the company's ability to remain open minded about improving upon a procedure that has been executed for several years, even as the company has changed hands and grown in different directions. Additionally, research was narrowed down to a common variable that influences all drug, hormone, and protein programs. The readiness and flexibility of current production based animals were used for this trial and are active in producing product headed directly to our customers who can already begin reaping the benefits due to the positive outcomes of this study.

6.2 Limitations of Research Trial

As the study progressed some limitations arose. Tracking site reactions are tricky as they are based on a biased opinion of one observer. Sometimes it can be difficult to define the difference between the beginnings of a site reaction and one that is almost healed or has left a remaining scar or cyst. Both present as a small hard lump. Only time is an indicator of whether they will progress or not. Also, physical observations are very laborious and adjusting for time and assistance are crucial to ensuring all data is collected in a close proximity time frame. Additionally, more test bleeds would have provided more insight into titer responses and potential production opportunities but due to the lab already balancing a regular heavily impacted work load, the base line standard 6 month titer tests were all that could be obtained for this study.

6.3 Future Research

After the completion of the research trial a few factors came to mind if the company were to repeat, test bleed sooner than 6 months, and hopefully tighten down the window of initial immune response to decrease lead times for production animals. Also, with regards to the sight reaction study, the sheep are often sheared twice a year. At the beginning of this study they were sheared just prior to the initial immunization and then again after 6 months. The wool was allowed to grow back at its normal rate and immunizations took place according to protocol. Further research may keep some of those areas clipped for the entirety of the injection process to decrease any other foreign bodies introduced to the subcutaneous region of the lumbar area. This may also play a role in decreasing sight reactions and would be in addition to decreasing adjuvant use. Another idea that would help the selection of animals for production purposes would be finding a genetic marker in successful production animals to further investigate if a certain marker is association with

antibody production for the various antigen/adjuvant combinations. This would increase accuracy immensely and cut down on several costs from labor, feed, maintenance, laboratory materials, and animal cost.

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