

**DETERMINING IMMUNOGLOBULIN TRANSFER AND THE
RELATIONSHIP TO CALF HEALTH AT CAL POLY DAIRY
THROUGH THE ASSESSMENT OF SERUM PROTEIN
CONCENTRATION**

A Senior Project

Presented to the Faculty of the Dairy Science Department and the

Animal Science Department

California Polytechnic State University, San Luis Obispo

In partial fulfillment

of the Requirements for the Degree

Bachelor of Science

By,

Kevin M. McEvelly

March 2012

ABSTRACT

The objective of this study was to determine the success of current calf management protocols at the Cal Poly dairy by measuring Immunoglobulin (Ig) uptake at 48-hours post-partum, and by following mortality rate through weaning. By measuring serum protein concentrate (SPC) and using it as a direct indicator of Ig uptake we were able to grade the rate of passive immunity. Serum protein concentrate levels were obtained using a refractometer, and were measured in degrees Brix ($^{\circ}\text{Bx}$). Blood samples were acquired via jugular venipuncture at the 48-hour mark from a mixed breed, mixed gender sample comprised of both Holstein and Jersey breeds of Cal Poly calves ($n=73$). Samples were collected over three winters (Jan.-Mar.). With 7 total calves lost out of the original sample, the true mortality rate is 9.6%. Due to incomplete or missing data on 16 of the calves within the sampling range, our sample size was corrected to $n=57$, and the mortality rate amended to 12.3%. A scoring system was adapted to assign grade values to $^{\circ}\text{Bx}$ recorded for each sample. Ranges of $^{\circ}\text{Bx}$ to grade and description are: 7.0-5.5 $^{\circ}\text{Bx}$ =A “Ideal”, 5.4-5.3 $^{\circ}\text{Bx}$ =B “Borderline”, 5.2-5.0 $^{\circ}\text{Bx}$ =C “Poor”, 4.9-4.5 $^{\circ}\text{Bx}$ =D “Inadequate”, <4.5 $^{\circ}\text{Bx}$ = F “No Colostrum”, and >7.0 $^{\circ}\text{Bx}$ = “Likely Dehydrated”. Results of the data collection showed 70.1% of calves fell within ideal range, 1.75% of calves were borderline, 1.75% of calves displayed poor uptake, 3.5% of calves received scores of inadequate uptake, and 1.75% showed scores of no uptake. The remaining 21.5% were likely dehydrated, alluding to the idea that calves did not initially receive adequate amounts of Ig. As a cost of inadequate transfer of immunity, calves are more susceptible pathogens known to cause diarrhea. Diarrhea leads less water in the blood translating into a higher percentage of protein concentrated in the serum. In an effort to discover

statistically significant correlations between our results and pre-weaning mortality rate, statistics were run using the PROC PROBIT feature of the SAS program. No statistically significant conclusions could be reached when correlating SPC scores against breed, gender, or date of birth. When using the same model to compare actual SPC values against pre-weaning mortality rate, our data approached statistical significance ($P>0.058$). Nevertheless, these observations are congruent with the precept that adequate amounts of Ig lead to a decrease pre-wean mortality.

Table of Contents

ABSTRACT	ii
INTRODUCTION	1
BACKGROUND	2
Clonal Selection and Immunoglobulin	2
Passive Immunity	6
Diseases Associated with Neonates Lacking Ig	8
MATERIALS AND METHODS	13
Cal Poly Dairy	13
Parturition and Colostrum Administration	14
Sampling	15
RESULTS AND DISCUSSION	16
CONCLUSION	19
ACKNOWLEDGEMENTS	20
REFERENCES	21

List of Tables

Table 1: Total Serum Protein by Refractometer (mg/dL)	16
Table 2: Results of Initial SAS Analysis	17
Table 3: Results of Final SAS Analysis.....	18

List of Figures

Figure 1: Clonal Selection (de Castro and Von Zuben, 2000)	4
Figure 2: Anamnestic Response (Isomura et al, 1985)	5
Figure 3: Percentile Breakdown Based on SPC Values	17
Figure 4: Calf Survival Curve	18

INTRODUCTION

As dairies worldwide continue to make progress towards maximum production, optimal health, and environmental stability, it is important not to overlook calf management as a means to critically affect each of these factors. Through proper management of calf health, greater control can be exerted over mortality rate and spread of disease can be minimized. With greater interest and value now impressed on calf management protocols, I became interested in the existing protocols at Cal Poly Dairy. Through sampling and analysis of total serum protein, my objective was to determine current baselines of calf health at Cal Poly Dairy with intent to explore areas for potential improvements. My hope is that the data and presentation of information in the following pages may be used as a stepping-stone in an ultimate effort to provide superior health to calves at our facility and others like it. Additionally, my goal is to provide an accurate and scientifically founded assessment on calf health at Cal Poly dairy with intent being to provide a usable breakdown of data allowing improvements to be made. With all the work to be done on a dairy, the easier this can be made, the more likely it is to be utilized to its full potential benefit.

Blood samples were collected from calves to assess their serum protein concentration and ultimately to determine uptake of Ig. This paper begins first with a review on theory and principles of clonal selection. With the hopes of properly elucidating basic immunology with respect to our current context, the discussion will continue with the significance of Ig. Concepts of both clonal selection and Ig will then be applied into the concept of passive transfer of immunity. The section following will examine several common diseases affecting neonatal calves as well as treatment plans

and methods of prevention. Following necessary coverage of contextual material, the study performed will be covered in greater detail, followed by results and a discussion. The final section will be marked by acknowledgements followed by concluding remarks.

BACKGROUND

Clonal Selection and Immunoglobulin

In order to best understand passive immunity transfer, it is essential to obtain a brief but profound understanding of the mechanisms involved in immunology.

Immunology is the far-reaching spectrum of biomedicine that deals with the physiological functions of the immune system under conditions of health and disorder; the recognition of self versus non-self (Janeway et al., 2001). An Athenian named Thucydides first theorized early concepts regarding immunity in 430 B.C. In the fallout of a great plague, he wrote:

“The same man was never attacked twice- never at least fatally. And such persons not only received the congratulations of others, but themselves also, in the elation of the moment, half entertained the vain hope that they were for the future safe from any disease whatsoever.” –Thucydides (431 B.C.E.)

The connection had been made that the body was resilient to the plague after surviving, and the earliest concept of immunology began to flourish. Later advancements led to the greater understanding that this resiliency is due to the body’s recognition and adaptation to a previously encountered antagonist. Edward Jenner, often called “the father of immunology”, demonstrated this great progress by creating a lifesaving smallpox vaccine in 1796 (Hopkins, 1983). In that century, this disease was known to have infected

approximately 60% of the British population, with a 20% kill-rate within the infected population (Voltaire, 1733). After an initial inoculation, Jenner proved through repeated trials that his patients no longer suffered any signs of infection even upon subsequent introductions to the widespread disease (Hopkins, 1983). Through progress over the last 200 years we now know that our survival depends on the body's ability to successfully carry out these concepts in immunology, and to defend itself against non-self antagonists.

Antigens are the natural antagonists to the immune system. Any molecule that activates a defensive immunological response within an animal is known as an antigen (Freeman, 2008). Once the immune system recognizes the presence of an antigen, a response is launched starting with the activation of B-lymphocytes, (a subpopulation of cells derived from the bone marrow of the animal), and T-lymphocytes, (a subpopulation of cells derived from the thymus) (Freeman, 2008). The role of B-lymphocytes is fundamental in the context of immunity because they are responsible for the detection and response to antigens within the system. T-cells also provide a life-sustaining role in the immune system however their role does not include the production of antibodies, thus their mechanisms and position within the immune system will not be detailed in this review (Janeway et al. 2001). Upon recognition of an internal threat, B-lymphocytes locate and respond to antigens through the production of antibodies on the surface of their cells. Antibodies are binding proteins specific to the antigen antagonizing the system. Once the antibody is present on the surface of the B-cells, it can then bind to receptor proteins on the surface of the specific antigen and signal dormant lymphocytes to become activated (de Castro and Von Zuben; de Castro and Von Zuben). B-cells divide to yield clones which are then either used to respond to residual antigens, or they are kept in

dormant state of torpor as a memory cell poised to rapidly and effectively identify similar infection upon reintroduction (Forsdyke, 1995). This response of cloning memory cells is called clonal selection, and can be visually represented in figure 1 below. As B-cells manufacture binding proteins specific to the antigen they then are able to proliferate and differentiate into either memory cells as we've previously discussed, or into plasma cells for immediate use in an immunological response.

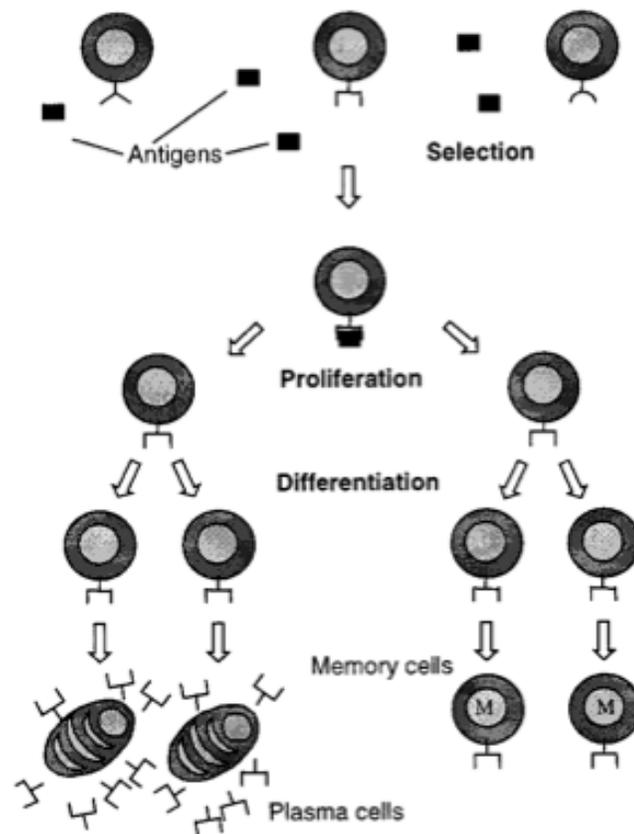


Figure 1: Clonal Selection (de Castro and Von Zuben, 2000)

With each subsequent encounter with the same antigen, cloned B-cells only become more effective at identifying and responding as part of the anamnestic response (de Castro and Von Zuben, 2000; Isomura, 1985). The anamnestic response and the concept of clonal

selection can be well explained by figure 2 below. The figure displays data illustrating the Ig levels in a primary infection group of infants introduced to rotavirus relative to that same group following re-infection four weeks later (Isomura, 1985).

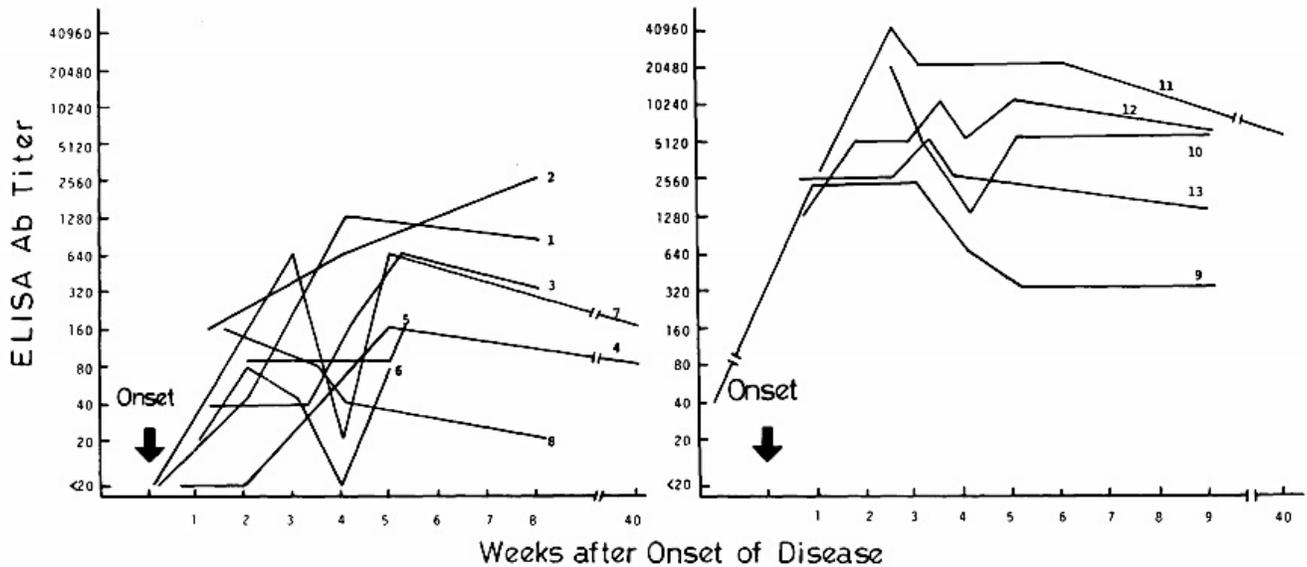


Figure 2: Anamnestic Response (Isomura, 1985)

The Enzyme-linked Immunosorbent Assay (ELISA) titer is a method used in this case to measure the presence of antibody in response to the same strain of rotavirus particles. As shown, in weeks following initial introduction to the rotavirus, infants reintroduced weeks after onset display a much higher production of antibodies, and a more rapid response to the virus due to the launch of the anamnestic response (Isomura, 1985).

A greater understanding and definition of both antibodies and Ig was achieved in the aftermath of a study conducted in 1956. A study using a radioactively labeled antigen allowed scientists to isolate and purify antibodies on the surface of B-cells (Co et al, 1985) Analysis of the antibodies showed that they had identical structure to free-floating antibodies in the blood previously produced by B-cells upon first contact (Co et al, 1985).

This led to the discovery of Ig and their use as defense against similar antigen (H. Eisen, and Sirisinha 1971). To summarize, Antibodies or Ig, over the course of a lifetime, continually provide support and protection against immunological threats; they are the central cog in the acquired immune response and play an essential role in the body's natural defenses.

Passive Immunity

Due to the unique nature and orientation of ruminant placental physiology, a brief overview of the form and function must be reached as a precursor for the understanding of passive immunity and its essential roles. The ruminant placental attachment is classified as cotyledonary epithelialchorial based on both the shape and points of interface between maternal and fetal components-- epithelial referring to the dam's endometrial epithelium, and chorial referring to the fetal chorion (Senger, 2005). Cotyledons formed from the trophoblast layer of the developing embryo and join maternal contributions from lumen of the uterus known as caruncles. The cotyledon-caruncle complexes, termed placentomes, are the sites of transient metabolic interchange between the dam and the conceptus for the term of the pregnancy. Forming at week five into parturition, placentomes are selective barriers preventing the crossover of potential antagonists (Senger, 2005). While these sites allow for the diffusion of nutrient building blocks, (glucose, amino acids, vitamins, and minerals), and the exchange of gases, maternal cells do not exchange, and neither do large proteins. While this protective barrier prevents transfer of disease, it comes at the cost of preventing Ig (which are large proteins) from being actively transferred from the maternal blood into the blood of the fetus.

Because maternal Ig does not traverse into fetal blood supply pre-partum, it is absorbed from the blood stream into the mammary gland of the dam in the several weeks leading up to parturition, reaching a peak 1-3 days pre-partum (Devery et al, 1979; Beam et al., 2009). This antibody-enriched lacteal secretion is known as colostrum. Colostrum is a nutrient and antibody rich milk meant for the calf as a means of survival in early life. Neonatal ruminants are born without a developed immune system and thus depend on the Ig found in colostrum to survive long enough to produce their own antibodies. If left without colostrum, without the ability to defend against foreign antigen the new-born will invariably be lost (Beam et al., 2009). Ingram and Smith (1965) were able to see that 30 days after parturition, calves begin to actively create their own antibodies. Without any colostrum, a calf will begin to produce its own immunities as early as day five following parturition (Hopkins and Quigley 1997), however I firmly believe that even on the cleanest farm the calf would have minimal chance of survival.

Calves must be given an adequate dose of colostrum with an adequate concentration of Ig if they are to survive long enough to actively produce their own immunities. The first 24 hours post-partum the calf is most receptive to the absorption of the large Ig proteins. The lumen of the small intestine is lined with pores called enterocytes which remain open in the first 24 hours (Bey et al, 2007). During this “open” period, Ig are pinched off into the blood stream using pinocytosis (Bey et al, 2007). Following the first 24 hours, the enterocytes steadily begin to seal themselves off to reduce vulnerability and the likelihood of harmful exposure (Bourc'his and Zamudio 2010). Between hours 36 and 48, the calf will cease to show absorption of Ig, and more drastic measure must be taken to ensure the calf will survive (Bey et al, 2007; Fowler et

al, 2009). For the purpose of this paper the focus will remain on practices to optimize the collection and administration of quality colostrum to the calf within a timeframe to allow for optimal absorption of Ig.

Diseases Associated with Neonates Lacking Ig

The ability to recognize and accurately treat specific diseases in calves is an asset that never fails to be overlooked. Calf neonates are most susceptible to disease if they are not given enough initial immunity or Ig. Regardless of the success or failure of the passing of immunities, calves are still vulnerable to disease from birth throughout their remaining lives. For this reason I am allocating a portion of my introduction to briefly cover the causes, symptoms, and treatments of several basic diseases. The conclusion of this section will include the best-known ways to prevent such diseases from proliferating. Through greater understanding these summaries may be used as tools to optimize calf health, and minimize avertable loss.

Of the many diseases that pose threats to calves worldwide, I am choosing to refine the focus to four of which I believe to be the most prevalent: Calf Enteritis (Scours), Calf paratyphoid (CP), Coccidiosis, and Pneumonia.

Calf Enteritis (CE) is caused by a diverse range of bacteria and viruses. While exposure to these diverse pathogens may be prevalent, infection can be minimized through the sufficient transfer of Ig (Beam et al, 2009). When the immune system is compromised, or there is no immunity to combat infection, the natural enteropathic bacteria within the primitive gut of the newborn then become a source of infection (Bey et al, 2007). Typically seen as culprits of this disease are *Escherichia coli*, rotavirus, coronavirus, and *Cryptosporidium parvum* (Merck, 2010). While any one of these

pathogens is known agents in the cause of CE, most cases are multifactorial. More than one of these pathogens, (and a range of others not listed), could be collaborate agents in the cause of this disease. The result is diarrhea, dehydration, and varying intensities of weakness. Calf Paratyphoid is very similar to CE, however the causes are limited to strains of *Salmonella typhimerium* in most pre-weaned calves.

At the subclinical level the aftermath of these diseases can be relatively mild. The clinical level is where long-term negative implications can be witnessed. There are degrees and factors making each case unique, however calves experiencing clinical enteritis notably exhibit a slower rate of gain, and an overall decrease in productive life relative to their healthier counterparts. This data is supported in research conducted by van der Fels-Klerx and others who were able to note a 2% drop in first lactation milk production, as well as an average month delay in calving age, and an overall decrease in bodyweight when CE calves were compared against healthy herd-mates. The high-energy needs of a developing calf must be met to maximize health later in life. Compromising the nutrient uptake not only hinders productive life but, in more severe cases, leads to greater susceptibility to re-infection, and a predisposition to abortion (Fels-Klerx et al, 2001; Heinrichs and Heinrichs, 2011).

Diarrhea as a result of both CE and CP is classified as either hypersecretory, or malabsorptive. Hypersecretory diarrhea is characterized by large amounts of fluid being mobilized into the small intestine, which exceeds the resorptive capacity of the mucosa within lining the small intestine. Malabsorptive diarrhea is when the mucosa are damaged or impaired from carrying out their main function of water absorption. The results can be devastating. After 2-3 d of CE at the clinical level, the villi and microvilli responsible for

the absorption of nutrients can atrophy which can rapidly lead to mortality if left untreated (Merck, 2010).

Symptoms and signs begin with watery stools that can contain mucous, leading into a state of dehydration (Merck, 2010). Calf paratyphoid cases are typically differentiated by yellow odorous diarrhea (Merck, 2010). Eyes are an excellent indicator of general lucidity. Sunken eyes can usually be linked to dehydration and mild to severe depression depending on the intensity level. Clinical dehydration is marked by a 6% decrease in a calf's body weight (Merck, 2010). Drooping and cold ears are another qualitative form of quick assessment. Elevated temperature in the 24 h after onset is another confirmation that you may have a calf with CE, however temperature has been known to re-stabilize after the 24-48 hr mark (Heinrichs and Heinrichs, 2011). While all these factors are relatively surface level, without running labs on fecal samples, or evaluating the state of the small intestine at abattoir, it cannot be concluded without a doubt that scours or CE is the source of illness.

Treatment should begin with oral electrolyte therapy to promote rehydration (Merck, 2010). If calves are recumbent or unable to stand for long periods their dehydration has reached $\geq 8\%$ and an IV of electrolytes is the best course of action (Merck, 2010). Isotonic sodium bicarbonate (13g/L) should be used in cases where calves are acidotic, and the addition of anywhere between 25-50 g of dextrose or glucose added to the bicarbonate can help to combat hypoglycemia which usually follows dehydration $>6\%$ (Merck, 2010). As for treating the infections, consulting your veterinarian will offer the best course of action seeing as there are many avenues of antibiotic one could pursue. Tetracycline and amoxicillin are used throughout dairies in California to treat CE with

varying results (Merck, 2010). Treating on a hunch can be ineffective. Drugs such as meglumine, indomethacin, loperamide, and diphenoxylate have proven to be effective as treatment as anti-inflammatory and anti-secretory treatments however they have not yet passed clinical trials to successfully show treatment of CE in calves (Merck, 2010). Overtreating can contribute to bacterial resistance and the prevalence of super-resilient strains of pathogenic bacteria onsite (Cirz et al 2005).

Coccidiosis is a parasitic infection that causes symptoms similar to CE. The main species known to cause coccidiosis is *Eimeria* (Merck, 2010). While CE is typically a neonatal disease, onset of coccidiosis is typically seen after the first 3 weeks of life. Bloody diarrhea is a common indicator and symptom. As the parasites begin to proliferate within cells of the small intestine, they prepare for life in extra-cellular environment by producing oocysts and merozoites; seeds of the parasite which allow survival outside the body. Once proliferation reaches maximum density within intestinal cells, host cells rupture to release those “seeds” into the environment allowing for the spreading of the parasite into other susceptible animals (Elsheikha and Khan, 2011). The rupturing of cells in the lumen of the small intestine is what leads to the presence of blood. In growing groups of calves, coccidiosis is characterized by a noticeable decrease in feed efficiency in subclinical stages, by tenesmus and muscular tremors in more clinical cases; tenesmus defined as the feeling of constantly having to pass stool (Merck, 2010). These phantom bowel movements are marked by discomfort and restlessness in calves. Coccidiosis is known to be a “self-limiting” disease meaning that recovery can be spontaneous without the utilization of drug therapy. Once the coccidian have completed the multiplication stage, they undergo mass die-out and recovery can begin (Elsheikha

and Khan, 2011). While some calves die due to coccidiosis, many die due to secondary bacterial infection. Diarrhea and dehydration causes a depression of the immune system, which allows other pathogens to propagate and affect the systemic health of the animal. Respiratory infections are the leading cause of death in calves despite the fact that many occasions they are a secondary infection (Merck, 2010).

Among those respiratory infections is pneumonia. A common respiratory disease caused by a wide range of pathogenic microorganisms, but most often isolated are: *Pasteurella multocida*, *Mannheimia haemolytica*, Parainfluenza virus and *Mycoplasma bovis* (Merck, 2010). Each of these pathogens are so challenging to control because of their adaptations making them very stable in the environment. If the immune system does not achieve adequate development, or becomes compromised due to a primary infection, pneumonia is an incredibly effective killer (Fels-Klerx et al, 2001, Bey et al 2007). Through periods of stress and weaning there are also opportunities for the immune system to erode, and for the susceptibility of infection to increase (Bey et al 2007). Symptoms at the clinical level include fever of 40-41°C, and a mucopurulent discharge from the eyes, nose, or both. This discharge will vary in color, but is typically clear and translucent, or off-white to green. Respiratory rate greater than 40 breaths per minute is an excellent indicator however ambient temperature and climate must be considered. In clinical cases of pneumonia, dyspnea marked by rapid shallow breaths can also be marked as an observable symptom, usually matched with an elevated heart rate, and a moist cough. While the cough can vary in intensity, isolating that calf and keeping them under close watch is important in the prevention of contact with healthier herd-mates. The best treatment begins with early recognition. Antibiotics used should be ones

with the greatest effect on the previously mentioned gram-negative bacteria (such as Excenel). An important concept to prevent relapse is to continue administration of the antibiotic for the entire prescribed time period (Merck, 2010).

The best attempt to minimize each of these diseases is to ensure calves are getting adequate amounts of colostrum in the first 24 and 48 hours of life. Once immunity has been successfully transferred, staying observant is vital. Good management practices are key. Group calves by age and maintain clean ventilated housing for them. Additionally, if using individual structures for each calf, ensure they are far enough away from one another to prevent the potential communication of disease. Additionally, isolation of sick calves from healthy ones can reduce the spread of disease amongst the more vulnerable.

MATERIALS AND METHODS

Cal Poly Dairy

California Polytechnic State University (CP) is located in on the central coast of California in San Luis Obispo approximately 200 miles north of Los Angeles, and 11 miles east of the Pacific Ocean. San Luis Obispo is known to have a cool Mediterranean climate with temperatures averaging 18° C during winter months, (December through March), with temperatures no higher than 28° C during the remaining months, and an average of 24 inches of rain annually. The dairy is located on the northwest portion of campus where the 250 mixed milking herd of Holsteins and Jerseys reside in addition to 230 young-stock raised onsite. The herd is milked twice daily at 3 A.M., and 3 P.M.. The milking cow barns are located on the far west end of the facility, while the hospital and maternity pens are located closest to the parlor. These pens were designed in an

orientation that would allow for greater attention to be placed on the animals that need it most.

Parturition and Colostrum Administration

Once a cow begins exhibiting signs of parturition, we make the attempt to move her into an individual pen where external stress can be minimized and greater measures in care and cleanliness can be taken. Bedding in the individual parturition enclosures typically consist of dry straw-hay. Following parturition, the dam is allowed to lick her calf clean before the calf is pulled and given its own temporary quarters. This practice allows for greater stimulation of blood flow throughout the calf and has been positively linked to improved Ig absorption (Marina and Weary, 2007). Navels are dipped in a 7% tincture of iodine from a dipping cup a minimum of two times providing a visible coating over the exposed area and initiating the first major hurdle in prevention of infection.

Colostrum is milked from the dam, and measured for relative quality using a colostrometer. Because of the high statistical relationship between the presence of Ig in colostrum and high specific gravity, the quality of colostrum through the specific gravity is a quick and effective tool utilized at the Cal Poly dairy (Fleenor and Stott, 1980; Donovan et al, 1986). A refractometer is used as another method of analysis determining colostrum quality often used congruently as another method of scoring colostrum. Degrees brix scores >22% indicate high levels of antibodies in the colostrum sample, while scores 18-21% are used for second and third feedings, and <17% indicate an inadequate amount of antibodies in the sample.

Once records of colostrum quality are made, calves are bottle-fed following the rule of four quarts of high quality colostrum within four hours of being born (Jaster,

2008). This ensures maximum absorption of large Ig proteins into the blood of the calf. In rare cases where calves are reluctant to drink from bottles, they are tube-fed as a measure to ensure maximum rate of survival.

Sampling

At 24-48 hours post-partum, calves were restrained in two person teams to ensure safe handling and comfort of calves. Using 12 cc syringes and 20-gauge one-inch needles, blood samples were collected by jugular venipuncture into non-evacuated tubes. Blood was then immediately taken to the onsite lab for analysis. Samples of blood were centrifuged at 1745-x g to achieve separation of serum and red blood cells. Using a dropper, serum was then collected and spread onto the surface of a refractometer to assess total serum protein in mg/dL. Relative values are listed below in Table 1 (French et. Al, 2000). Total serum protein levels been recorded for each calf in study and will be used to assess the percentage of calves that are within ideal range as an indicator of how successful the current calf-raising protocols are as they stand at the CP dairy. For the population of calves that fall outside normal range, causes will be identified and potential areas of improvement will be assessed and suggested as a means to maximize the health of the herd, and to minimize calf loss. The statistical method used to determine correlations within our collected data is the PROC PROBIT function of SAS, which is used to model binary outcome variables. Variables cross-analyzed against pre-weaning mortality rate include: breed, gender, or date of birth, SPC grade, and SPC score in °Bx.

Table 1: Total Serum Protein by Refractometer (mg/dL)

Total Serum Protein by Refractometer (mg/dL):	Grade:
<4.5= No Colostrum	F
4.5-4.9= Inadequate	D
5.0-5.2= Poor	C
5.3-5.4= Borderline	B
5.5-7.0= Ideal	A
>7.0= Likely Dehydrated	--

RESULTS AND DISCUSSION

Results began with a broad look at the overall mortality rate. Post-collection results of all 73 calves were assembled into a spreadsheet and listed by ID. Seven calves total were lost out of 73 giving us a mortality rate of 9.58%. While all calves were used to determine true mortality rate within this sample of data, 16 calves were not utilized in final analysis due to missing or incomplete information. With that understood, our sample number n=57, and our 7 calves lost translates into a calf loss of 12.3% within our usable range of data. Records on the usable total (n=57) includes: date of birth, bodyweight at time of sampling, Serum Protein Concentrate (SPC), gender, and breed. Results continued with a breakdown of calves into groups by grade. Calves were broken down into groups by grades based on SPC values adapted in table 1 above. Figure 3 illustrates the breakdown using grades adapted in table 1.

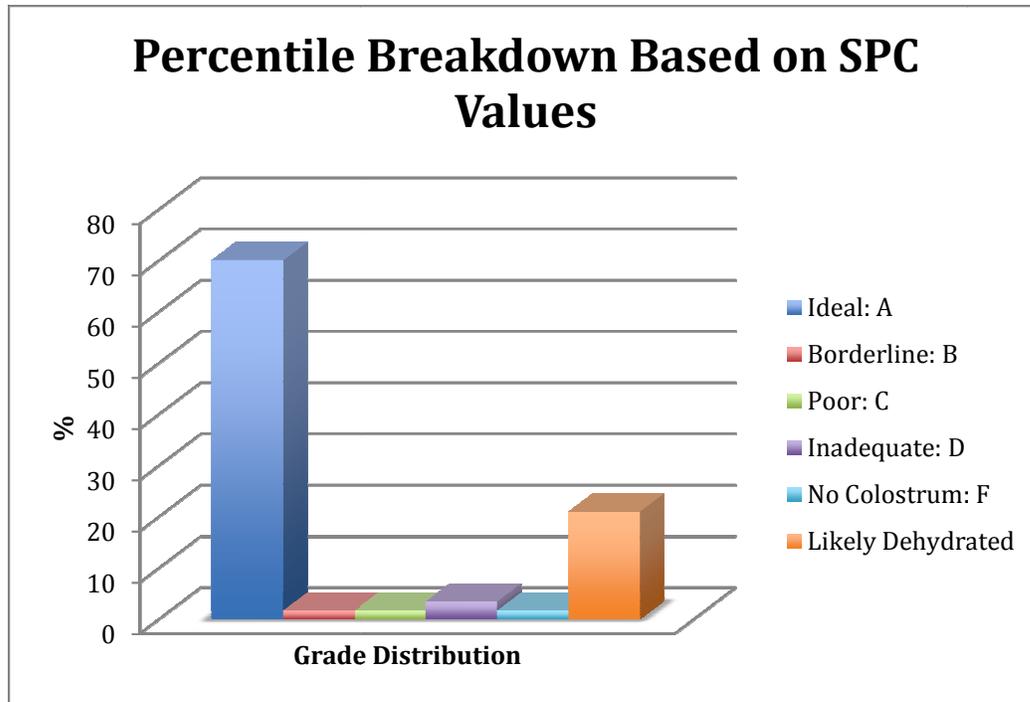


Figure 3: Percentile Breakdown Based on SPC Values

Subsequent statistics were run to determine potential correlations to life or death rate with factors including: Date of Birth, Breed, Sex, SPC Grade, and Sex. Results of a cross-factorial analysis shown below in table 2 showed no statistically significant relationship between the above-mentioned factors affecting life or death rate.

Table 2: Results of Initial SAS Analysis

Effect	Degrees Freedom	Wald Chi-Square	Pr>Chi-Square
Breed	1	0.0108	0.9171
Sex	1	0.5918	0.4417
Grade	5	2.5436	0.7699

When Life or Death rate was analyzed against SPC as a quadratic function, results approached statistical significance ($P < .1$). Table 3 shows Chi-squared values for the function used, while Figure 6 below displays those results graphed. Using the PROC

PROBIT of SAS program, the inputted value for alive at weaning was “1”, and the corresponding value for death before weaning was “0”. Calf survival increases towards the value of 1 as SPC values increase towards the ideal of 5.5-7. At values > 7.1 there is a notable decrease in our survival curve.

Table 3: Results of Final SAS Analysis

Effect	Degrees Freedom	Wald Chi-Square	Pr>Chi-Square
Breed	1	0.9667	0.3255
Sex	1	1.1296	0.2879
SPC	1	3.5716	0.0588
SPC*SPC	1	3.5788	0.0585

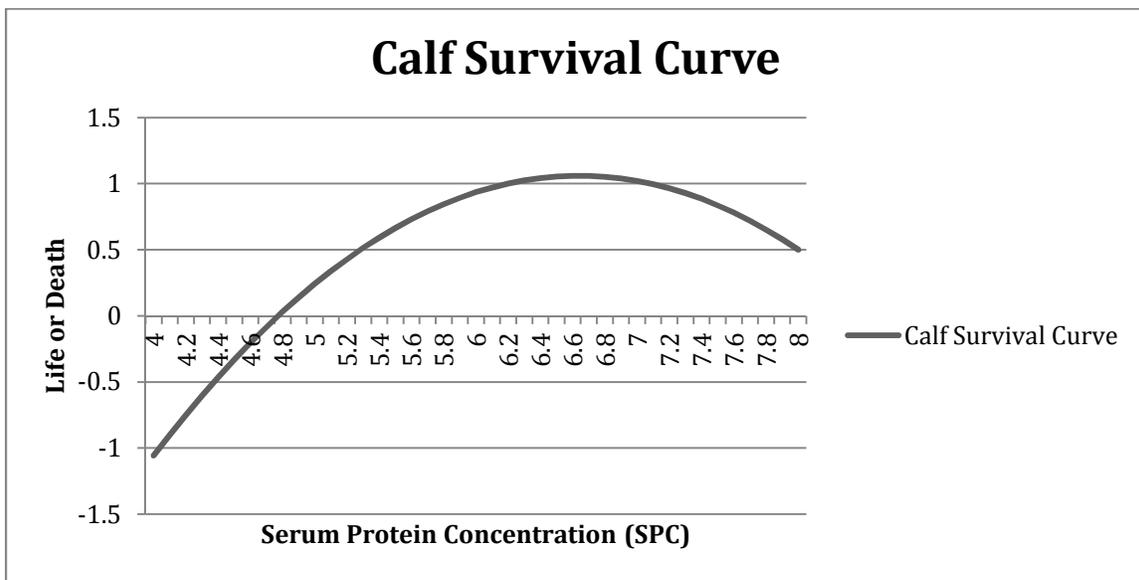


Figure 4: Calf Survival Curve

When calves become diuretic, they are mobilizing fluids from their body as fallout of having a compromised small intestine. Understandably this leads to a decrease in water within blood serum, and an increase in concentration of the SPC. Our data confirms that calves with low SPC lead to an increased mortality rate. This could be likely caused by respiratory illness and complications associated with those diseases. As

our curve breaches a 7.1 SPC concentration and continues upward we notice a trend leading back towards “0”, or an increase in mortality rate. This also alludes to a low initial uptake of Ig. Immuno-compromised calves that contract a disease as a result of low initial Ig who begin to scour will exhibit a greater SPC concentration and the increased mortality rate we observe.

CONCLUSION

Many factors contribute to the health of a neonatal dairy calf. Of these factors, most notable include the proper and timely administration of quality colostrum. As our findings illustrate, Cal Poly calves who failed to absorb adequate amounts of Ig were more likely to respond fatally to diseases ranging from Calf Enteritis (Scours) and Calf paratyphoid (CP) to Coccidiosis, and Pneumonia. While other preventative measures are previously detailed above, the best preventative measures are the proper utilization and coverage of a 7% tincture of iodine as a navel dip, and the timely administration of high quality colostrum. In closing, I would like to see this project be taken further to really allow for the scope of the study to broaden. Better records of calves must be taken to truly pursue this next step, however I believe it to be vital in the successful understanding of what it will take to improve. Details on diseases and weights will affect future studies as in the steps following students will be able to determine correlations between neonatal diseases in individual calves, to data on those same individuals with consideration to: age of puberty, age of calving, and production relative to their herd-mates.

ACKNOWLEDGEMENTS

I'd like to acknowledge and express appreciation to Dr. Bruce Golden for his technical assistance and his statistical acumen, Dr. Ed Jaster and Rich Silacci for inspiring and cultivating my love of cows; the lessons learned and many hands on experiences will not easily be forgotten. To Dr. Leanne Berning: I am, and will remain grateful for her unrelenting guidance and support throughout this project, and throughout my undergraduate career.

Lastly, I'd like to thank my mom and dad. Without them I can say with certainty that none of this would have been possible.

REFERENCES

- Bey, R., H. Swan, J., Fetrow, H., Chester-Jones, S., Wells, and S., Godden. 2007. Passive Transfer of Immunoglobulin G and Prewaning Health in Holstein Calves Fed a Commercial Colostrum Replacer. *J. Dairy Sci.* 90:3857-3866.
- Beam, A. L., J. E. Lombard, C. A. Koprak, L. P. Garber, A. L. Winter, J. A. Hicks, J. L. Schlater. 2009. Prevalence of Failure of Passive Transfer of Immunity in Newborn Heifer Calves and Associated Management Practices on US Dairy Operations. *J. Dairy Sci.* 92:3973-3980.
- Bourc'his, D., and N., Zamudio. 2010. Transposable elements in the mammalian germline: a comfortable niche or a deadly trap?. *Heredity.* 1:92-104.
- Castro, L. N., F. J. Von Zuben. 2000. An Evolutionary Immune Network for Data Clustering. *IEEE Eng Med Biol Mag.* 4:84-89.
- Castro, L. N., F. J. Von Zuben. 2002. Learning and Optimization Using the Clonal Selection Principle. *IEEE Eng Med Biol Mag.* 6:239-251.
- Cirz, R. T., J. K., Chin, D. R. Andes, V. de Crécy-Lagard, W. A. Craig, et al. 2005. Inhibition of Mutation and Combating the Evolution of Antibiotic Resistance. *PLoS Biol.* 3:1024-1033.
- Co, M. S., G. N. Gaulton, H. D. Royer, M. I. Greene. 1986. Anti-idiotypic antibodies as probes of cell surface receptors. *Mol Cell Biochem.* 65:5-21
- Crawley, R., Thucydides. *History of the Peloponnesian War.* 431 B.C.E.
- Devery, J. E., B. L. Larson, H. L. Heary. 1979. Immunoglobulin Production and Transport by the Mammary Gland. *J. Dairy Sci.* 63:665-671.
- Donovan G. A., L. Badinga., R. J. Collier., C. J. Wilcox and R. K. Braun. 1986 Factors Influencing Passive Transfer in Dairy Calves. *J. Dairy Sci.* 69:754-759.
- Eisen, H. H., S. Sirisinha. 1971. Autoimmune-like antibodies to the ligand-binding sites of myeloma proteins. *Proc. Natl. Acad. Sci. USA* 68:3130-3135.
- Elsheikha, H. M., N. A. Khan. 2011. *Essentials of Veterinary Parasitology.*
- Fleener, W. A and G. H. Stott. 1980. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrums. *J. Dairy. Sci.* 63:973-977.
- Forsdyke, D. R. 1995. The Origins of the Clonal Selection Theory of Immunity. *FASEB J.* 9:164-166.

- Freeman, S. 2008. Biological Science. University of Washington, San Francisco, CA.
- French, P., J. D. Quigley, III, and R. E. James. 2000. Effect of pH on absorption of immunoglobulin G in neonatal calves. *J. Dairy Sci.* 83:1853-1855.
- Fowler, M., B.J., Nonnecke, T.E. Johnson, R.L. Horst, M.R. Foote, and B.L. Miller. 2009. Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of preruminant calves. *J. Dairy Sci.* 92, 12: 6134-6143.
- Heinrichs, B., and A., Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94(1):336-341.
- Hopkins, B. A., J. D. Quigley. 1997. Effects of method of colostrum feeding and colostrum supplementation on serum IgG concentrations in neonatal calves. *J. Dairy Sci.* 80:979-983
- Hopkins, D. R. Princes and Peasants: Smallpox. 1983. Pages 79-87. The University of Chicago Press. Chicago, IL.
- Ingram D. G., A. N. Smith. 1965. Immunological Responses of Young Animals II: Antibody Production in Calves. *Can. Vet. Jour.* 6:226-232.
- Isomura, S., S. Matsuno, H. Yamaguchi, S. Inouye, M. Yamaguchi, T. Morishima, S. Suzuki. 1985. Anamnestic Response in Fecal IgA Antibody Production after Rotaviral Infection of Infants. *J. Infect. Dis.* 152(2):398-400.
- Jaster, E. H. 2008. "Dairy Cattle Nutrition". DSCI 301. California Polytechnic State University.
- Janeway C. A., P. Travers, M. Walport, M. J. Schlomchik. 2001. Immunobiology. 5th ed. New York, NY.
- Marina A. G., D. M. Weary. 2007. Maternal Behavior in Cattle. *Horm Behav.* 52:106-113.
- Merck & Co. 2010. Merck Manual of Veterinary Medicine. 10th ed. Whitehouse Station, N.J.
- Senger, P. L. 2005 Pathways to Pregnancy and Parturition. 2nd ed. Current Conceptions. Redmond, OR.

van der Fels-Klerx, H. J., R. B. Huirne, , J. T. Sorensen, A. W. Jalvingh. 2001. An economic model to calculate farm-specific losses due to bovine respiratory disease in dairy heifers. *Prev. Vet. Med.* 51(1-2):75-94.

Voltaire. *Letters on The English or Lettres Philosophiques: 1733.* 2008. Pages 27-31. Rockville, M.D.