

Use of ENDOVAC-Bovi® in Pre-Weaned Calves

A Senior Project

Presented to

The Faculty of the Dairy Science Department

California Polytechnic State University, San Luis Obispo

In Partial Fulfillment

Of the Requirements for the Degree

Bachelor of Science

By

Jena Glasgow

December, 2010

© 2010 Jena Glasgow

Abstract

The objective of this experiment was to determine the effects of the vaccine ENDOVAC-Bovi® on pre-weaned calves. Data regarding the health status of individual calves was collected by calf feeders at the Cal Poly Dairy, and written down in a communal log book. Calves were divided into a treatment group and a control group at birth. The control group received standard vaccinations, and the treatment group was given the additional vaccine on day 1, 4, and 10. A 1 mL injection was given i.m. in the thigh muscle. Treated calves had fewer days off feed initially, but after 1 week control calves had less days off feed. The average days off feed for control were 0.6, and for the treatment group was 1.75. In conclusion, a reduction in morbidity and mortality could not be demonstrated, and more experiments with this vaccine should be done. Further experiments could provide more conclusions if the experiment was conducted on a larger scale, with more calves involved. Also, treatment protocols should be followed better to provide less variation in the way the calves are raised.

List of Tables

Table

1. Days off Feed.....Page 13
2. Average Days off Feed.....Page 13
3. Calf Records from Field.....Page 14

List of Figures

Figure

1. Absorption over time in a calf.....Page 5
2. S.C. Injection.....Page 8
3. Graph of calves off feed.....Page 14

Table of Contents

Abstract	i
List of Tables	ii
List of Figures	iii
Introduction.....	1
Literature Review.....	2
Gram Negative/Positive bacteria	2
Gram Negative Associated Calf Diseases.....	2
Immunopathologic Mechanisms	3
Type I Reactions	3
Type II Reactions	4
Type III	4
Type IV Reactions	4
Passive Immunity via Colostrum	5
Active Immunization	6
Use of vaccines in livestock.....	7
Administration of Vaccines	8
Immunostimulants.....	9
Materials and Methods.....	11
Materials	11
Methods.....	11
Results and Discussion	13
Conclusion	15
Appendix.....	17
Citations	18

Introduction

Arguably, the most important part of a dairy is the calf raising program. This importance stems from the fact that, without it, there can be no mature dairy cows to produce milk. So, dairy producers must do everything in their power to ensure healthy calves are produced, whether they are produced on their own dairy, or at a separate specialized calf raising facility. Part of this entails a good vaccination protocol to help immunize calves against pathogens that could make them sick. Sickness in turn causes calves that are poor doers, and cannot be bred at the optimal age for productivity and profit. A good vaccination program can help offset these problems.

There are many different vaccines and vaccine protocols that one can use for immunizing calves. Choosing the correct one for your calves could be the difference between healthy, high performing calves, and sick, low performing calves. Vaccines usually go through trials, where they are proven to show benefits to the calf. This senior project investigated the vaccine ENDOVAC-Bovi® to see if it helped protect calves against Gram negative associated diseases. ENDOVAC-Bovi® offers cross protection for three major types of disease pathogens. They include *E. coli*, *Salmonella*, and *Pasteurella*. Because this vaccine covers against a wide array of bacterial pathogens, and not just one specifically, it could prove valuable in a calf vaccination program. ENDOVAC-Bovi® is a mutant bacterin-toxoid of *Salmonella* origin that uses an antigen that is devoid of the outer O side chains. This means, because Gram negative pathogens cell walls are very similar, the antigen provides broad spectrum protection against 99% of Gram negative organisms. The vaccine also has an immune stimulating adjuvant mixed in, to provide an even better protection to vaccinated animals.

Literature Review

Gram Negative/Positive bacteria

In bacteria, there are two general types of cell wall. Gram positive bacteria have a plasma membrane enclosed by a thick cell wall with extensive peptidoglycan. Peptidoglycan is a substance created by cross-linked carbohydrate strands. Gram negative bacteria also have a plasma membrane, but it is surrounded by a two component cell wall. The inner layer is a thin gelatinous layer containing peptidoglycan, and the outer layer is a phospholipid bilayer. Classification of bacteria into either positive or negative status is accomplished by Gram staining a microorganism. The dyes used in a Gram stain react differently with the cell walls, and therefore cause positive and negative microorganisms to turn a different color. (Freeman, 2007)

Gram Negative Associated Calf Diseases

Calf morbidity and mortality are often linked with Gram-negative bacterial diseases. (Deluyker et al, 2004) Most of the damage caused by these diseases is created by endotoxins released from the Gram-negative bacteria. Gram-negative associated diarrheas and pneumonias are often complicated by endotoxins excreted from the bacteria. (Sprouse et al, 1990) These toxins elicit an expansive response with symptoms ranging from diarrhea to the possible life threatening status of endotoxic shock. Calfhood scours was the cause for >62% of mortality in pre-weaned heifers. (Baumrucker et al, 2010) Diarrhea caused by Gram-negative bacteria causes hypersecretion of water within the intestine. In turn, this causes the intestinal wall cells to produce a huge amount of excess fluid in the intestine, trying to flush out excess toxin. This is what is believed to cause the condition of scours. (Jones and Heinrichs, 2006) Pneumonia can cause a reduction in first lactation milk yield by 2.2% and cause heifers to calve two weeks later than if they had not had pneumonia. (Grimmet, 2010)

Immunopathologic Mechanisms

The immune system's job in the body is to discriminate between self and non-self. This distinction must be made so that the body is protected against potentially pathogenic microorganisms, or other foreign material. The immune system differentiates between the self and non-self, and then eradicates the non-self material with an assortment of mechanisms. These include inactivation of biologic agents, lysis of foreign cells, agglutination or precipitation of molecules or cells, or phagocytosis of foreign materials. Dendrite cells are responsible for synthesizing the correct immune response, and these cells are considered an immunologic sensor. According to the Merck Veterinary Manual, "they are scattered throughout the body, sense the environmental stimuli, and convey this information to naïve lymphocytes to tune the relevant immune response." These responses can sometimes create significant tissue damage, and are classified as immune-mediated disease. There are 4 general types of these diseases- Type I, II, III, and IV. (Merck, 2008)

Type I Reactions

The first type of reaction is anaphylactic and allergic reactions in the body. This entails an antigen binding to IgE antibodies, which causes the discharge of chemical substances from mast cells and basophils. Mast cells produce biologically active mediators, which include histamine, leukotrienes, eosinophilic chemotactic factor, platelet activating factor, kinins, serotonin, and proteolytic enzymes. The aforementioned chemicals cause a contraction of smooth muscle in the vascular system. The type of antigen dictates the severity of the reaction,

and causes IgE antibodies to be produced. Type I reactions can have numerous causes, ranging from venom from insects to vaccines, drugs, foods, and blood products. (Merck 2008)

Type II Reactions

According to the Merck Veterinary Manual, “Type II reactions occur when an antibody binds to an antigen present on the surface of the cell. This antibody-antigen complex then can activate the complement pathway, resulting in cell lysis or, through and antibody-mediated or complement-fragment-mediated receptor binding of a phagocytic cell, an antibody-mediated cytotoxicity.” This type of reaction’s triggering mechanism is not well known, but a combination of external factors and genetic predisposition can lead to this disorder.

Type III

Type III reactions occur mostly in the joints, skin, kidneys, lungs, and the brain. According to the Merck Veterinary Manual, “Type III reactions occur when antigen-antibody complexes are deposited along the endothelium.” A neutrophilic inflammatory response and vascular damage ensues, caused by the reaction directly and indirectly. This type of disease develops because of a presence of soluble antigen that corresponds with a continuous production of antibody. When there is more antigen than antibody, complexes are deposited along endothelial cells. It is not know why deposition occurs, but in the end it causes vasculitis, which is the inflammation of the blood or lymph vessel. The original source of the antigen cannot be determined most times, and the disease is unidentifiable. (Merck, 2008)

Type IV Reactions

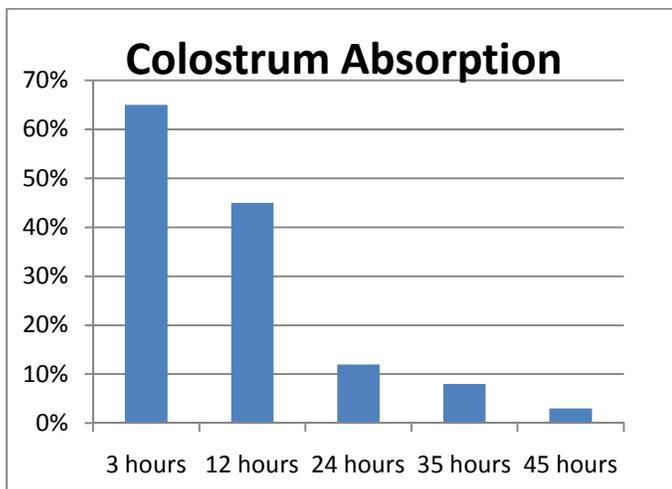
Type IV reactions are cell-mediated immune reactions. According to Merck Veterinary Manual, “The infiltration of mononuclear cells and the elaboration of a variety of substances from these cells result in the pathologic processes of cell-mediated immune reactions.” Some

examples of antigens that cause this are bacteria or parasites, occasional viruses, chemicals, and possibly cell antigen. Because this can occur in any type of organ, the disease signs will vary. (Merck, 2008)

Passive Immunity via Colostrum

Colostrum is the first milking from a cow that has just given birth to a calf. This first milking provides the first line of defense for the calf's immune system, and is essential for protection from environmental microorganisms. In cattle, transfer of IgG to the neonate is accomplished solely by the ingestion of colostrum because *in utero* transfer does not occur. (Baumrucker et al, 2010) Colostrum also provides nutrients to increase metabolism and stimulate digestive activity. (Jones, Heinrich 2006) For the first 24 h after birth, the calf can absorb whole antibodies across the intestinal membrane and into the bloodstream directly. This type of protection is called passive immunity, and it protects the calf from outside pathogens up until it can make its own immune system function to a higher degree. (Baumrucker et al, 2010) There is a rapid decrease in the calf's ability to absorb the immunoglobulins, so colostrums should be fed within 1 h of birth. (Agrilabs, 2010) (See Figure 1 below)

Figure 1: Absorption over time in the calf.



There are many factors that affect how much colostrum is needed by a calf to acquire adequate immunity. These factors can range from the calf's size and efficiency of IgG absorption, to how high of a bacteria load is present in the environment and directly in the colostrums. (Jones, Heinrich 2006) The antibodies in colostrum are also able to fight pathogenic organisms well after the first 24 h after, though the antibodies will not be passed through to the bloodstream. Instead, the antibodies line the intestinal tract, interfering with pathogen adherence. However, if bacteria enter the intestine first, this coating action is ineffective. High quality colostrum is considered to have an IgG level of over 50 mg/ml, with a common range of 20-100 mg/mL. (Jones, Heinrich 2006)

Active Immunization

According to the Merck Veterinary Manual, "Active immunization involves administration of antigen(s) derived from an infectious agent so that an animal mounts an acquired immune response and achieves resistance to that agent." If correctly used, vaccines can be helpful in minimizing the spread of infectious disease. There are some criteria to follow when deciding whether a vaccine should be used in a health program. The genuine cause of the disease should be known. Many bacteria are secondary causes of a disease, and vaccination should be used to prevent the primary causes of diseases. The risks of vaccination must not exceed those caused by the disease itself. (Merck, 2008) The concept of herd immunity should be considered also. Herd immunity refers to "increased resistance of a group because of the presence of some immune animals within the group, which reduces the probability of a susceptible animal encountering an infected one", according to the Merck Veterinary Manual. This causes the spread of infectious disease to be sluggish or possibly impeded. The perfect

vaccine for active immunization should give long lasting, potent immunity to the animal. The vaccine should be safe, inexpensive, stable, and adaptable to large scale. It should also be noted that a good nutritional program should be in place concurrently with a vaccination program. Energy is diverted to the immune system when a vaccine is introduced, and if calves are not receiving adequate nutrition, this can cause decreased weight gain. (Agrilabs, 2010)

Use of vaccines in livestock

The vaccination of animals on a widespread basis is the most successful method to protect farm animals from disease and prevent losses. (Singh, O'Hagan 2003) The body responds to antigens by producing antibodies specific to the antigen, or by producing specialized T cells. A vaccine stimulates the immune system to produce these products to protect the animal from the specific disease, and results in "the development of an immune response to the inducing antigen". (Merck, 2008) There is always a need to develop new and improved vaccines, because of problems including emergence of new diseases, re-emergence of "old" diseases, and the spread of antibiotic resistant bacteria. (Singh, O'Hagan 2003) With vaccines, one is attempting to protect the animal from a disease introduced by a viral or bacterial agent. (Yancey, 1993) Production of vaccines is under the regulation of the government system. Companies that produce vaccine are inspected by regulatory authorities to ensure that standards are upheld and the facilities and methods used are up to par. Vaccines should be tested for safety and potency according to federal regulations. (Merck, 2008)

Administration of Vaccines

Common methods of administering vaccine are s.c. (subcutaneous) or i.m. (intramuscular). The method of s.c. puts the vaccine underneath the skin layer, but not in the muscle, as it should stay above. This is done by tenting the skin and inserting the needle into the tent flap. (See Figure 2.)

Figure 2. S.C. injection



The method of i.m. puts the vaccine straight into the muscle, and is the more common method used for administering vaccines. There are also other ways to dispense vaccine, including intranasal, aerosolization, or through feed and drinking water. For convenience, it is common to use mixtures of organisms in single vaccines. (Merck, 2008) This mixed type of vaccine saves time, but may not be as economical as a single organism version because there may not be a problem with all the organisms that a multiple vaccine is protecting against. Also, antigens can compete against one another, so it is important to make sure the type of vaccines you are mixing will not compete against one another causing a weak reaction to both the antigens in the vaccine. Mixing of vaccines without professional advice is not advised because of this.

Immunostimulants

In general, immunostimulants are classified as substances, either drug or nutrient, that activate or increase the activity of the immune system. There are two broad classifications regarding immunostimulants. The first class, specific immunostimulants, works in the immune system to provide an antigen-specific immune response. Examples include many common vaccines. The other class is non-specific immunostimulants. These immunostimulants do not have a correlation to antigenic specificity, and they provide an increased response of components of the immune system. (Merck, 2008) Typically, non-specific immunostimulants are adjuvants, as is the case with the immunostimulant component of ENDOVAC-Bovi®.

Adjuvants have many uses that improve immune response to vaccine antigens. These uses include increasing the immunogenicity of weak antigens, enhancing the speed and duration of the immune response, and modulating antibody avidity, specificity, isotype or subclass distribution. Also, adjuvants can stimulate cytotoxic T lymphocytes, promote the induction of mucosal immunity, enhance immune responses in immunologically immature or senescent individuals, decrease the dose of antigen in the vaccine to reduce costs, and help to overcome antigen competition in combination vaccines. (Singh, O'Hagan 2003) The way adjuvants work is still not completely understood, because it is hard to discern which immune reaction is caused by the adjuvant. A class of adjuvant active compounds has been identified. That produces a direct immunostimulatory effect on immune cells from bacterial DNA. (Singh, O'Hagan 2003) According to Macdonald, "Injection of cell wall preparations of various bacteria results in the following:

1. Increase in macrophage production,

2. Increase in phagocytic activity and rate,
3. Increase in natural killer cell activity,
4. Induced production of cytokine proteins such as interleukins and interferons.”

(McDonald, 1995)

Materials and Methods

Materials

Most of the materials needed to perform this experiment are already in use at a dairy or calf feeding operation. The first and most important thing needed is the actual vaccine itself. ENDOVAC-Bovi® (NAC #11260011, US Patent #5641492) is available only through a veterinary prescription, and has a 60 d withdrawal. The vaccine must be refrigerated. It is not recommended for use in septicemic or mastitic cattle. This vaccine can be purchased in 40, 100, or 200 mL vials. The 100 mL vial size was used in this experiment. Also, appropriate sized single use, sterile needles and syringes will be needed to administer the vaccine. The needle sized used was 18 g, and the needle length was 1 inch. The syringes used had a 3 mL capacity. For each calf vaccinated according to the experimental protocol, three needles and three syringes are used. The next thing needed is a person who is knowledgeable in calf health that can monitor calves in the experiment for sickness. In this experiment, that person was the calf feeder that was working the shift on a particular day. An established treatment protocol should be in practice so when calves are treated they are all on a level field.

Methods

The experimental group consisted of 25 calves born from January 13, 2010 to March 4, 2010. Two breeds of dairy cattle were represented, Holstein and Jersey. Calves of both sexes were part of the experiment. There were two experimental groups, a control group and a vaccinated group. The control group consisted of calves that were given standard vaccinations according to the Cal Poly Dairy's management protocol. These vaccinations included TSV-2®, a modified live virus given intranasal to protect against respiratory diseases, and a Bovine

Ecolizer vaccine to protect against *E. coli* given orally. Both were given 30 minutes prior to colostrum being fed. The treatment group was given the standard vaccines, and in addition, the vaccine ENDOVAC-Bovi®. The dosage was 1 mL given intramuscular in the thigh muscle, and was given on the day the calf was born. Additional shots of the vaccine were given at 4d and 10d, with dosage being 1 mL given in the thigh muscle.

Calves of both treatment groups were fed twice daily with a 20 percent fat, 22 percent protein Cargill® brand Snowflake milk replacer. The main ingredient in this powder was non-fat dried milk. Approximately 0.5 lbs of powder were fed to each calf, each feeding. The powder was reconstituted with five pints of 110°F water and mixed for a ten min. Milk was fed in bottles throughout the experiment. Feeding times were 5:30 a.m. and 5:00 p.m. Calves had access to water and calf starter grain around day three of their life, which was provided in small buckets. Buckets were washed once a day, and feed and water were replaced after they were cleaned. Calves were housed in hutches bedded down with straw. Calf feeders were responsible for identifying, diagnosing, and treating calves when they were ill. All observations were recorded in a mutual record book, which included documenting temperature and consistency of milk, new treatments, and calves that did not eat or looked sick. (See Appendix for treatment protocol) A calf was determined “off feed” if it did not come up to eat when bottles were placed out. Calves were monitored until they were eight weeks old, at which point they were weaned from milk. Results were then documented into an Excel spreadsheet which included the calf I.D. number, date of birth, breed, sex, treatment, days off feed, and dead/alive.

Results and Discussion

During the experiment, three calves died, one from the control and two from the experimental group. With respect to morbidity, 9 treatment calves were off feed at some point during the experiment, indicating sickness. Only 8 control calves were off feed during the experiment. (See Table 3) For all the calves, there was a total of 36 d off feed, with 21 being from the treatment group and 15 from the control group. (See Table 1) There was an average of 1.44 d being off feed for all the calves, 1.75 d for the treatment group, and 0.6 d for the control group. (See Table 3) Though the treatment group and control group were off feed the same amount of times in the first week, during the seven subsequent weeks, the treatment group was off feed more. (See Figure 2)

Table 1. Days off Feed

	day 0-2	day 3-5	day 5-6	day 7+
total				
treatment	3	2	3	6
total control	4	3	1	3
total	7	5	4	9

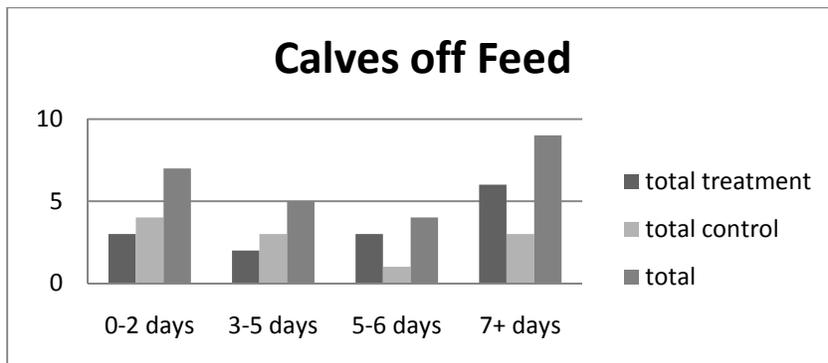
Table 2. Average Days off Feed

	days	average
total	36	1.44
treatment	21	1.75
control	15	0.6

Table 3. Calf Records from Field

CALF ID	DOB	BREED	SEX	TREATMENT	DAYS OFF FEED
10392	1/13/2010	J	M	vaccinated	didn't eat-1/14(new calf, tubed), 1/15(new calf, tubed), 1/19(bloat, tubed, 8 cc Pen) 1/20(scour, no appetite, t
2245	1/13/2010	H	F	control	
20339	1/14/2010	H	M	control	1/31(scours, del)
427	1/22/2010	J	F	vaccinated	1/28(bloat, tubed bicarb and water, 5 cc Pen) DEAD 2/2
2446	1/26/2010	H	F	control	1/27(no app, dehydrated, Del) 1/28(dehydrated, 5 cc Ex) 2/2(scours, 5cc Ex and tubed Del)
2447	1/29/2010	H	F	control	2/17(5 cc Pen, Del) 2/24(scours, Del)
10393	2/1/2010	J	M	control	2/2(new, tubed, 3cc vit B) 2/3(tubed milk, fastrack) 2/4(tubed milk)
2448	2/3/2010	H	F	vaccinated	2/4(Del) 2/5(3 cc Ex, electrolytes) 3/26(scours, wheezing, 5 cc Pen)
428	2/3/2010	J	F	vaccinated	
2449	2/6/2010	H	F	vaccinated	
20340	2/8/2010	H	M	control	2/13(lethargic, 5cc Ex, Del)DEAD 2/14
10394	2/8/2010	J	M	control	
10395	2/12/2010	J	M	control	
10396	2/15/2010	J	M	control	2/16(5 cc Pen)
20341	2/16/2010	H	M	vaccinated	2/17(5cc Pen) 2/20(bloat, 5cc pen) DEAD 2/21
2450	2/19/2010	H	F	vaccinated	2/28 (5cc Ex)
429	2/19/2010	J	F	control	
2451	2/21/2010	H	F	vaccinated	2/27(lethargic, 5 cc ex, electrolyte) 3/5(del) 3/13(scours, del)
20342	2/25/2010	H	M	vaccinated	3/6(lethargic, 5cc ex) 3/7(tubed Del) 3/13(no trtmnt)
2452	3/1/2010	H	F	vaccinated	
2453/20343	3/1/2010	H	M	control	3/20(scours, del)
10397	3/1/2010	J	M	control	3/2(no trmt) 3/5(no trtmnt)
20344	3/3/2010	H	M	vaccinated	3/18(8 cc Pen, bloat) 3/19(5cc Ex)
2454	3/3/2010	H	F	vaccinated	3/12(tubed del) 3/17(lethargic, 5cc Ex)
20345	3/4/2010	H	M	control	

Figure 3. Graph of Calves off Feed



Conclusion

In conclusion, the data suggests that there was not a reduction in calf mortality or morbidity from using ENDOVAC-Bovi®. Data suggested that the vaccine improved health of the calves during the first week of life, with fewer experimental calves sick for the first five days. However, after the five days, this advantage was erased with more days off feed noted for the treatment group. This could be because the vaccine has been only administered once at this point, and perhaps has not caused an immune challenge to cause visible illness yet. Once the second dose is administered on d4, it may cause the immune system to be challenged, and that is why we see more days off feed in the treatment group. The calves could be followed and monitored throughout to lactation to see if the vaccine would help these calves ward off Gram-negative caused mastitis.

The average amount of days off feed also contributes to the conclusion of the vaccine not improving mortality or morbidity. For the 13 control calves, the average days off feed were only 0.6 days for the eight week period of monitoring. This means that the control calves only were off feed for half a day, or one feeding of milk. Conversely, the treatment calves were off feed for 1.75 days, so they missed almost two days of feed during the eight week experiment. Treatment calves had double the amount of days off feed than control calves, and were even higher than the average days off feed for all the calves combined, at 1.44 days.

More treatment group calves died than control group. This could be caused from a variety of issues though, and it cannot be concluded that the calves died or didn't die because of this vaccine. It should be noted that established treatment protocols were not always followed correctly, and therefore could have had a major effect on both the measurement of days off feed

and mortality. Further experiments should be done, with an established protocol set and mandated, to be able to see if this vaccine has a negative effect on calves. Poor management techniques could be a major factor in why this vaccine seemed to have a negative effect on calves, so this should be rectified and the experiment redone to further be able to make conclusions on the effect of ENDOVAC-Bovi® on pre-weaned calves.

Appendix

Sick Calf Protocols:

Scours:

If not eating or calf is eating slowly: administer 5cc Polyflex or Pen-1-Pro and put on deliver txt regardless of how loose stool is;

Very lose watery stool, eating fine, greater than 3 days old: put on deliver

Deliver protocol: 1st shift: 2 scoops deliver and 4pts H₂O

2nd shift: 1 scoop deliver and 4 pts H₂O

3rd shift: 1 scoop deliver and 4 pts milk

Coughing/Pneumonia:

*coughing before milk is set out for calves

Young calves: check temp, look for and note any nasal or ocular discharge, administer 5cc Polyflex or Pen-1-Pro; leave note for next calf feeder to keep an eye on calf and watch appetite

Super Hutch/Far Barn calves: check temp, look for and note any nasal or ocular discharge, administer Nuflor per **Sub-Q** instructions on bottle; leave note for next calf feeder to keep an eye on calf and watch appetite

Bloat:

5cc pen1pro orally and handful of bicarb in mouth; try and get calf to get up and walk around or to at least sit sternally; if the calf looks dehydrated, call me so I can tube it water; if the calf appears like it's severely bloated and is at death's door do not hesitate to call myself, the herdsman or Rich

Citations

1. AgriLabs. 2010. Good colostrum: a solid foundation for herd health and profits. Bovine Health Watch Fall 2010 Edition.
2. AgriLabs. 2010. Successful recipe for immunity includes proper mix of vaccines and nutrients. Bovine Health Watch Fall 2010 Edition.
3. Baumrucker, C.R., Burkett, A.M., Magliaro-Macrina, A.L., Dechow, C.D. 2010. Colostrogenesis: Mass transfer of immunoglobulin G1 into colostrum. *J. Dairy Sci.* 93:3031-3038
4. Deluyker, H.A., Rossitto, P., Van Oye, S.N., Cullor, J.S. 2004. Efficacy of an *Escherichia coli* mutant strain bacterin in the protection of calves from endotoxin disease caused by subcutaneous challenge with endotoxins from *Escherichia coli*. *Vaccine*. Volume 23, Issue 5, Page 709-717.
5. Grimmet, H. 2010. Pneumonia in calves. *International Dairy Topics*. Volume 9, Issue 6, Page 34.
6. Jones, C., Heinrichs, J. 2006. *Calf Care*. ISBN 0-932147-48-8. Fort Atkinson, WI.
7. Macdonald, F. 1995. Use of Immunostimulants in Agricultural Applications. Page 97-103 in *Proc. Of Alltech's 11th Annual symposium*.
8. Singh, M., O'Hagan, D.T. 2003. Recent advances in veterinary vaccine adjuvants. *Australian Society for Parasitology Inc.* doi:10.1016/S0020-7519(03)00053-5
9. Sprouse, R.F., Garner, H.E., Lager, K. 1990. Cross-protection of calves from *E.coli* and *P. multocida* endotoxin challenges via *S. tryphimurium* mutant bacterin-toxoid. *Agri-Practice*. Volume 11, Issue 2, Page 29-34.
10. Yancey, R.J. Jr. 1993. Recent advances in bovine vaccine technology. *J. Dairy Sci.* 76:2418-2436