3-31-2022

Monitoring the Immune Status of Calves at the Agricultural Education Center

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J. N. Andrews Honors Program

Andrews University

HONS 497

Honors Thesis

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03/31/2022

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Abstract

Each year at the Andrews University Agriculture Education Center calves born to the resident cows and calves that are purchased are raised by the students in the animal science program. In each of the previous two years that calves have been raised, morbidities and mortalities have been observed. In order to prevent any calves from dying and reduce the rate of illness this research project was designed to monitor the calves' immune status. By doing weekly blood draws, the total serum protein levels were determined and used as a standard for their health. Body temperature, food and water intake, and activity levels could be used as health indicators.

Introduction

Unlike humans, animals do not receive immunoglobulins (Ig's) transplacentally *in utero:* they receive them in the colostrum, the first milk. The calf receives these Ig's has whole molecules during a 24-hour window after birth when the small intestine is able to absorb them. The transfer of Ig's in the colostrum to the calf is termed "passive transfer." If the calf does not receive the colostrum in the first 24 hrs after birth the Ig concentration in the blood is too low and does not provide protection from pathogens. Ideally the colostrum should be fed in the first 6 hours after birth. (Figure 1).

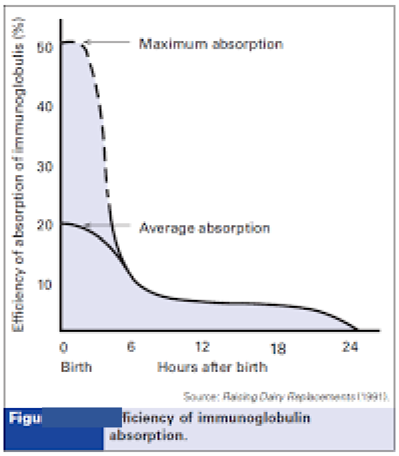


Figure 1 The efficiency of immunoglobulin absorption by the neonatal calf. *(https://www.extension.iastate.edu/dairyteam)*

At about 2 weeks of age, the calves' active immunity will start developing and the passive immunity (PI) will begin weakening. (Figure 2). Passive immunity is crucial because if they do not receive PI they will not have any immunity until their active immunity develops. Active immunity is the natural immunity developed over time due to exposure to different pathogens.

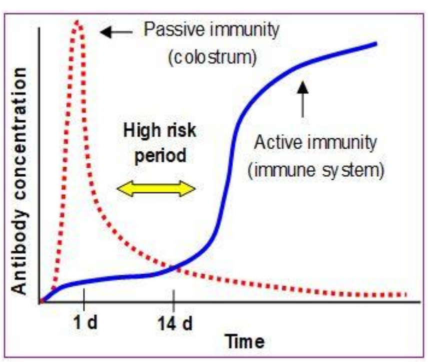


Figure 2: The transition from passive immunity to the development of active immunity in calves. *(https://www.alltech.com/blog/calving-considerations-3-tips-early-nutrition)*

The calves in this study will be housed at the A.U. Agriculture Education Center (AEC). They will be composed of two populations: 1) the calves born to resident cows and 2) the purchased calves. Those calves which are born at AEC are expected to show higher levels of serum immunoglobulins since they will have received their colostrum in a timely manner and have not experienced the stressors that the purchased calves will have experienced. The purchased calves will come from a large dairy farm about 1.5 hours from the AEC. The stressors that these calves will experience include 1) possibly not receiving colostrum within the first 6 hours after birth, 2) traveling via trailer, 3) a new environment (people, animals, surroundings), and 4) possible exposure to different pathogens/diseases. The stress experienced by the calves will weaken their immune systems increasing morbidities (Hulbert and Moisa).

The goal of this research project is to observe the relationship of serum protein (STP) levels in the blood of the young calves, both resident and purchased, at the Agriculture Education Center (AEC) and their health status during the 8-week period they are fed milk.

Methodology

Each fall semester calves from two different sources are raised by the Animal Science students at the A.U. Agriculture Education Center: those born to the resident cows and those born at another farm and brought here. The calves born at the AEC will be weighed and monitored from the time they are born. For these calves, the blood will be drawn within the first week following parturition (birth). For the purchased calves, the blood will be drawn from the time they arrive. After the initial blood draw, the blood will be taken weekly for 8 weeks to determine serum total protein (STP) levels. Bovine STP levels are based on a successful passive transfer level of 5.2 g/dL (Hernandez et. al) the sample type is serum, and the detection method is done using a refractometer. Data collected from the calves will include weekly blood for STP level analysis, daily rectal temperatures, daily milk and grain intake, weekly body weights, and any incidence of disease such as diarrhea or pneumonia. This data will be analyzed to determine any correlation of health status/morbidity and serum immunoglobulin levels in the calves. All other health indicators will be recorded when the calves are cared for twice daily.

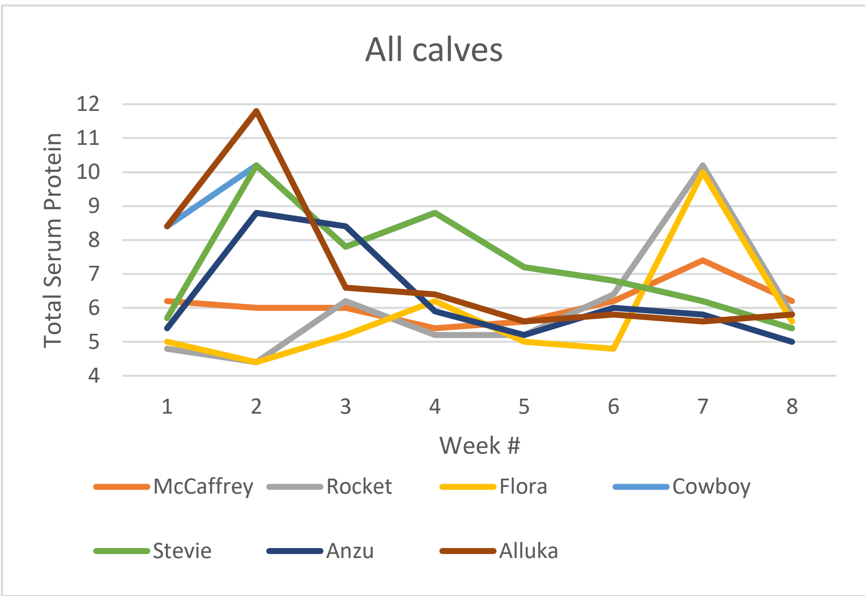
By correlating the STP levels with health indicators, it will be possible to determine if there is a pattern to when the calves are getting sick. The expectation is that there will a higher rate of sickness at about two weeks of age. If there is a pattern to calf morbidity, further research can be conducted to ascertain the cause or causes. Thus, when combined with the other indicators of calf health, I will be able to diagnose morbidity with a high level of certainty.

Blood will be drawn weekly for 8 weeks with two additional blood draws taking place when the calves reach two weeks of age. A blood draw will be taken on the day of two weeks of age, if possible, and two days on either side of two-week marker. The blood will then be spun down using a centrifuge and the total serum protein will be used as an indicator for antibodies in the blood. Greater than 5.2 g/dL (Hernandez et. al) is considered successful passive transfer.

Results

Based on the STP levels seen in each of the calves over the period of 8 weeks, the native calves were healthier. Both calves born at the AEC had higher STP levels throughout the 8 weeks with Cowboy's levels being significantly higher. All seven calves saw an increase in their STP levels at the same time on October 31. Each of the calves STP levels had dropped back down to their normal levels by the next week. Because the calves were born or purchased at the same time, the October 31 peak shows up at different weeks in the calves' growth. This can be seen in Figure 1.

Fig. 1



When the two groups of calves are compared a similar trend can be seen. The October 31st data is outlier and does not follow the trends. Without that spike in STP levels each of the calves saw high STP levels at birth and then a slow decrease through 8 weeks of age. This is as expected. The calves should have high levels of immunity from the passive immunity received through the colostrum which would decrease until their active immunity develops at about 2 weeks of age. From 2 weeks of age the calves should see steady or small fluctuations in their STP levels dependent upon their health.

The two native calves saw STP levels between 5.8 and 8.0 g/dL. This was a much smaller range compared with the purchased calves. This is likely due to them being healthier and receiving consistent, quality care from birth. As seen in figure 2, both figures native calves saw some degree of change in their STP levels. Both calves saw an unusual spike in their STP on

October 31st. Because the calves were born at different times the spike appears at 2 weeks of age for Cowboy and at 7 weeks of age for McCaffrey. There was no clear evidence for what caused the spike but was assumed to be an error in the samples or measurement process due to all the samples in that week being affected. Comparing figure 2 with figure 3 shows the significant changes in STP levels in the purchased calves that is not present in the native calves. The native calves showed much more consistency in STP over the period of 8 weeks.

Fig. 2

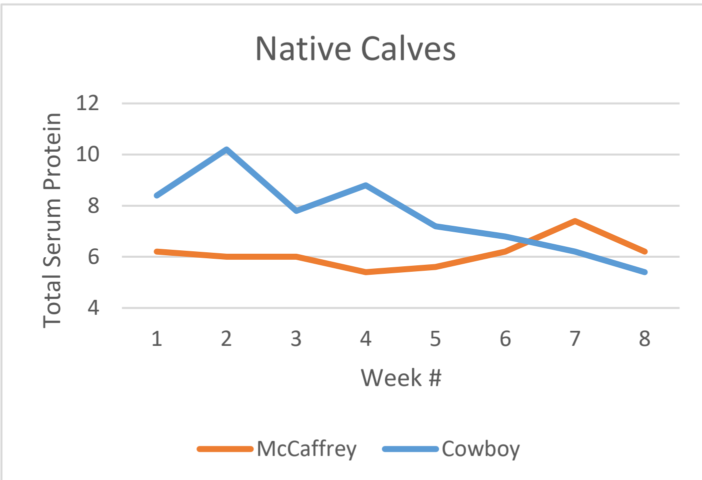
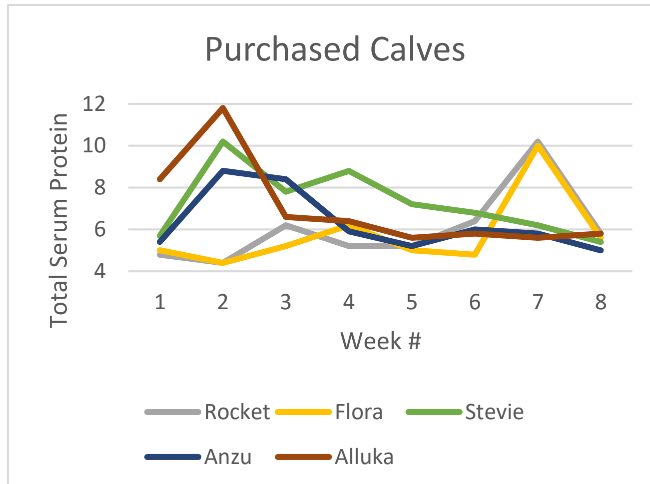


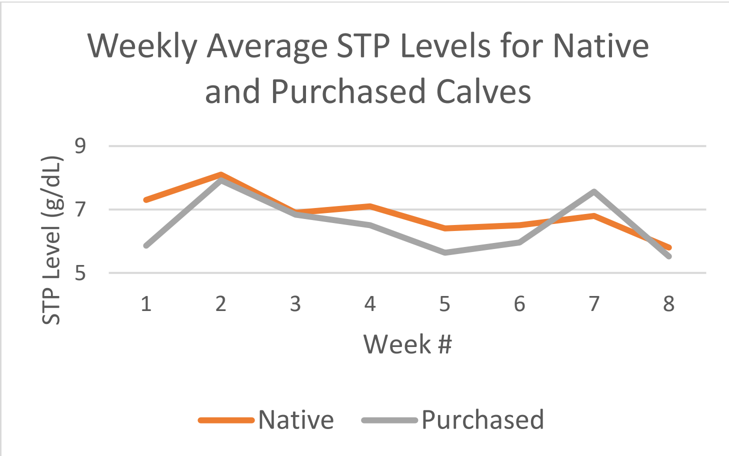
Fig. 3



When looking at the two groups of calves, those born in September and those born in October, similar trends are seen. In the September calves, McCaffrey, the native calf, saw more consistency in the STP level than the two purchased calves. The spike seen in all the September calves' STP levels at week 7, was not as drastic in the native calf as it was in the purchased calves. Although the spike in STP was at 2 weeks of age due to the age difference it is very similar to what is seen with the September calves. All the purchased calves saw very large spikes versus the native calf saw only a minor spike in the STP. When looking at graphs of the average STP of the native and purchased calves, the native calves had higher levels in all but one week, as seen in figure 4. That one week happened to be when one outlier spikes occurred.

One calf out of the seven studied presented morbidities. Looking at the STP levels in this calf as compared with the others did not present obvious reason to suspect that the calf was sick. The serum protein levels were slightly lower than that of the calf that was purchased at the same time, Rocket and Flora respectively. As with the other purchased calf, the STP level spiked on October 31st before dropping back down to similar levels seen before October 31st. However, the STP of Rocket, the sick calf, was on the rise before the STP spiked significantly, indicating that he was already beginning to recover.

Fig. 4



Discussion

The hypothesis of this project is that 1) the serum total protein (STP) levels of the native calves would be consistently higher than those of the purchased calves, and 2) that the STP levels would be positively correlated to the health of the animal could not be supported with this pilot study due to the low numbers of calves involved although the two native calves did average higher STP levels. Follow-up studies next year using the same protocol would be recommended in order to increase the number of calves in this project and to be able to apply statistical analyses to the data. Raising purchased calves is always a challenge due to their unknown immune status and the unavoidable stressors they experience during transportation to a different farm. The more information we can collect from these calves, the better we will be in keeping them healthy.

This project was a pilot study due to the low numbers of calves available for comparison and thus statistical tests could not be conducted. This discussion will be mainly descriptive. All the calves except two, Rocket and Flora, had a STP level of > 5.2 g/dl throughout the 8 weeks of blood sampling which is considered the minimum for successful passive transfer of antibodies from colostrum to the neonatal calf. Both of these were purchased calves and were from the same farm as the other purchased calves whose STP levels were satisfactory. It has been documented that up to 19.2% of all calves have failure of passive transfer even when fed the same colostrum as other calves which has successful transfer. (Beam et. al.) This may have been what happened with these two calves. One of these calves, Rocket, developed a nutritional myopathy, "white-muscle disease," which was not related to his STP during the second week he was here, and he was treated and recovered. The native calves had higher STP levels at week 1 than did all but one of the purchased calves (Alluka). All the calves showed a decrease in STP from weeks 3-6 which would be expected since the passive immunity was diminishing, and their active immunity was just starting to develop.

Only one calf presented a case of morbidity of the seven that were observed. The calf is believed to have contracted white muscle disease. Because of the specific nature of this disease, it did not present a significant change in the STP levels of that calf. Enzootic muscular dystrophy, white muscle disease, is associated with dams that are fed rations with low amounts of α-tocopherol. The milk contains a high proportion of unsaturated fatty acids, thereby increasing the amount of vitamin E required (Blaxter, 2007). Experiments have shown that Se is as effective as is vitamin E in preventing the disease (Sharman, Blaxter & Wilson, 1959; Blaxter, McCallum, Wilson, Sharman & Donald, 1961). The calf believed to have white muscle disease was treated as such and recovered. Overall, the calf that did present morbidities had lower STP levels than some of the calves but had similar levels to that of the purchased calves that arrived at the same time.

Based on the data collected from the blood draws, the STP levels were, on average, higher in the calves born at the AEC as compared with the calves that were purchased. This is expected due to a variety of factors affecting the health of the calves. The STP was also more consistent in the native calves indicating better health. The fluctuations seen in the purchased calves was likely due to health factors causing them to be healthier at some points. Further research can be conducted to determine the significance of the STP levels seen in native and purchased calves as well as to determine possible causes for the variations seen in the STP levels. Continued research would look at more purchased calves to determine if similar trends are seen as in this pilot study. Depending on the results of these studies, other research could be done in an effort to determine possible causes of the health issues and ways to prevent morbidity and mortality in the calves.

Acknowledgements

Thank you to Dr. Koudele, Dr. Holly Shaffer, Dr. April Habenicht, and Dr. Alison Dawson for their advice and support.

Bibliography

Chase, C. C., Hurley, D. J., & Reber, A. J. (2008). Neonatal immune development in the calf and its impact on vaccine response. *The Veterinary clinics of North America. Food animal practice*, *24*(1), 87–104. https://doi.org/10.1016/j.cvfa.2007.11.001

This research article focuses on calves' immunological response as the calves develop and the components of passive immunity. The article discusses the effects of maternal immunity on how calves develop specific immunity and vaccine strategies for developing protection

against pathogens in calves. The research performed in this article follows a similar path I plan to perform in my research. This article states the importance of colostrum in passive immunity, "The ingestion of colostrum is essential for providing neonates with immunologic protection during at least the first 2 to 4 weeks of life." I plan to test the level of immunoglobulins in calves throughout development and the pathogens in their blood. This will allow me to compare the calves' passive immunity and active immunity and the time in between.

Filteau, V., Bouchard, E., Fecteau, G., Dutil, L., & DuTremblay, D. (2003). Health status and risk factors associated with failure of passive transfer of immunity in newborn beef calves in Québec. *The Canadian veterinary journal = La revue veterinaire canadienne*, *44*(11), 907–913.

This study was done to determine risk factors associated with failure of passive transfer immunity (FPT). Physical exams were performed on normal calves and blood samples collected for measurements of serum concentration of immunoglobulin (Ig) G_1. They found that calves born in a stanchion-stall were more likely to shows FPT. They also found the cold-stressed may have a slower rate of intestinal absorption and may also be reluctant to stand and suckle voluntarily but birth month was not associated with FPT. The final consideration mentioned was that of quality calf environment and management practices, and monitoring dams' BCS. This provides further information as to possible causes for calf sickness in Andrews University calves. These are factors that must be considered if some of the calves have FPT.

Hulbert, L. E., & Moisá, S. J. (2016). Stress, immunity, and the management of calves. *Journal of Dairy Science*, *99*(4), 3199–3216. https://doi.org/10.3168/jds.2015-10198

This article describes the study of different types of weaning methods used and how it may impact a calf's oral behavior pre-weaning. Calf stressors are important to understand to reduce morbidity and mortality. This article also studied commingling strategies and nutritional supplements that may help with the transition from individual to group housing. The aim of this article was to optimize calves' health and well-being at the early stages of life to improve their long-term health and welfare. I will use this article as supplemental material in studying stressors and how Andrews University calves are handled in order improve our methods. I will study how our calves develop passive and active immunity and determine stressors in their early environment and how those stressors can be reduced to maintain their health.

Lombard, J., Urie, N., Garry, F., Godden, S., Quigley, J., Earleywine, T., McGuirk, S., Moore, D., Branan, M., Chamorro, M., Smith, G., Shivley, C., Catherman, D., Haines, D., Heinrichs, A. J., James, R., Maas, J., & Sterner, K. (2020). Consensus recommendations on calf- and herd-level passive immunity in dairy calves in the United States. *Journal of Dairy Science, 103*(8), 7611–7624. https://doi-org.ezproxy.andrews.edu/10.3168/jds.2019-17955

This study brought together various academic, extension, and industry specialists in the calf health arena to discuss the evaluation and possible revision of the Transfer of Passive Immunity (TPI) for dairy calves. 4 different options were evaluated with a different number of categories. The option selected separated calves into 4 different risk groups depending on their IgG levels. These groups <10.0 g/L, 10.0 to 14.9 g/L, 15.0 to 19.9 g/L, 20.0 to 24.9 g/L, >25.0 g/L. These were rated as poor, fair, good, and excellent, respectively. This article is important to my research because it will inform me as what how healthy the calves at the AEC are based on their IgG levels. These standards are for dairy calves as that is the area in which the article was focused, however, it does mention the TPI levels for beef calves as well. These are: >24 g/L or <24 g/L. Failure of passive transfer (FPI) has been generally labeled as <10 g/L.

Lora, I., Gottardo, F., Contiero, B., Dall Ava, B., Bonfanti, L., Stefani, A., & Barberio, A. (2018). Association between passive immunity and health status of dairy calves under 30 days of age. *Preventive Veterinary Medicine*, *152*, 12–15. https://doi.org/10.1016/j.prevetmed.2018.01.009

In this study, Italian dairy cattle were observed to evaluate the association between passive immunity and health status within 30 days of age under field conditions. Blood serum samples were taken for the assessment of Ig and fecal samples were taken to determine bacteria present. They found that FPT showed in increased risk of diarrhea and mortality. Low passive immunity levels were also associated with early age onset of disease and potentially the need for antibiotic treatment for recovery. This article serves as a source for my research because it shows the importance of proper passive transfer. From this research, I can know that decreased Ig's in the AEC calves is a significant indicator of morbidity.

Priestley, D., Bittar, J. H., Ibarbia, L., Risco, C. A., & Galvao, K. N. (2013). Effect of feeding maternal colostrum or plasma-derived or colostrum-derived colostrum replacer on passive transfer of immunity, health, and performance of preweaning heifer calves. *Journal of Dairy Science*, *96*(5), 3247–3256. https://doi.org/10.3168/jds.2012-6339

This article studied the effects of different types of colostrum. The forms studied in this trial were maternal colostrum, a plasma-derived colostrum, or colostrum-derived colostrum. At birth calves were assigned different groups and then were tested for things such as serum total protein, serum IgG concentrations, apparent efficiency of absorption, and mortality rates. Given the conditions of the trial, maternal colostrum was found to be superior. This trial can inform my research as to the most effective types of colostrum and what benefits they may have. This can then be applied to Andrews University calves for better health.

Trotz-Williams, L. A., Leslie, K. E., & Peregrine, A. S. (2008). Passive Immunity in Ontario Dairy Calves and Investigation of Its Association with Calf Management Practices. *Journal of Dairy Science*, *91*(10), 3840–3849. https://doi.org/10.3168/jds.2007-0898

This article studies the effects of farm techniques such as calf management and details of colostrum feeding. The numbers of serum total protein in calves were used to compare the effects of the management techniques. The results showed serum TP ranging from 3.5 to 9.8 g/dL. This implied management techniques could affect the passive transfer of maternal immunity. This is important for my research because it shows how external factors can be applied to improve calf and reduce the chance of risk of failure of passive transfer (FPT).

Poulsen, K. P., Foley, A. L., Collins, M. T., & McGuirk, S. M. (2010). Comparison of passive transfer of immunity in neonatal dairy calves fed colostrum or bovine serum-based colostrum replacement and colostrum supplement products. *Journal of the American Veterinary Medical Association*, *237*(8), 949–954. https://doi.org/10.2460/javma.237.8.949

This study was done to test to the effects on the transfer of passive immunity when using bovine serum-based colostrum replacement and colostrum supplement products as compared with natural colostrum. The study included 287 neonatal heifer calves from 8 different farms. The study found that the calves that received natural colostrum had significantly higher levels of IgG and total protein levels as compared with the calves that received the colostrum replacement and colostrum supplement. No difference was detected between calves that received adequate levels of passive transfer of immunity. This article informs my research by describing and articulating the adequate levels of passive transfer of immunity. It also describes the effects of supplemental or replacement colostrum should the AEC ever need to use it.

Todd, C. G., McGee, M., Tiernan, K., Crosson, P., O'Riordan, E., McClure, J., Lorenz, I., & Earley, B. (2018). An observational study on passive immunity in Irish suckler beef and dairy calves: Tests for failure of passive transfer of immunity and associations with health and performance. *Preventive Veterinary Medicine*, *159*, 182–195. https://doi-org.ezproxy.andrews.edu/10.1016/j.prevetmed.2018.07.014

This study was performed to evaluate the tests used to determine failure of passive transfer based on calf health and performance and to describe the epidemiology of morbidity and mortality in suckler beef and dairy calves under Irish conditions. The results showed that calves with lower passive immunity test results are at a higher risk of experiencing a negative health event or poor performance. It also found that passive immunity test results were lower for beef calves than for dairy calves. This further informs my research regarding the results of failure of passive transfer and effect is has on calves. It also shows that beef calves have a higher incidence of failure of passive transfer than dairy calves.

Lee, S.-H., Jaekal, J., Bae, C.-S., Chung, B.-H., Yun, S.-C., Gwak, M.-J., Noh, G.-J. and Lee, D.-H. (2008), Enzyme-Linked Immunosorbent Assay, Single Radial Immunodiffusion, and Indirect Methods for the Detection of Failure of Transfer of Passive Immunity in Dairy Calves. Journal of Veterinary Internal Medicine, 22: 212-218. https://doi.org/10.1111/j.1939-1676.2007.0013.x

The goal of this study was to determine the agreement between the Enzyme-Linked Immunosorbent Assay (ELISA) and single radial immunodiffusion (SRID) and to compare the diagnostic performance of ELISA with indirect methods, in detection of failure of passive transfer. 115 dairy calves were observed from 23 calf-rearing facilities. The agreement between SRID and ELISA was 94%. The supports my research because it shows the accuracy of the ELISA test which I will be using to test for IgG's in the calves' blood.

Swan, H., Godden, S., Bey, R., Wells, S., Fetrow, J., & Chester-Jones, H. (2007). Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. *Journal of dairy science*, *90*(8), 3857–3866. https://doi.org/10.3168/jds.2007-0152

This study was performed because 40% of dairy heifers failure to receive adequate IgG levels. Thus, they determined to look at the difference between plasma-derived colostrum replace and maternal colostrum. To determined the difference they looked at the blood serum total protein levels and IgG concentrations. This supports my research because I will be using serum total protein instead of IgG concentrations and it shows that the tests can be interchangeable. It also shows a high incidence of failure of passive transfer something that is possible in the calves I will be observing.

Hernandez, D., Nydam, D. V., Godden, S. M., Bristol, L. S., Kryzer, A., Ranum, J., & Schaefer, D. (2016). Brix refractometry in serum as a measure of failure of passive transfer compared to measured immunoglobulin G and total protein by refractometry in serum from dairy calves. *The Veterinary Journal*, *211*, 82–87. https://doi.org/10.1016/j.tvjl.2015.11.004

This trial was done to compare different tests and their accuracy as a measure for failure of passive transfer. The study found the refractometer method, measuring serum total protein, to have equal sensitivity (100%) and similar specificity (80.4%) as compared to (89.2%) in other tests. This shows that using serum total protein as a test for failure of passive transfer is effective. This supports my research because I will be using serum total protein as an indicator instead of IgG concentrations due to the much simpler methods. This article shows that the switch can be made without concerns about the accuracy of the results.

Beam, A. L., Lombard, J. E., Kopral, C. A., Garber, L. P., Winter, A. L., Hicks, J. A., & Schlater, J. L. (2009). Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *Journal of Dairy Science*, *92*(8), 3973–3980. https://doi.org/10.3168/jds.2009-2225

This study looks at the prevalence of failure of passive immunity in calves, measure to be 19.2% percent of dairy calves in this study. They also looked at effects of management practices on failure of passive transfer. They found several management practices to increase the odds of failure of passive transfer. This informs my research because the ultimate goal is to determine whether management practices at the Agriculture Education Center can improve calf health. However, this research study will not specifically look at this issue.

Blaxter, K. (1962). Muscular dystrophy in farm animals: Its cause and prevention. *Proceedings of the Nutrition Society*, *21*(2), 211-216. doi:10.1079/PNS19620034

This study looked at muscular dystrophy in different farm animals and the causes and methods of prevention. The study included muscular dystrophy in calves and why they might get it and how to treat it. This informs my research because one of the calves being observed was believed to have muscular dystrophy and was treated as such using information described in this study.