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A Comparison of Methods for On-Farm Determination of Failure of Passive Transfer of Immunoglobulin to Dairy Calves

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A comparison of methods for on-farm determination of failure of passive transfer of immunoglobulin to dairy calves

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ABSTRACT

Two commercially available, qualitative, on-farm test kits (Midland BioProducts Inc., Boone, IA), utilizing either serum or whole blood to evaluate failure of passive transfer (FPT) of immunoglobulins, were evaluated using 38 Holstein calves. Results from the kits were compared with refractometry determination of serum proteins and radial immunodiffusion determinations specific for IgG (RID; Triple J Farms, Bellingham, WA). Blood samples were collected immediately following birth before first colostrum feeding and at 48 h. At birth, serum protein concentrations averaged 4.52 g/dl and IgG averaged 8.6 mg/dl, respectively, for refractometer and RID. Forty eight hours after feeding colostrum, serum protein concentrations averaged 6.02 g/dl and IgG concentrations were 2129.3 mg/dl. Feeding colostrum increased serum protein and IgG concentrations at 48 h ($P < 0.01$). Serum protein concentrations determined by refractometry and serum IgG determined by RID were positively and significantly correlated ($r^2 = 0.78$, $P < 0.01$) and the relationship is characterized as: serum protein, g/dl = 0.0007 mgIg5/dl + 4.5726. Adequate immune transfer was assumed when serum IgG concentrations were greater than 1,000 mg/dl or FPT with IgG less than 1,000 mg/dl.

Using samples of blood from calves collected prior to feeding colostrum, the accuracy of the on-farm plasma kits for adequate passive transfer was 100% (n = 29). The accuracy of the whole blood kits for assessing adequate passive transfer of IgG on samples from newborn calves was 95.5% with 4.5% false positives (n = 22). On blood samples from calves fed colostrum, the whole blood kits presented 4.5% false negative readings and 0% false positives (n = 22). On the colostrum-fed calves, the plasma kit predicted passive transfer with 100% accuracy (n = 30).

Thus, dairy producers can use these qualitative assessment tools to ensure that calves that test adequate for passive transfer do indeed have adequate blood IgG concentrations and avoid FPT in calf rearing systems.

Key words: Calves, Dairy, Immunoglobulin, Passive transfer

Abbreviations: **FPT**, failure of passive transfer; **Ig**, immunoglobulin; **PK**, plasma kit; **RID**, radial immunodiffusion; **WBK**, whole blood kit

INTRODUCTION

According to McVicker (2002), the economic value of passively acquired immunity in bull Holstein calves is $23.04 per calf. One can assume that the value of a healthy immune system in heifer calves would be significantly higher. Thus, the ability to rapidly and accurately assess the immune status of newborn dairy animals is extremely important to the dairy producer. The only way to assess the immune status is to measure the level of circulating antibodies in the animal's bloodstream.

Antibodies are globulins that are synthesized under a directing influence in vertebrate animals. They are responsible for the serological specificity of serum because of their ability to react with antigens in some observable way (Gray, 1970). Antibodies that comprise the majority of the neonate's immune system are called immunoglobulins (**Ig**). Goldsby, et al (2000) define immunoglobulins as an antibody or a heavy or light polypeptide chain that is part of an antibody molecule. There are three major classes of Ig in cattle: IgG_1 and IgG_2, IgA, and IgM.

The IgM molecule is the first to appear in response to immunization. Its presence in the bloodstream is transient and limited in amount, failing to exceed 5-8% of the circulating immunoglobulins. After a short interval in the immune response, IgM is overtaken and replaced by IgG production, possibly by inhibitory antibody feedback to antigen-sensitive IgM lymphocytes. The IgG eventually comprise 75-80% of the circulating antibody (Gray, 1970). The IgG_2 makes up approximately two-thirds of the IgG in serum (Butler, 1973). All species examined produce IgG and IgM, but may vary in their ability to produce the remaining classes (Gray, 1970).

Neonates rely on colostrum for passive immunization, or the acquisition of immunity by receipt of preformed antibodies rather than by active production of antibodies after exposure to an antigen (Goldsby, et al, 2000). "Colostrum is a source of immune components and nutrients to the neonate and contains more protein, immunoglobulins, non-protein nitrogen, fat, as, vitamins and minerals than milk does. Because some vitamins do not cross the placental barrier, colostrum is the primary source of these nutrients for the calf after birth" (Quigley, 1998).

Immediately after birth, neonate mammals possess the ability to absorb these large protein molecules through the intestinal epithelial cells. Eventually these cells will lose their ability to absorb immunoglobulins. This is what is known as closure (Stott, et al, 1979). "Intestinal epithelial cells lose their ability to absorb intact macromolecules after about 24 h because of the maturation of the cells and development of the intracellular digestive apparatus" (Quigley, 1998). "From birth until the maximum concentration of

immunoglobulin in serum is reached is the period of absorption. Following the peak concentration, there is a gradual decline in serum content due to discontinuance of absorption and, presumably, to the reflected catabolism of the immunoglobulin in serum and/or transfer to other metabolic pools, making the peak quite evident" (Stott, et al, 1979).

In a study looking at the immunoglobulin transfer in calves, conducted by Stott, et al (1979), "The age at first colostrum feeding influenced closure as indicated by differences in the mean closure time among the age groups for each immunoglobulin class. The trend appears linear, with closure time earlier with calves fed at 0 h and the period of absorption increasing with each increment of age up to 24 h when the final colostrum feeding was initiated. The data indicate that as feeding of colostrum is delayed, the estimated time for closure is also delayed. However, since the coefficient is less than 1.0, the length of time that the calves absorbed the immunoglobulin decreased as time to the initial feeding was delayed. Hence, in calves fed initially at birth, (0 h), closure occurs at approximately 21 h for IgG, 23 h for IgM, and 23 h for IgA. However, if feeding is delayed until 24 h after birth, then closure occurs at 33, 31 and 32 h for IgG, IgM and IgA, respectively. Thus, the length of time the calf is actually absorbing colostrum is reduced from about 21 h to about 8 h.."

Serum IgG concentrations of less than 10 g/L are termed failure of passive transfer (**FPT**). In a study conducted by the National Animal Health Monitoring System, 40% of all calves sampled between 24 and 48 h had IgG concentrations below the recommended level of 10 g/L and over 25% had less than 6.2 g/L. That study indicated that over half of the deaths of calves with serum IgG concentrations less than 10 g/L were attributed to lack of IgG intake (Quigley, 1998). Things that influence the amount of IgG absorbed are: sex of the calf, age at first feeding, body weight, amount of IgG consumed, and colostrum quality (Quigley, 1998). "Colostrum must have a minimum IgG_1 concentration of 35.2 mg/ml to provide 100 g of IgG_1 in 2.84 L. Similarly, for feedings of 1.89 L or 3.78 L, the minimum IgG_1 concentrations were 52.9 and 26.5 mg/ml, respectively" (Pritchett, et al, 1994).

The development of a healthy immune system is important for neonate dairy heifers to survive to maturity. Knowing this, a method to assess the immune status of heifers on the farm is needed. Midland BioProducts, Boone, IA, developed two on-farm test kits that qualitatively assess whether the animal has FPT or adequate immune transfer. One of these kits uses whole blood and the other uses serum. The objective of this project was to determine the accuracy of these kits for diagnosing FPT in newborn calves. The hypothesis was that producers who use these kits will be able to confidently rely on the results of these kits, thus allowing proper steps to be taken to care for animals with FPT.

MATERIALS AND METHODS

Thirty-eight Holstein and Brown Swiss calves born at the South Dakota State University Dairy Research and Training Facility, Brookings, SD, were used for this trial. Information collected, in regards to the general status of the calves, was calving difficulty score, birth weight, amount of colostrum fed, method of colostrum feeding, and source

of colostrum. Hematocrit readings, to assess the clinical dehydration level of the calves, were also obtained. The kit results were compared to refractometry and a radial immunodiffusion assay (RID) that was donated by Triple J Farms, Bellingham, WA. The RID results were considered the "gold standard" for comparison and that is how final accuracy determinations were made.

Jugular vein blood samples were drawn following birth before first colostrum was fed, and again 48 h later using an 18-gauge needle and 20-ml syringe. For plasma collection, 10 ml of blood were placed in a K_3 EDTA vacutainer and inverted several times to allow mixing of the anti-coagulant materials and blood and then immediately placed in a cooler with ice packs until the sample could be tested immediately at the lab. For serum collection, another 10-ml of the sample were placed in a vacutainer without additives and allowed to clot at room temperature for 30-90 min. After sufficient clotting, serum collections were centrifuged at 1200 x g for 10 min. The serum was then frozen at -20°C until analyses could be performed. Prior to testing with the Midland Quick Test Kit Plasma Calf IgG (**PK**), samples were placed in a test tube rack and left at room temperature for approximately 30 min. After completion of the test kits, the samples were refrozen.

The Midland Quick Test Kit Whole Blood Calf IgG test (**WBK**) was performed upon arrival at the lab. This test used the whole blood from the K_3 EDTA sample. Upon completion of the test kit, the blood was then centrifuged at 1200 x g for 25 min. The plasma portion was collected and frozen at -20°C.

Figure 1 shows contents of the kits: a test cartridge, dilution vial and pipettes. The kits are simple to use. All the producer has to do is obtain a blood sample and, depending on whether the kit is for whole blood kit or plasma, the serum is separated. After the serum or whole blood is obtained, a filled pipette of the sample is placed into the dilution vial and the pipette is flushed several times to mix the sample and ensure quantitative

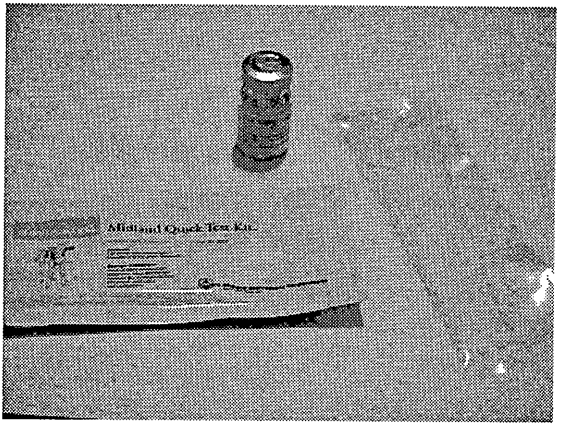

Figure 1. Midland BioProducts Test Kits for evaluation of passive transfer of IgG in calves. Kits are available for whole blood or plasma. Each kit contains a test cartridge, dilution vials and pipettes.

transfer. The same pipette is then used to transfer the diluted sample onto the test cartridge. After waiting at least 20, and not more than 40 min, results can be obtained.

These kits are a qualitative test. They specify adequate or inadequate passive transfer. Inside the cartridge there is a complexing agent specific for IgG molecules. If there are inadequate amounts of IgG in the sample (> 10 mg/ml), the IgG does not complex completely with the complexing agent and reacts with the immobilized "T" and "C" lines. Two lines became visible; however, if there were adequate amounts of IgG (< 10 mg/ml), the IgG complexes with the agent, the complex migrates through the "T" indicator, and only one line is visible. The kits have with a built-in control mechanism. Regardless of adequate transfer or FPT, the "C" line should develop. If it fails to develop, it means the test was erroneous. **Figure 2** shows what the cartridges look like after completion. The top cartridge shows adequate immune transfer and the bottom indicates FPT.

Colostrum was tested for IgG concentrations, with Quick Test Colostrum Kit (Midland BioProducts, Boone, IA), and a colostrometer to insure that calves were receiving adequate amounts of IgG in the colostrum they received. If frozen colostrum was fed, the method and temperature of thawing was recorded, the temperature of the colostrum was obtained before sampling and before testing with the kit, and a colostrometer reading was obtained. To adjust the colostrometer reading to achieve a corrected reading at 20°C, a formula developed by Mechor et al, (1991), of (uncorrected reading - 13.2 + (0.8 temperature (°C)) was used.

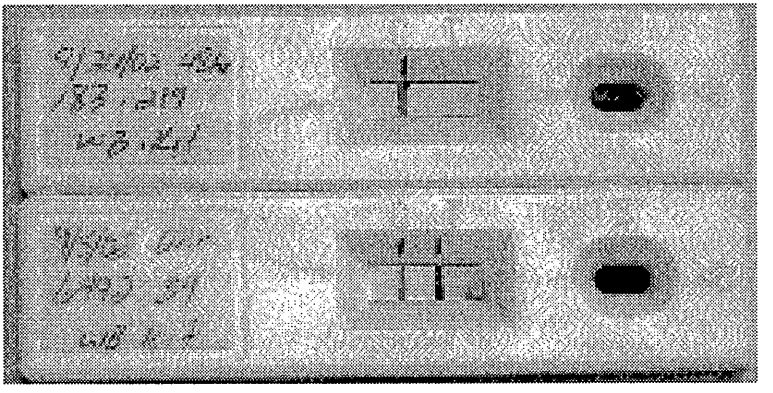

Figure 2. Completed test kits. A single line at "C" (upper strip) signifies IgG concentrations greater than 1,000 mg/dl and adequate immune transfer. Appearance of two lines at "C" and "T" (lower strip) signifies failure of passive transfer.

To determine the accuracy of the whole blood and plasma kits, both kits as well as refractometer readings, were compared with a radial immunodiffusion (**RID**) assay, which is quantitatively specific for IgG. The refractometer (**Figure 3**) measures total serum proteins in g/dl. It assumes that IgG is the largest portion of the total protein. By refractometry, serum protein concentrations of greater than 5 g/dl are considered indicative of adequate immune transfer. To use the refractometer, a droplet of serum is placed on the glass and the slipcover is placed on top. To obtain the serum protein concentration, one must look through the ocular piece and read the appropriate scale. The RID assay (**Figure 4**) worked on the basis of antibodies reacting to a particular antigen. The wells on the plate were each filled to capacity with plasma or serum and were allowed to incubate for 24 h. During that time, the antigen diffused through the agarose gel that had a specific antiserum for IgG. A circle developed until equilibrium between the antigen and antibody was reached. The diameter of the ring was a direct function of the IgG concentration.

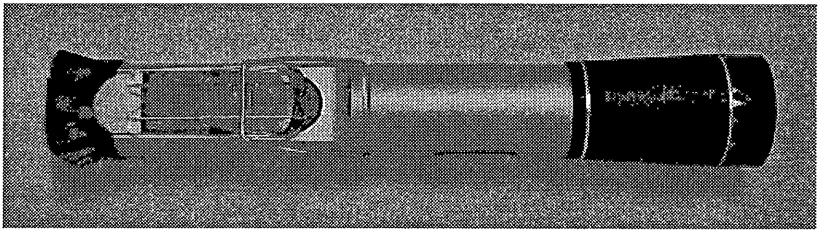

Figure 3. Refractometer. A drop of plasma is placed on the glass and density of the plasma is read on a scale inside the ocular piece. This density is converted into concentration of protein in the sample. Concentrations of less than 5 g/dl of protein indicate failure of passive transfer of IgG.

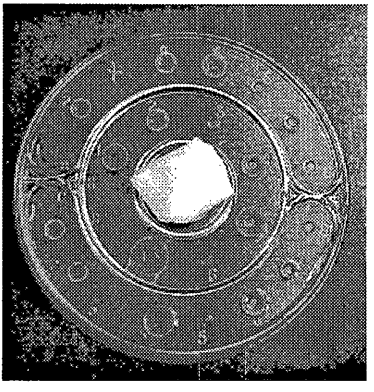

Figure 4. Completed radial immunodiffusion assay (Triple J Farms, Bellingham, WA) for determination of plasma IgG.

RESULTS AND DISCUSSION

Serum protein concentrations, as determined by refractometry, at birth and at 48 h are shown in **Figure 5**. The increase in proteins at 48 h can be attributed to the IgG that were absorbed from colostrum before gut closure occurred.

The IgG concentrations, as determined by RID, are shown in **Figure 6.** At birth, there are virtually no IgG in the calf, which is to be expected since IgG must be obtained through passive transfer from the dam. After colostrum was fed, IgG increased to 2100 mg/dl of serum.

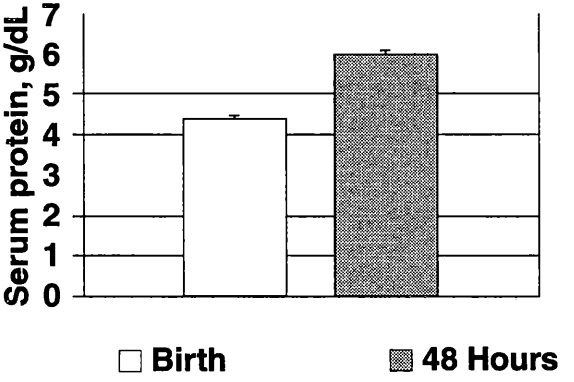

Figure 5. Serum protein concentrations at birth and at 48 h after feeding colostrum to Holstein calves as determined by refractometry. Concentrations of serum proteins less than 5 g/dl indicate failure of passive transfer of IgG.

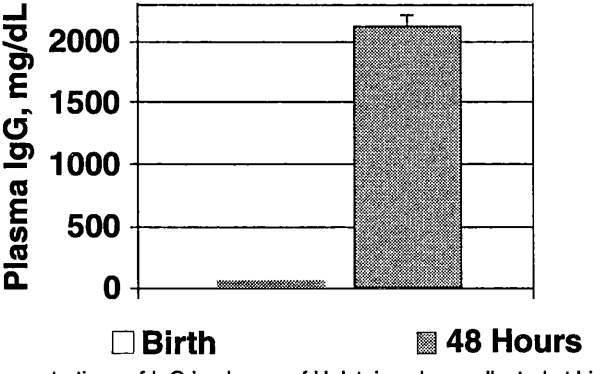

Figure 6. Concentrations of IgG in plasma of Holstein calves collected at birth and at 48 h after feeding colostrum as determined by RID assay. Concentrations of less than 1,000 mg/dl are considered failure of passive transfer of IgG.

Figure 7 shows the relationship between serum protein levels and IgG concentrations as a regression model. This project suggests that there is a moderate linear relationship between total protein and IgG levels in the serum, as $r^2 = 0.7807$. The equation for the slope of the line is $y = 0.0007x + 4.5726$ where y = serum protein in gl/dl and x = IgG in mg/dl.

To test the accuracy of the kits, results from the kit determinations were compared with RID. Additionally, refractometry and RID were compared with each other to test the accuracy of the refractometry. In all comparisons, RID was considered to be the "gold standard." Results were categorized as either false positives or false negatives. False positive was defined as when either the kits or refractometer reported adequate immune transfer and RID reported FPT. False negatives were defined as when either the kits or refractometer suggested FPT and RID reported adequate immune transfer.

For comparing the accuracy of refractometry to RID there were 27 samples obtained at birth. Refractometry yielded one false positive for an accuracy rate of 96.3%. At 48 h of age, there were 31 samples obtained and testing with refractometry resulted in 0 false negatives and 1 false positive for an overall accuracy rate of 96.8%.

Comparison of the WBK to RID with 22 samples collected at birth resulted in one false positive reading. Accuracy was 95.5%. Analysis by PK of the 29 samples collected at birth resulted in no false negatives or false positives, for an accuracy rate of 100%. Comparing samples collected at 48 h after feeding colostrum, 22 of the WBK analysis resulted in one false negative and 0% false positives, for an overall accuracy rate of 95.5%. Of 30 samples collected at 48 h that were tested with PK, there were no false negatives or false positives, a 100% accuracy rate.

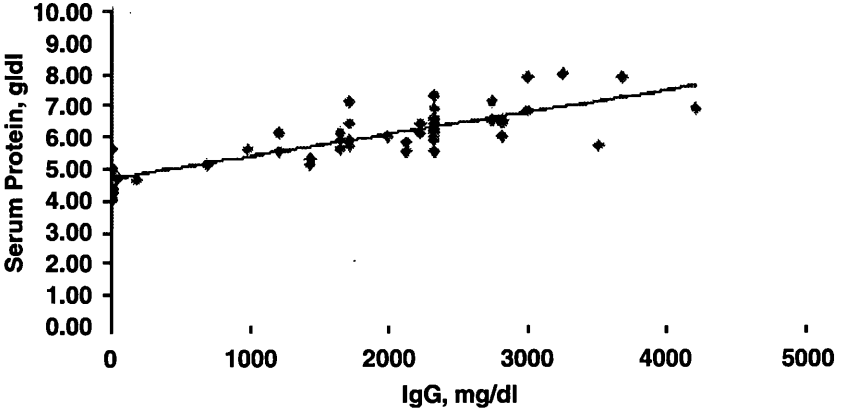

Figure 7. Regression model comparing serum protein levels obtained by refractometry and IgG levels obtained by RID assays. $r^2 = 0.7807$ and slope is $y = 0.0007x + 4.5726$. This suggests that there is a linear relationship between IgG levels and total serum protein levels; however, it also illustrates a less than perfect relationship for reliance upon refractometry for measurement of IgG concnetrations in blood.

CONCLUSIONS AND IMPLICATIONS

The plasma kits tested in this study were 100% accurate, and the whole blood kits were 95.5% accurate when compared with RID, a quantitative measure of IgG. Refractometry was 96.6% accurate when compared with RID. Use of the plasma kits provides producers with a highly accurate tool for assessment of adequate immune transfer in young calve. Use of the whole blood kit provides an assessment tool that is as accurate as refractometry, yet does not require separation of plasma or serum from blood. Based upon these results, both dairy and beef producers can confidently rely on the results of these kits when assessing the immune status of their newborn calves. These new kits provide producers with another tool in their production toolbox and allow them to take the appropriate steps to successfully manage those calves with FPT.

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