Short communication: Validation of methods for practically evaluating failed passive transfer of immunity in calves arriving at a veal facility

D. L. Renaud, T. F. Duffield, S. J. LeBlanc, and D. F. Kelton¹

Department of Population Medicine, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT

Providing a sufficient quantity of high-quality colostrum to male and female calves soon after birth is critical to reduce the risk of disease and mortality. Practical tests have not been validated to determine failed passive transfer of immunity upon arrival at veal facilities. There are many challenges to validation, including the lack of information on the age of the calf and the high prevalence of dehydration. The objective of this study was to validate a semiquantitative IgG antibody test using whole blood and a digital refractometer using serum to determine passive transfer of immunity status. A total of 149 Holstein calves were evaluated upon arrival at a milk-fed veal facility for dehydration status and had blood drawn to evaluate passive transfer of immunity. Serum IgG determined by radial immunodiffusion was used as the gold standard for the validation of the tests, and a cut-off point of <1,000 mg/dL of IgG was used to indicate failed passive transfer of immunity. Serum total protein (STP) was evaluated using a digital refractometer (Misco Palm Abbe no. PA202x, Misco, Solon, OH), and a semiquantitative test (ZAPvet Bovine IgG test, NOWDiagnostics, Toronto, Ontario, Canada) was used on whole blood. A nonparametric receiver operating characteristic curve was generated to compare STP and IgG levels. Sensitivity, specificity, positive predictive values, and negative predictive values were calculated for STP and the semiquantitative IgG test. A total of 31 calves (21%) had serum IgG <1,000 mg/dL. Twelve percent of calves were showing signs of clinical dehydration when assessed upon arrival. The serum total protein (STP) was very well correlated with the concentration of IgG ($R^2 = 0.75$). The STP cut point to determine passive transfer was ≥ 5.1 g/dL, yielding a sensitivity of 84% and a specificity of 90%. The semiquantitative antibody test on

whole blood performed poorly, with a sensitivity of 77% and a specificity of 44%. This study demonstrates that serum total protein is a reliable measure for evaluating passive transfer of immunity and can be used despite a high prevalence of dehydration.

Key words: male calf, serum total protein, failed transfer of passive immunity

Short Communication

Providing a sufficient quantity of high-quality colostrum to newborn calves is an integral component of calf management because failed passive transfer of immunity (FPT) in calves is associated with an increased risk of disease (Postema and Mol, 1984; Godden, 2008; Pardon et al., 2015) and mortality (Renaud et al., 2018). Despite the known importance of feeding colostrum, FPT is estimated to be common among calves entering the veal industry (Wilson et al., 2000; Pardon et al., 2015).

No rapid point-of-care tests have been validated to assess passive transfer of immunity upon arrival to veal facilities, making it difficult for producers to identify high-risk calves upon arrival and avoid source dairy farms that may have poor colostrum management. Radial immunodiffusion (RID) is the gold standard method for determining passive transfer of immunity (Beam et al., 2009); however, blood must be sent to a referral laboratory for this technique to be performed. Serum total protein (STP) determined by refractometry has been shown to be well correlated with immunoglobulin concentrations measured by RID in dairy calves (Naylor and Kronfeld, 1977), but it has not been validated in calves upon arrival at veal facilities, where the age of the calves is unknown and many calves are dehydrated (Renaud et al., 2018). A new semiquantitative antibody test (ZAP test) has been validated using serum from dairy calves; however, it has not been used on whole blood (Elsohaby and Keefe, 2015). Utilizing this test on whole blood may allow for a quicker point-of-care decision and reduce the amount of time required to determine passive transfer of immunity status. The objective of this study was to validate a semiquantitative IgG test (ZAPvet Bovine IgG test, NOWDiagnostics,

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¹Corresponding author: dkelton@uoguelph.ca

Table 1. Description and prevalence of clinical dehydration scores upon arrival to a milk-fed veal facility (n = 140)

Dehydration score	Description	Prevalence, % (n)
0	Skin tent returns to normal in <2 s, eyes bright and alert, strong suckle ($\leq 5\%$ dehydrated)	87.9 (123)
1	Skin tent returns to normal in 2 s, eyes not sunken, good suckle (6–8% dehydrated)	10.7 (15)
2	Good suckle, eyes slightly sunken, skin tent returns to normal in 2–4 s (8–10% dehydrated)	1.4 (2)
3	Mild depression, sternal recumbency, moderately sunken eyes, skin tent returns to normal in 4–8 s, tacky mucous membranes with poor suckle (10–12% dehydrated)	0 (0)
4	Profound depression, absent suckle, lateral recumbency, eyes deeply sunken, skin tent returns to normal in >8–10 s, dry mucous membranes (>12% dehydrated)	0 (0)

Toronto, Ontario, Canada) using whole blood and a digital refractometer (Misco Palm Abbe no. PA202x, Misco, Solon, OH) using serum for determination of FPT.

This study was conducted in cooperation with a milk-fed veal producer and in accordance with the University of Guelph Animal Care Committee requirements (animal use protocol no. 3453). A total of 149 Holstein male calves were sampled in August 2016 upon arrival at a milk-fed veal farm.

For sample size calculations, the prevalence of FPT ($<1,000$ mg of IgG/dL) upon arrival was expected to be 38% (Trotz-Williams et al., 2008), and the sensitivity and specificity of STP as a test to identify calves with FPT were predicted to be 85 and 75%, respectively. Using the method described by Buderer (1996) and assuming that the clinically acceptable width of the 95% confidence intervals for sensitivity and specificity was to be no larger than 10%, the sample size required was determined to be 123 calves.

Calves were evaluated for dehydration by a single assessor (Renaud et al., 2018) using skin tent, attitude, and suckle reflex (Table 1). Calves were considered dehydrated if they had a dehydration score of ≥ 1 . Whole blood was collected by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle into sterile plastic vacuum tubes without anticoagulant and into a sterile 3-mL syringe without anticoagulant. Immediately following collection, whole blood was dispensed according to manufacturer instructions onto the semiquantitative IgG test using a syringe. The test was kept on a flat surface and read by comparing the intensity of the test line against the referent line 15 to 20 min following the application of the whole blood. If the ZAPvet test line was lighter in color than the referent line, the calf was considered to have FPT. However, if the test line was similar or darker in color than the referent line, the calf was considered to have successful passive transfer of immunity (Elsahaby and Keefe, 2015). The vacuum

tube blood samples were allowed to clot and then were centrifuged at $1,500 \times g$ for 15 min at approximately 20°C. Serum was separated and placed on the measuring surface of the digital refractometer to estimate STP. The remainder of the separated serum was stored at -20°C until submission to the Saskatoon Colostrum Company (Saskatoon, SK, Canada) for measurement of IgG by RID as described by Chelack et al. (1993).

All statistical analyses were completed using Stata 14 (StataCorp LLC, College Station, TX). Data were imported from Microsoft Excel (Microsoft Corp., Redmond, WA) into Stata 14 and checked for completeness. Serum IgG determined by RID was used as the gold standard for the validation of the tests, and a cut-off point of $<1,000$ mg/dL of IgG was used to indicate FPT (Godden, 2008). A nonparametric receiver operating characteristic curve was generated to compare STP and IgG levels and to determine the sensitivity and specificity of STP to classify cases of FPT (Dohoo et al., 2009). The cut-off point for STP was selected to weigh sensitivity and specificity equally to limit the effect of both false-positive and false-negative diagnoses (Florkowski, 2008). Sensitivity, specificity, positive predictive value, and negative predictive value were also calculated using the ZAPvet Bovine IgG. To evaluate the correlation between RID and STP, simple linear regression analysis was conducted with STP as the predictor and IgG concentration as the outcome of interest. Using a *t*-test, the STP in dehydrated calves (dehydration score ≥ 1) and the STP in calves that were not dehydrated were compared to evaluate the effect of dehydration on STP.

A total of 31 calves (21%) had serum IgG $<1,000$ mg/dL. The mean ($\pm$ SD) concentration of IgG in the serum samples (n = 149) was 1,989 mg/dL ($\pm 1,057$ mg/dL), ranging from 110 to 5,730 mg/dL. The mean ($\pm$ SD) concentration of STP (n = 149) was 5.6 g/dL (± 0.7 g/dL), ranging from 4.1 to 7.9 g/dL. The concentration of STP was well correlated with the concentra-

Table 2. Test characteristics relative to serum IgG measured by radial immunodiffusion of a semiquantitative IgG test (ZAPvet Bovine IgG test, NOWDiagnostics, Toronto, ON, Canada) using whole blood and by a digital refractometer (Misco Palm Abbe no. PA202x, Misco, Solon, OH) using serum from 149 Holstein calves arriving at a veal facility

Test	Cut point (g/dL)	Sensitivity ¹ (%)	Specificity ² (%)	PPV ³ (%)	NPV ⁴ (%)
ZAPvet	—	77	44	27	87
Digital refractometer	5.1	84	90	68	96

¹The probability of testing positive for failed passive transfer of immunity (FPT) using the ZAPvet test or digital refractometer given that the calf has a level of IgG <1,000 mg/dL as measured by radial immunodiffusion.

²The probability of testing negative for FPT using the ZAPvet test or digital refractometer given that the calf has a level of IgG ≥1,000 mg/dL as measured by radial immunodiffusion.

³Positive predictive value: the probability that the calf has a level of IgG <1,000 mg/dL given that the calf tested positive for FPT according to the ZAPvet test or digital refractometer.

⁴Negative predictive value: the probability that the calf has a level of IgG ≥1,000 mg/dL given that the calf tested negative for FPT according to the ZAPvet test or digital refractometer.

tion of IgG, with STP explaining 75% of the variation in the level of IgG (Figure 1). The receiver operating characteristic curve for these data had an area under the curve of 0.92, which indicates the probability that a randomly selected positive individual has a greater score than a randomly selected negative individual (Dohoo et al., 2009). Assuming that the optimal cut-off point is the point where sensitivity and specificity are at a maximum, the cut-off point for passive transfer of

immunity was determined to be a score of ≥5.1 g/dL, which correctly classified 89% of calves (Dohoo et al., 2009) with an apparent prevalence of FPT of 28%. The test characteristics are summarized in Table 2.

A total of 17 (12%) calves were identified with clinical dehydration upon arrival (Table 1). Despite the number of calves with dehydration, dehydration did not influence STP levels, with dehydrated and nondehydrated calves having a mean ± standard deviation concentra-

Figure 1. Relationship between serum total protein (STP) and IgG measured in 149 calves upon arrival to a veal facility.

tion of 5.6 ± 0.9 and 5.6 ± 0.7 g/dL, respectively ($P = 0.87$). As determined by the ZAP test, the apparent prevalence of FPT was 36% (54/149 calves tested). The test characteristics are summarized in Table 2.

Serum total protein measured by a digital refractometer was found to be a good predictor of the level of IgG despite dehydration being present upon arrival in more than 10% of calves. The semiquantitative test on whole blood was found to not be a reliable method for determining passive transfer of immunity status. A possible limitation of the study was the use of a single threshold derived from dairy replacement calves of <1,000 mg/dL of IgG for determination of FPT status. Renaud et al. (2018) demonstrated that a higher threshold of 1,670 mg/dL of IgG improved the ability to predict mortality. Thus, the traditional threshold of <1,000 mg/dL may need to be re-evaluated on veal calves to determine what the optimal cut point is for predicting both mortality and morbidity.

Providing a simple and easy test for veal producers is necessary to identify source farms that have colostrum management issues. The RID assay for IgG is time consuming and expensive, thus limiting the ability to practically apply it on farm. The STP of calves can be easily measured and is an excellent on-farm tool for herd monitoring (Weaver et al., 2000). It can also be evaluated without the use of a centrifuge, where the serum is simply allowed to clot (Wallace et al., 2006). Hence, it could be used by veal producers as a mechanism to provide feedback to dairy producers regarding colostrum management to facilitate behavior change. A cut-off point of 5.1 g/dL would maximize the sensitivity, thus minimizing the number of false negatives.

It has been speculated that calves suffering from dehydration would have higher STP (Tyler et al., 1996). In our study, clinical dehydration was found in some calves upon arrival but did not influence the level of STP. To better evaluate the true effect of dehydration on STP measured upon arrival, a greater variation in the levels of dehydration and greater sample size are required.

Despite showing efficacy when assessing FPT on serum (Elsohaby and Keefe, 2015), the semiquantitative IgG test used on whole blood was not valuable in identifying veal calves with FPT. The test had moderate sensitivity, but the low specificity would lead to a large number of false positives. Small changes in the intensity of the test line relative to the referent line used indicate FPT and the difficulty in placing the correct volume of blood into the application zone were challenges with the interpretation of the test. Under ideal circumstances, it would have been useful to evaluate this test on both

whole blood and serum; however, the number of tests available for this project was limited.

This study demonstrates that, despite calves arriving with clinical dehydration and unknown age, STP is an accurate method for evaluating FPT as identified using RID. It also demonstrates that the semiquantitative IgG test, which has been shown to be accurate when applied to serum, is not sufficiently accurate in determining passive transfer of immunity status when used on whole blood.

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