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

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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

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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 (≤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 mucus 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)

IgG test (ZAPvet Bovine IgG test, NOWDiagnostics, 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 FTP (<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 FTP 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 (Elsohaby and Keefe, 2015). The vacuum tube blood samples were allowed to clot and then were centrifuged at 1,500 × *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 (±SD) concentration of IgG in the serum samples (n = 149) was 1,989 mg/dL (±1,057 mg/dL), ranging from 110 to 5,730 mg/dL. The mean (±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 concen-