Determination of Passive Immunity in Calves

Louis J. Perino
University of Nebraska - Lincoln

R. James Sutherland
Texas A&M Veterinary Medical Diagnostic Laboratory

Neal E. Woollen
U.S. Meat Animal Research Center
Determination of Passive Immunity in Calves

Louis J. Perino, R. James Sutherland, and Neal E. Woollen

Introduction

Calves passively acquire a significant and vital portion of their immune protection from disease through consumption of the first milk (colostrum). The immunoglobulins (antibodies) that are contained in colostrum will help protect the calf from disease for the first several months of life. This process is called passive immunoglobulin transfer.

Failure of passive immunoglobulin transfer (FPT) is a serious and ongoing problem in calves. Although many factors that contribute to FPT have been examined, it continues to be an obstacle to profitability. Calves that do not receive adequate colostrum are at increased risk of infection from a variety of disease-causing organisms.

Several methods of detecting FPT have been described. Evaluating the status of passive immunity in calves is hindered by deficiencies in the available testing technologies. The most accurate means to assess FPT is determining concentrations of serum immunoglobulin. The predominant type of immunoglobulin transferred from the cow to the calf through colostrum is immunoglobulin G (IgG). Direct measurement of serum concentrations of IgG is usually accomplished using radial immunodiffusion. The value of this test is limited by the high cost involved, the technical expertise required, and the lack of relevance of the test results after the 24 to 48 hr required for the test to run.

Several indirect methods of determination are available. These include zinc sulfate turbidity, sodium sulfite precipitation, glutaraldehyde coagulation, and serum refractometry. These are indirect measurements of the immunoglobulin levels of the calf and therefore are subject to artifact readings due to aberrations in hydration status, total blood protein levels, and other blood attributes. Some of the above tests (zinc sulfate turbidity, sodium sulfite precipitation, and glutaraldehyde coagulation) require the transport of test tubes and reagents to the field. These three tests are semi-quantitative and provide estimates of minimal levels or ranges of serum immunoglobulin levels. Refractometry is simple, quick, and inexpensive, but considered the most inaccurate estimator of immunoglobulin status.

Gamma-glutamyltransferase (gamma-GT) is a membrane-associated enzyme located in multiple sites throughout the body. Gamma-GT is located primarily in cells that have absorptive or secretory functions. Serum level of gamma-GT is recognized as a useful clinical indicator of liver disorders in many species. Activity of gamma-GT in colostrum has been reported to be high in a number of species, including dogs, sheep, cattle, and human beings. In many of these species, serum activity of gamma-GT in neonates that have consumed colostrum is elevated. However, this is not true in all species, with horses being a reported exception.

The purposes of this study were to characterize the activity of serum gamma-GT in newborn calves before and after suckling and to explore the usefulness of serum gamma-GT as an indicator of FPT in calves.

Procedure

Blood samples were collected from the calves of 48 four-breed composite heifers (1/4 Red Poll, 1/4 Hereford, 1/4 Pinzgauer, 1/4 Angus) at the time of birth and at 1 day of age. Serum was harvested from the blood, frozen, and stored for later assay.

At birth, calves received an ear tag, oral rotavirus and coronavirus vaccine, and their navels were treated with iodine. At approximately 60 days of age, and 3 wk before weaning (approximately 5 mo of age), the calves were vaccinated with multivalent clostridial and leptospiral vaccines. A modified-live virus vaccine containing infectious bovine rhinotracheitis and bovine virus diarrhea viruses was also given 3 wk before weaning.

Health status of the calves and cause of morbidity were determined by trained animal caretakers under veterinary supervision. Unusual cases were referred to the veterinary staff for diagnosis.

Serum concentrations of IgG were determined using a commercial radial immunodiffusion kit (VMRD RID Kits, VMRD, Pullman, Washington). The upper and lower limits of detection were 3,300 and 412 mg/dl, respectively. Serum total protein values were assessed with a refractometer. Activity of gamma-GT in serum was measured by automated spectrophotometry using a commercially available kit (gamma-GT reagent 44074, Ciba-Corning Diagnostics Corp, Oberlin, Ohio).

Correlation coefficient, means, percentages, and standard deviations were generated with a commercial microcomputer spreadsheet program (Lotus Development Corp, Cambridge, Massachusetts). Mantel-Haenszel Chi-squares, relative risk, and Kappa values were calculated using a public-domain microcomputer epidemiologic statistic program (USDA Inc, Stone Mountain, Georgia).

Results

Paired serum samples were obtained from 48 calves. Activity of gamma-GT was elevated in calves that sucked colostrum. The degree of elevation was proportional to the amount of colostrum consumed, as indirectly indicated by serum concentrations of IgG. Calves sucking colostrum had 10.0 and 1.3 times greater serum concentrations of IgG and protein, respectively, and a 26 times greater serum activity of gamma-GT, compared to concentrations at birth. At birth the avg serum concentrations of IgG and protein were 131 mg/dl and 3.9 g/dl, respectively, and serum activity of gamma-GT was 28 IU/L. After 24 hr these values had increased to 1,400 mg/dl, 5.0 g/dl, and 734 IU/L, for the same respective parameters.

Calves were classified as having FPT, PFPT, and normal passive transfer, on the basis of concentration of serum...
IgG detected by radial immunodiffusion. Twenty-one percent of calves had FPT (Table 1).

Serum IgG concentrations, serum protein concentrations, and serum activity of gamma-GT were related (Figure 1). The correlation coefficient between IgG and gamma-GT was 0.41. The correlation coefficient between IgG and protein was 0.77.

Significant differences were detected in the morbidity between calves classified as having FPT, PFPT, and normal passive transfer (Table 2). The calves with FPT had a 9.5 times greater risk of becoming classified as sick prior to weaning compared with calves with PFPT and normal passive transfer ($P=0.0004$). The causes of morbidity were variable (Table 2), suggesting a generalized immunodeficiency.

The sensitivity and specificity of a cut-off value of 200 IU gamma-GT/L serum for diagnosing FPT were 80% and 97%, respectively. The sensitivity and specificity of a cut-off value of 4.2 g protein/dl serum for diagnosing FPT were 80% and 100%, respectively. The Kappa values for diagnosis of FPT using serum concentrations of IgG versus serum activity of gamma-GT, IgG versus protein, and gamma-GT versus protein were 0.72, 0.86, and 0.79, respectively.

In summary, serum activity of gamma-GT is elevated in 24 hr old calves that have consumed colostrum; therefore diagnostically valuable of elevations of gamma-GT for hepatic pathology is limited during at least the first wk of life for a calf that has received an adequate amount of colostrum.

The least expensive and most rapid indicator of passive immune status in this study was determination of concentrations of serum total protein, serum activity of gamma-GT, IgG versus protein, and gamma-GT versus protein were 0.72, 0.86, and 0.79, respectively.

Serum activity of gamma-GT also gave reliable indications of concentration of passive immunity but such determinations were more costly and time consuming to determine than those used for serum protein. Serum activity of gamma-GT is not susceptible to changes in other serum analytes and is less susceptible to artifacts caused by dehydration.

Determination of either gamma-GT serum activity or protein serum concentration was less expensive and gave results sooner than radial immunodiffusion for IgG. Determination of both would be useful in determining the success or failure of colostral management in groups of bovine neonates. The value in applying these tests lies in evaluation of groups of calves. Failure of passive transfer is a management problem and the prevalence of subsequent infection depends largely on the success of the colostral management. The role of these tests lies in testing healthy calves in the range of one to seven days of life. A minimum of ten calves should be sampled since the greater the sample size the less sensitive and more specific a test can afford to be.

Once effective methods of identifying calves that have experienced failure of immunoglobulin transfer have been validated, cattle producers can use these methods as management tools. It too many calves are found to have experienced failure of immunoglobulin transfer, producers can alter their management. Individual calves that have experienced failure of immunoglobulin transfer can receive special treatments such as supplementary colostrum and additional vaccinations. Evaluation of the efficacy and cost effectiveness of such interventions are part of the ongoing research in this project.

Table 1—Number of calves and avg serum IgG, gamma-glutamyltransferase (gamma-GT), and total protein (TSP) values at 24 hr after birth for calves classified as failure of passive transfer (FPT), partial failure of passive transfer (PFPT), and normal.

<table>
<thead>
<tr>
<th>Classification</th>
<th>serum IgG levels</th>
<th>FPT &lt;800 mg/dl</th>
<th>PFPT 800-1,600 mg/dl</th>
<th>Normal &gt;1,600 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calves</td>
<td>10</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Avg IgG mg/dl</td>
<td>449.0*</td>
<td>1,272.0</td>
<td>1,990.0</td>
<td></td>
</tr>
<tr>
<td>Avg gamma-GT IU/L</td>
<td>154.0</td>
<td>706.0</td>
<td>1,049.0</td>
<td></td>
</tr>
<tr>
<td>Avg TSP g/dl</td>
<td>4.0</td>
<td>5.0</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>

*Includes eight calves with IgG concentrations below 412 mg/dl for which 411 mg/dl was used to determine the mean.

Table 2—Clinical diagnoses of sick calves classified as failure of passive transfer (FPT), partial failure of passive transfer (PFPT), and normal at 24 hr after birth.

<table>
<thead>
<tr>
<th>Classification</th>
<th>serum IgG levels</th>
<th>FPT &lt;800 mg/dl</th>
<th>PFPT 800-1,600 mg/dl</th>
<th>Normal &gt;1,600 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIAGNOSIS:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Keratoconjunctivitis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Omphalophlebitis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TOTAL SICK</td>
<td>5*</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>TOTAL AT RISK</td>
<td>10</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*Values differ from other values in row ($P<.05$).