Development of an ELISA for Detection of Bovine Pregnancy

Katherine Velek; Shona Michaud; Kate Boucher; Nevena Djuranovic; Lori Plourde; Anna Rice, *PhD*
IDEXX Laboratories Inc., Westbrook, ME 04092-2040

Introduction

Accurate and timely detection of pregnancy in dairy cows is an essential component of today's reproductive management programs. Veterinarians and farmers use early detection of non-pregnant (open) cows to enable faster re-breeding and shorten the calving interval, thereby maximizing milk production and revenue for the farm. The cost for each additional day that a cow is open is estimated to be up to $5 per day. IDEXX Laboratories, Inc. is developing an ELISA for the accurate detection of pregnancy as early as 28 days post-breeding, which provides veterinarians and dairy farmers with another tool for the early identification of open cows.

Materials and Methods

The IDEXX bovine pregnancy ELISA detects the presence of early pregnancy-associated glycoproteins (PAGs) in bovine serum or plasma as a marker for determination of pregnancy in cows. The assay uses an anti-PAG antibody coated onto the solid phase to bind PAGs that may be present in the sample. A second anti-PAG antibody, coupled with biotin is used as the detection reagent along with streptavidin-horseradish peroxidase (SA-HRP). TMB substrate is used as a colorimetric indicator for PAG containing samples, and the enzymatic reaction is stopped with an acid stop solution. After reading the plate at 450nm, wells with color development above the assay threshold are considered positive, indicating a pregnant animal, while wells with little or no color development indicate non-pregnant animals.

Preliminary evaluations of the bovine pregnancy ELISA have been conducted with serum and plasma samples obtained from approximately 50 bull bred Holstein heifers. Blood samples were taken every three days post-breeding for the first 45 days of pregnancy and then every two weeks until calving. After calving, samples were taken from the cows once weekly for 10 weeks. Transrectal ultrasound was performed between days 27 and 35 post-breeding to confirm pregnant status, and once weekly thereafter through 60 days of pregnancy. Fifteen control animals were not bred, and were bled every three days for 30 days.

Samples were tested on the IDEXX bovine pregnancy ELISA to determine the accuracy of pregnancy detection between days 25 and 30 post-breeding as well as the specificity of results obtained after a cow has delivered a calf.

Results

Specificity results were 100%; none of the open cows were considered pregnant by the ELISA. Samples from confirmed pregnant cows were considered positive by ELISA at least 95% of the time by day 27 after breeding. After 30 days post-breeding, 100% of the cows determined to be pregnant by ultrasound indicated positive results on the ELISA. Samples tested after calving suggest that the results of the ELISA return to baseline levels 30-60 days postpartum.

Significance

Preliminary evaluations of the IDEXX bovine pregnancy ELISA indicate that the test could be a useful adjunct to existing reproductive management programs. It offers a reliable method to distinguish between pregnant and open animals 27-30 days after breeding, and throughout the course of pregnancy. Veterinarians could use the test during regular herd checks to evaluate individual animals when it is too soon after breeding to palpate accurately. In addition, veterinarians may also recommend the test for use when farmers do not have regular access to a veterinarian or an experienced palpator. As with any diagnostic test, the IDEXX bovine pregnancy ELISA should be used under the guidance of a veterinarian as part of the farm's overall health and reproductive management program.