Evaluation of Leptospiral Proteins in the Serodiagnosis of Equine Leptospirosis
Brittney Beigel and Ashutosh Verma

1 College of Veterinary Medicine, Lincoln Memorial University, Harrogate, TN 37752
2 School of Mathematics and Science, Lincoln Memorial University, Harrogate, TN 37752

ABSTRACT
In this study, we evaluated usefulness of several leptospiral proteins in serodiagnosis of leptospirosis. Leptospiral proteins Lcn, Thermolysin, and Qlp19 are infection-associated immunogenic proteins that are expressed early in the infection and are conserved among pathogenic species. These features make these proteins attractive candidates for use in the diagnosis of leptospirosis. Indirect ELISA’s, using recombinant Lcn, Thermolysin, or Qlp19 as the antigen were standardized via checkerboard titration and used for the screening of sera from healthy and infected horses for the presence of anti-leptospiral IgG antibody isotypes. Preliminary results demonstrate high levels of protein-specific antibodies in MAT-positive sera, but not in MAT-negative healthy controls. Further studies are underway to evaluate correlation between the recombinant protein-based ELISA and MAT.

INTRODUCTION
Leptospirosis, caused by pathogenic Leptospira spp., is a zoonotic disease that affects a wide range of animal species, including horses. Equine leptospirosis is commonly associated with spontaneous abortion in mares and equine recurrent uveitis (ERU). Microscopic agglutination test (MAT) is the current gold standard in the serodiagnosis of leptospirosis, but it is labor-intensive, requires maintenance of a battery of live cultures, and is subjective in interpretation.

METHODS
ELISA: Microtiter plates were coated with recombinant protein in bicarbonate buffer (pH 9.6). The next day, after washing, plates were blocked with 5% dry milk. Diluted serum samples were added to each well, incubated, and then washed to remove unbound material. Bound IgG was detected using horseradish peroxidase-conjugated Protein G. The cutoff value was defined as the mean absorbance value for negative controls plus two standard deviations.

MAT: Leptospiral serovars Pomona and GrippypHosa were used to perform MAT on the equine sera as described by Cole et al. Equal volumes of diluted sera were mixed with live leptospiral cultures, incubated at 30°C for 2 h, and examined under a dark-field microscope.

RESULTS
Figures 1 and 2: Recombinant protein serum antibody levels in horses as determined by ELISA. The cutoff value is the mean + 2SD of the absorbance values of healthy controls.

DISCUSSION/FUTURE DIRECTIONS
Preliminary results demonstrate high levels of Thermolysin and Qlp19 protein-specific antibodies in MAT-positive sera, but not in MAT-negative healthy controls. The screening of naturally infected and vaccinated sera for anti-leptospiral antibodies against LenC is ongoing. Further studies are underway to evaluate correlation between the recombinant protein-based ELISA and MAT. These preliminary results and analysis demonstrate the abilities of these proteins to serve as a serodiagnostic markers in the detection of leptospirosis in equines. Further assessments of these proteins will include screening for the presence of an anti-leptospiral IgM antibody isotype.

ACKNOWLEDGEMENTS
I would like to thank Dr. Verma and Joey Morgan for their continued support in the laboratory.
I would also like to acknowledge Lincoln Memorial University-College of Veterinary Medicine for providing financial support to complete this project this summer.