IDENTIFICATION OF 35-50kD PROTEINS OF STREPTOCOCCUS SUIS, SEROTYPE 2, BY SERA OBTAINED FROM VACCINATED PIGS THAT SURVIVED VIRULENT CHALLENGE

B.C. Lin[1], A. Hogg[1], and K. Tarasiuk[2]

[1] MVP Laboratories, Inc., Ralston, NE, USA; [2]National Veterinary Research Institute, Pulawy, Poland

Introduction

Streptococcus suis has become a worldwide cause of a variety of pig infections, including meningitis, bronchopneumonia, arthritis, pericarditis, endocarditis, polyserositis, septicemia, rhinitis and abortion (1). Attempts to control this disease with vaccines have been made by several researchers (2,3,5). However, a lack of knowledge about specific imunogenic components of S. suis makes it very difficult to evaluate the immune response and the level of protection induced in pigs after vaccination. An author had suggested cell wall proteins as one of the major imunogens (4). The aim of this study was to identify the special bacterial fractions that can only be detected by sera obtained from vaccinated pigs that survived virulent challenge.

Materials and Methods

Test Bacterin: Streptococcus Suis Bacterin, MVP Serial LS 101. Challenge Strain: S. suis, serotype 2 (MVP Reference Strain 7837). Test Animals: Thirty SPF pigs known to be free of S. suis infection were randomly selected from four litters at 10 to 12 days of age and allocated to two treatment groups, 20 vaccinates and 10 controls. Vaccination: One ml administered intramuscularly (IM) into the neck at 10 to 12 days of age, followed by a 2ml dose IM at 3 to 4 weeks of age. Challenge: All test pigs, vaccinates and controls were challenged 16 days following the second vaccination with a 1.0 ml intravenous dose containing 10^7 CFU of S. suis, serotype 2. Pig Sera: Serum samples were taken prior to each vaccination and prior to challenge. Serum samples were also taken at 10 days after challenge or just prior to death by euthanasia. SDS-PAGE and western blotting: A 20ml culture of S. suis, serotype 2 was centrifuged at 1000 G for 10 minutes and the pellet was resuspended in 1ml of cold lysis buffer (0.1M Tris pH 8.0, 0.8mg EDTA, 1mg lysozme). About 20 ul of the lysed cell suspension was loaded in each well of a minigel for electrophoresis. After electrophoresis and eletroblotting, blots were probed with pig serum.

Results and Discussion

The vaccinates were protected from a virulent challenge with 1 x 10^7 CFU of S. suis, serotype 2, while the controls showed clinical signs and necropsy lesions. Western blot analysis identified the special bands between 35 and 50 kD that were recongnized by all sera from pigs immunized twice and also by sera from pigs surviving virulent challenge. These special bands were never recognized by sera from pre-vaccinates and negative controls (Fig. 1). A band of 59 kD was frequently recognized by most of the pig sera including those pre-vaccinates, vaccinates, and survivors after challenge. These data suggest that these special 35-50 kD proteins of S. suis, serotype 2, may play a role in stimulating protective antibodies in pigs. The frequent recognition of 59 kD band by most of the pig sera may indicate the exposure of pigs to a common antigen shared by many Streptococcus species or a cross-reacting antigen. This also indicates the origin of difficulties encountered in serological study of S. suis by using a whole-bacterium preparation in ELISA.

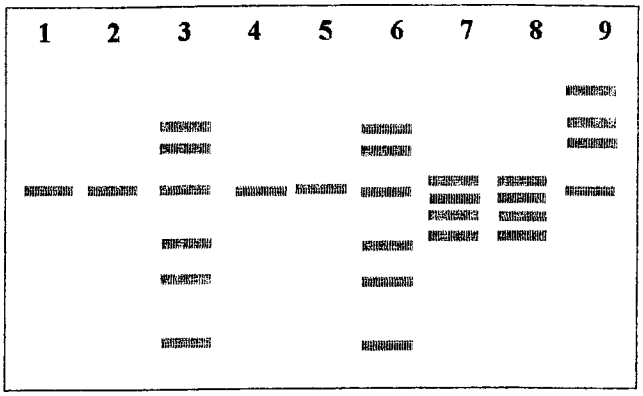

Fig. 1 Western blot of lysed S. suis, serotype 2, probed with negative pig serum (lane 1,2), pre-vaccinated pig serum (lane 4,5), and vaccinated pig serum (lane 7,8). Lane 3 and 6 are low range molecular weight marker (106kD, 80kD, 50kD, 35kD, 28kD, 20kD). Lane 9 is high range marker (205kD, 117kD, 80kD, 50kD).

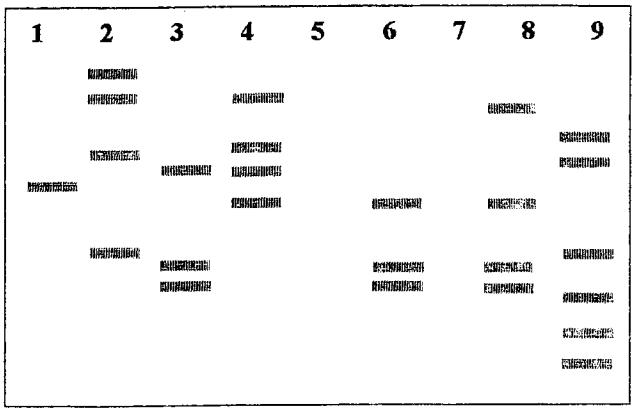

Fig 2 Western blot of lysed S. suis, serotype 2, probed with serum from unvaccinated control pig (lane 1), post challenge control pig (lane 3), pre-vaccinated pig (lane 4), two times vaccinated pig (lane 6), and vaccinated pigs after challenge (lane 8). Lane 2 is high range marker and lane 9 is low range marker.

References

1. Higgins, R., Gottschalk, M.J. (1990) Vet. Diagn. Invest. 2:249-252.
2. Holt, M.E., Enright, M.R., Alexander, T.J.L. (1988) Res. in Vet. Sci. 45:349-352.
3. Holt, M.E., Enright, M.R., Alexander, T.J.L. (1990) Res. in Vet. Sci. 48:23-27.
4. Holt, M.E., Enright, M.R. Alexander, T.J.L. (1990) J. Comp. Pathol. 103:85-94.
5. Ripley, P.H. (1983) IPVS 10:24-39.