# IMMUNOGENICITY STUDIES OF E. COLI BACTERIN FOR THE PREVENTION OF BABY PIG SCOURS

H.T. TUNG*
BEECHAM LABORATORIES
WHITE HALL, IL 62092 USA

Enteric colibacillosis caused by enterotoxingenic E. coli (ETEC) is characterized by diarrhea, dehydration and its related symptoms, and death. It has been well-established that ETEC are pathogenic because of two properties: adhesiveness and enterotoxingenicity. The adhesive ability resides in the minute hairlike structure, pili, that are protein in nature and are present on the surface of the bacterium. The pili enable ETEC to adhere and colonize the intestinal wall and thus establish infection. It has been well-established that most of the ETEC isolated from pigs contain one of K-88, K-99 or 987P antigens. The lack of antigenic relatedness among K-88, K-99 and 987P pilus antigens has been reported. The use of pili as vaccines to prevent ETEC-induced diarrheal disease has been practiced with good results. However, many bacterins are based on K-88 antigens, which only confer protection in approximately half the outbreak. A greater coverage of protection can be conferred from a bacterin containing all 3 pili antigens, but availability is limited. In this report, we described the efficacy evaluation of a multivalent E. coli bacterin in the prevention of baby pig scours caused by the ETEC.

An inactivated whole cell E. coli bacterin containing K-88, K-99 and 987P pilus antigens was produced, qualitatively and quantitatively analyzed for respective antigen content by a slide agglutination test, and adjuvanted with aluminum hydroxide gel. The bacterin was administered twice to 30 pregnant sows (15 IM and 15 SQ) at approximately 4 and 2 weeks prior to farrowing with 30 pregnant sows held as non-vaccinated controls. The vaccinated and the non-vaccinated pregnant sows were divided into 3 groups according to Table I. After farrowing, the piglets from respective sows were challenged according to the designated challenge serotype. All pigs were bled pre-vaccination, 2 weeks post-first vaccination and pre-challenge for micro-SN testing.

TABLE I: Experimental Design

| Vaccination Route | K-88 | K-99 | 987P |
|---|---|---|---|
| SQ | 5 | 5 | 5 |
| IM | 5 | 5 | 5 |
| Controls | 10 | 10 | 10 |

All piglets were challenged with designated virulent serotype E. coli at birth by oral inoculation at approximately $10^{11}$ organisms per dose. After challenge, the piglets were allowed to nurse their mother freely. Scour and death of each individual were scored according to Table II.

TABLE II: Scoring System for Diarrhea and Death

| Score | Scour or Death |
|---|---|
| 0 | None |
| 1 | Solid to slight diarrhea |
| 2 | Mild diarrhea |
| 3 | Runny, white diarrhea |
| 4 | Profused watery diarrhea |
| 5 | Death |

Piglets having a score of 2 for two or more consecutive days or piglets scoring >3 for one or more days in scours were considered as positive reactors to the challenge.

The challenge results based on both scour and mortality are summarized in Table III.

TABLE III: Challenge Results Based on Both Scours and Mortalities in the Baby Pigs From Vaccinated and Non-vaccinated Sows

| Treatment | K-88 | %P * | K-99 | %P | 987P | %P |
|---|---|---|---|---|---|---|
| SQ | 2/34 | 94.1 | 1/38 | 97.4 | 7/52 | 86.5 |
| IM | 0/41 | 100.0 | 5/40 | 87.5 | 3/41 | 92.7 |
| SQ + IM** | 2/75 | 97.3 | 6/78 | 92.3 | 10/93 | 89.2 |
| Control | 59/82 | 28.0 | 82/84 | 2.4 | 87/87 | 0.0 |

*%P signifies % protection.
**Overall analysis for all vaccinates.

The challenge results based on mortality alone are summarized in Table IV.

TABLE IV: Challenge Results Based on Mortality in the Baby Pigs From Vaccinated and Non-vaccinated Sows

| Treatment | K-88 | %M | K-99 | %M | 987P | %M |
|---|---|---|---|---|---|---|
| SQ | 2/34 | 5.9 | 1/38 | 2.6 | 7/52 | 13.5 |
| IM | 0/41 | 0.0 | 5/40 | 12.5 | 3/41 | 7.3 |
| SQ + IM** | 2/75 | 2.7 | 6/78 | 7.7 | 10/93 | 10.8 |
| Control | 47/82 | 57.3 | 56/84 | 66.7 | 83/87 | 95.4 |

*%M signifies % mortality
**Overall analysis for all vaccinates

The results of this study indicate that the multivalent E. coli bacterin consisting of K-88, K-99 and 987P pilus antigens is efficacious in the prevention of baby pig scours caused by ETEC. Based on both scours and mortalities after challenge as shown in Table III, the vaccinates demonstrated 97.3% protection against K-88 challenge, 92.3% protection against K-99 challenge and 89.2% protection against 987P challenge, whereas the controls had 72%, 97.6% and 100% morbidity respectively. In general, the IM route appears to be a better route of administration than the SQ route, although the latter is also satisfactory in inducing sufficient protection.

At the time of this write-up, serological testing is still in progress. We anticipate reporting the serological data at IPVS Congress, 1982.