

PATHOGENICITY

***ENTEROBACTER SAKAZAKII* INFECTION IN NEONATAL MICE**

(A. N. Richardson, S. Lambert, and M. A. Smith)

Enterobacter sakazakii has been associated with nosocomial infections in premature and very low birth weight human infants. The affected infants were exposed to *E. sakazakii* when fed with contaminated reconstituted powdered infant formula. In this study, experimental CD-1 suckling mice were challenged orally with reconstituted powdered infant formula inoculated with 10^9 and 10^{11} CFU *E. sakazakii* strain MNW2 on postnatal day 3. Deaths occurring immediately or less than 15 hours post-treatment were suspected to result from gavaging technique and were not included in the analysis. Twenty deaths occurred at least 15 hours post-treatment and were assumed to result from *E. sakazakii* infection. The remaining mice were euthanized and brains, ceca, and livers were excised and pooled in groups within each litter for culturing. *E. sakazakii* was isolated from brain, liver, and cecum tissues in animals treated with 10^{11} CFU as compared to brain and liver tissues in neonates administered 10^9 CFU. *E. sakazakii* was not found to be present in control tissues. One out of three litters at 10^9 CFU had neonatal deaths associated with *E. sakazakii* treatment whereas all litters (4/4) treated with 10^{11} CFU had at least three neonatal deaths. There was 17.8% lethality among pups administered 10^9 CFU and 34.8% lethality among pups given 10^{11} CFU as compared to 0.0% lethality among control pups. *E. sakazakii* infection in neonatal mice may be similar to that in premature human neonates because of their underdeveloped CNS at full-term birth. Thus neonatal mice may potentially serve as a model for *E. sakazakii* infection in premature and very low birth weight human infants.

MECHANISMS OF *LISTERIA MONOCYTOGENES* INDUCED-STILLBIRTHS IN PREGNANT GUINEA PIGS

(E. A. Irvin, D. Williams, S. Lambert, A. Richardson, and M. A. Smith)

Listeria monocytogenes has been identified as the causative agent in spontaneous abortions and stillbirths in humans after consumption of contaminated foods. Approximately 2500 cases occur each year in the U.S., and pregnant women are 20 times more likely to develop listeriosis than the general population. Previous studies indicate that pregnant guinea pigs are an appropriate model for human listeriosis. We hypothesize that pregnant guinea pigs orally exposed to *L. monocytogenes* have increased apoptosis and changes in cytokines in the placenta and that these are correlated with placental infection and stillbirth. Pregnant guinea pigs were treated orally on gestation day (gd) 35 with 10^5 , 10^6 , and 10^7 colony forming units (CFU) *L. monocytogenes* in whipping cream. Pregnancies were allowed to proceed normally until sacrifice on gd 56. Tissues were collected from mothers and fetuses for cytokine and apoptosis analyses. Tumor Growth Factor- β 1 (TGF- β 1) was analyzed in maternal serum using a human TGF- β 1 ELISA kit from R & D Systems. The presence of apoptosis in placentas was analyzed by PCR with an apoptosis-specific kit (PCR Kit For DNA Ladder Assay) from Maxim Biotech, Inc. Fetal lengths and weights were not affected by *L. monocytogenes* infection. Stillbirths occurred after treatment with *L. monocytogenes* at 10^6 and 10^7 CFU. Average fetal lengths were 8.2, 7.4, 7.9, 7.6 cm and average fetal weights were 55.8, 59.9, 58.6, 48.2 g for control, 10^5 , 10^6 , and 10^7 CFU, respectively. TGF- β 1 concentrations were not significantly different in maternal serum regardless of *L. monocytogenes* infection with 10.1 ng/mL, 11.3 ng/mL, 9.86 ng/mL, and 11.3 ng/mL for control, 10^5 , 10^6 , 10^7 CFU, respectively. Placentas positive for apoptosis showed a dose-dependent trend with 51% of placentas from control animals showing evidence of apoptosis based on PCR. Treated animals had 57%, 73%, and 87% of placentas positive for apoptosis after treatment with 10^5 , 10^6 , and 10^7 CFU *L. monocytogenes*. TGF- β 1 was not changed with infection of *L. monocytogenes*. Increases in apoptosis in placentas after treatment

with *L. monocytogenes* suggests infection-related changes occur in the placenta and this may impact fetal viability.

***CLOSTRIDIUM BOTULINUM* CELL NUMBERS FOR DETECTABLE BOTULINUM
TOXIN PRODUCTION IN BROTH AND FOODS**

(L. Ma, L. H. Thurber, C.-M. Lin, and M. P. Doyle)

The environmental factors affecting spore germination, growth and toxin production of *C. botulinum* are well documented. However, the number of *C. botulinum* vegetative cells at which botulinum toxin is detectable either in broth or foods has not been determined. Hence, the objective of this study was to determine vegetative cell numbers of *C. botulinum* present when toxin is initially detectable by the mouse bioassay.

Spores of two strains each of proteolytic or non-proteolytic *C. botulinum* strains were inoculated into Trypticase-peptone-glucose-yeast (TPGY), and mashed potatoes for proteolytic strains and canned tuna for non-proteolytic strains. Two inoculation levels (10 and 1000 spores/ml) and two incubation temperatures (15° and 31°C for proteolytic strains or 12° or 31°C for nonproteolytic strains) were used for the broth study to determine the effect of inoculum size and temperature on vegetative cell numbers of *C. botulinum* present when toxin is detectable. Studies with foods were conducted at 31°C with an inoculation level of 1000 spores/g. Inoculated samples were sampled every hour (in broth) or two hours (in foods) near toxin production times as determined in a preliminary study. At each sampling time, *C. botulinum* cell numbers in the samples were determined by plating, whereas botulinum toxin production was determined by the mouse bioassay. Results revealed that *C. botulinum* cell numbers were at 10⁵ to 10⁸ cfu/ml or g when toxin was first detected. Spore inoculation level (10 v. 1000 spores/ml or g) had little influence on cell numbers when toxin was initially detectable; however, a longer incubation time was needed for low level inoculum to grow and produce detectable toxin. Incubation temperatures (31°C vs. 12° or 15°C) had little influence on cell numbers when toxin was first detected; however, a longer incubation time was needed at low temperatures to reach detectable toxicity. Proteolytic *C. botulinum* cell numbers were higher in broth than in foods when toxin was initially detected. Longer incubation times were needed for foods than broth to reach detectable toxicity for both types of *C. botulinum* strains. Non-proteolytic strains produced toxin at earlier sampling times than proteolytic strains, both in broth and foods.