

PATHOGENICITY

DOSE RESPONSE OF *ENTEROBACTER SAKAZAKII* INFECTIONS IN CD-1 NEONATAL MICE (M.A. Smith)

Enterobacter sakazakii (*E. 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 orally challenged with a single dose of 0.1 ml reconstituted powdered infant formula inoculated with 10^2 to 10^{11} CFU *E. sakazakii* strain MNW2 on postnatal day 3. Deaths occurring immediately after or less than 15 hours post-treatment were suspected to result from gavage technique and were not included in the analysis. Twenty-six deaths occurred at least 15 hours post-treatment and were assumed to result from *E. sakazakii* infection. The surviving mice were euthanized and weighed on postnatal day 10. Brains, ceca, and livers were excised and pooled into 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 only brain and liver tissues in neonates administered 10^9 CFU. *E. sakazakii* was not found in control tissues. Three out of six litters at 10^9 CFU had neonatal deaths, whereas all litters (4/4) treated with 10^{11} CFU had at least three neonatal deaths. There was 14.5% lethality among pups administered 10^9 CFU and 34.8% lethality among pups given 10^{11} CFU as compared to no deaths 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.

DOSE RESPONSE, INFECTIVITY, AND STILLBIRTHS IN PREGNANT GUINEA PIGS INOCULATED WITH *LISTERIA MONOCYTOGENES*

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Listeriosis is a severe disease that results from the foodborne pathogen, *Listeria monocytogenes* and is responsible for ~ 2500 cases and 500 deaths each year. Pregnant women are 20 times more likely to develop listeriosis than the general population with adverse pregnancy outcomes including low birth weight, spontaneous abortions, stillbirths, or neonatal meningitis. The objective of this study was to determine an infective dose, infectivity, and corresponding stillbirths in pregnant guinea pigs. Pregnant guinea pigs (n = 4-11/dose) were treated orally on gestation day (gd) 35 with 10^4 to 10^8 *L. monocytogenes* CFU in sterile whipping cream. Pregnancies were allowed to proceed normally until sacrifice on gd 56. *Listeria* species were determined by direct plating tissue samples on selective media, and *L. monocytogenes* was confirmed using a chromogenic substrate test. Among the treated dams, there was a dose dependent relationship in the colonization of *L. monocytogenes* within tissue samples. *L. monocytogenes* cells were recovered from 64, 73, 90, and 100 percent of liver samples from animals treated with 10^5 , 10^6 , 10^7 , 10^8 CFU, respectively. Livers from each dose were examined microscopically after staining with H&E. No apparent lesions or chronic inflammation was seen in the control animals, but lesions were found in treated animals. At the lowest dose of 10^4 *L. monocytogenes* CFUs, some apoptotic hepatocytes and areas of minimal to mild acute inflammation were found. The lesions, in size and number, increased as the dose increased. In dams dosed with $\geq 10^6$ CFU, *L. monocytogenes* cells were cultured from 50% of the spleen and 33% of the gallbladder samples. Eleven of 34 dams treated with $\geq 10^5$ CFU delivered stillborn pups. *L. monocytogenes* cells were cultured from placenta, liver, and brain tissue from all stillbirths. However, the dams with nonviable fetuses shed *L. monocytogenes* for longer periods of time. Based on a log logistic model, the dose adversely affecting 50% of the pregnancies was approximately 10^7 *L. monocytogenes* CFU compared to that estimated from a human outbreak of 10^6 CFU. Listeriosis in pregnant guinea pigs can result in stillbirths, and the overall disease is similar to that described in non-human primates and in humans.

NEONATAL MICE AS MODELS FOR PREMATURE INFANTS FED *ENTEROBACTER SAKAZAKII*-CONTAMINATED INFANT FORMULA

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

Premature or very-low-birth-weight human infants exposed to *Enterobacter sakazakii* in reconstituted powdered infant formula may develop infections resulting in septicemia, necrotizing enterocolitis, meningitis, hydrocephalus, or death. Animal models are needed to estimate and understand the infectivity of *E. sakazakii* in

human infants. Due to their underdeveloped central nervous system at birth, the infection of neonatal mice with *E. sakazakii* may mimic that of premature human infants. Our objective was to compare the susceptibilities of three mouse strains to *E. sakazakii* strain MNW2 by observing mortality and infectivity. Timed-pregnant dams of the CD-1, BALB/C and C57BL/6 strains were obtained, acclimatized, and allowed to give birth naturally. At postnatal day (PND) 3 or 4, the pups were orally gavaged with a single dose of vehicle or 10^4 - 10^{12} colony-forming units (CFU) *E. sakazakii* strain MNW2 per ml reconstituted powdered infant formula. All pups surviving to PND 10 or 11 were sacrificed and brains, livers, and ceca excised and analyzed for the presence of *E. sakazakii*. *E. sakazakii* was isolated from 66.7%, 38.5% and 36.4% of brains, 60.0%, 30.8% and 45.4% of livers, and 26.7%, 0% and 18.2% of ceca from treated CD-1, BALB/C and C57BL/6 litters, respectively. No deaths occurred in any of the control groups for any mouse strain. Among the three strains, CD-1 appears to be the most sensitive demonstrating a dose-dependent response in mortality (10^{11} CFU resulted in 34.8% mortality). In C57BL/6 mice, mortality occurred only at the highest dose administered (4.2% at 10^{12} CFU). Although BALB/C mice had 19% mortality at 10^7 CFU, it was not dose-dependent. The results of this experiment suggest that the CD-1 mouse strain is the most susceptible to *E. sakazakii* infection and may serve as a potential animal model for the infection in human infants. (Funded by ILSI North America Technical Committee on Food Microbiology to MAS).

