

The Infant Rabbit as a Model of Pathogenicity for Vibrio parahaemolyticus

Principal Investigators: Udom Lecomboon, M.D., Ph.D.
Chiraphun Duangmani, M.D.
M. Talmage M'cMinn, CPT, MSC

OBJECTIVE: To determine if the infant rabbit could be used as a model for testing the possible pathogenicity of various strains of Vibrio parahaemolyticus.

BACKGROUND: The haemolytic activity of Vibrio parahaemolyticus on a special human or rabbit blood agar (Wagatsuma agar) is the primary biochemical property currently being used to determine the virulence of this organism. It is generally recognized that this organism does not produce a disease in laboratory animals resembling that in man. This laboratory has attempted to demonstrate pathogenicity of V. parahaemolyticus in adult rabbits, mice, monkeys, and gibbons and has not been able to obtain consistent results in any of these animals. The infant rabbit model has been used successfully in some pathogenic studies of V. cholera and preliminary investigations in this laboratory indicated that it was free of Coccidioides spp. which had interfered with our work in adult rabbit intestinal loops.

DESCRIPTION: A preliminary study indicated that the stomach contents of the infant rabbit markedly affected the response of the animal to inoculation of the halophilic V. parahaemolyticus. In feeding experiments, it was noted that in animals fed one ml. of an isolate obtained from a human diarrhea patient, the response was more pronounced after the animal's stomach had been purged with normal saline. This stomach washing technique often resulted in trauma to the small animal and we were often unable to insure the dosage of bacteria fed to the animal.

To overcome the disadvantages of such procedures, an intrainstestinal inoculation technique was developed. Infant rabbits, weighing 100–150 grams, were anaesthetized with ether and a small incision was made in the washed anterior abdominal wall. The ileum was withdrawn through the sterile incision. One ml. of test material was then carefully injected into the lumen of the ileum. The intestine was not tied or traumatized. A 27 gauge needle was used to inject the test material and extreme care was taken to insure that no leakage of the inocula occurred. After carefully returning the intestine to the peritoneum and closing the abdominal wall, the animals were deprived of food and water and closely observed for death, diarrhea, or any illness. Animals were sacrificed at 24 hours after the procedure. Cultures for V. parahaemolyticus were then made from the heart blood, small intestine and large intestine. Necropsy was performed on all animals. A group of three animals was used for each test material. Controls were injected with sterile, fresh media.

Preparation of the Inocula: Selected stock cultures were cultured on thiosulfate–citrate–bile salts–sucrose (TCBS) agar plates. A single colony was picked and inoculated into 20 ml. of brain–heart–infusion broth (BHIB) with 3% NaCl. This culture was incubated for 16–18 hours at 37°C. One ml. of the broth culture containing approximately 10^7 – 10^8 cells was used in the intrainstestinal injection. Cell free filtrates of used media were prepared by picking one typical colony of V. parahaemolyticus and inoculating it into 15 ml. of BHIB with 3% NaCl and incubating at 27°C for 16–18 hours. The cells were then removed by centrifugation and membrane filtration at 4°C. The cell free supernatant was then checked for sterility and dialyzed against a saturated sucrose solution for 24 hours at 4°C. The final concentration of the dialyzed solution was one fifth of its original volume.

RESULTS: Twenty strains of V. parahaemolyticus isolated from patients with acute gastroenteritis and 9 strains obtained from natural sources (sea water, sand, sea foods) were used as cell broths to test for pathogenesis in infant rabbits. Data obtained from our experiments are presented in Table 1.

Control animals inoculated with BHIB with 3% NaCl failed to demonstrate any of the characteristics noted in the table. Diarrhea (frequent, watery stools) occurred in 30.3% of those animals inoculated with isolates from human diarrheal stools while only 15.1% of those animals inoculated with natural isolates demonstrated any symptoms of acute diarrhea. Animals inoculated with human isolates had large amounts of fluid and gas in the large intestine in 83.3% of the animals tested and in the small intestine in 36.4%. Almost one half (48.5%) of the animals inoculated with natural isolates had fluid in the large intestine and 18.2% had small intestines grossly distended with fluid and gas.

Table 2 shows the results of postmortem cultures onto TCBS agar from the infant rabbits. Cultures from the peritoneum revealing V. parahaemolyticus were used as evidence of leakage of the inocula from the intestine. These animals are not included in this report. All animals that died had positive heart blood cultures of V. parahaemolyticus. These data indicate that those isolates of the organism from human diarrhea appear to be more adapted to survival in the animal intestine than those from natural sources.

Table 3 shows the results of intrainestinal inoculation with concentrated cell-free used broth. It is clear that this filtrate, in which V. parahaemolyticus was grown for 16-18 hours, contains some toxic substance (s) which caused 50% of the inoculated animals to have positive symptoms of acute diarrhea. 80% of the animals inoculated with filtrate from human isolates of the organism had gross fluid and gas in the large intestine. 17 of the 35 animals had fluid and gas in the small intestine. All postmortem cultures of heart blood and small and large intestine were negative, as were specimens of the inocula that were cultured at the time of injection. While we note that the broth control produced gas in the large intestine of 4 animals, the reaction was not so pronounced nor as frequent as with the used broth.

DISCUSSION: After numerous attempts to demonstrate pathogenicity of V. parahaemolyticus in a variety of ways, these results indicate that the infant rabbit ileum may be an acceptable model for additional research. Japanese investigators, basing their work on haemolysis on Wagatsuma media (the Kanagawa phenomenon), have suggested that isolates from natural sources are not pathogenic. In our hands, the Kanagawa phenomenon has been rather universal in all strains isolated in Thailand (see elsewhere in this report). This has left us without an acceptable method of distinguishing pathogenic and nonpathogenic strains, an important factor in any epidemiological study we may undertake. Attempts to demonstrate pathogenicity by use of the Di Test in adult rabbits were extremely erratic due to the rather universal contamination of the rabbits with Coccidioides spp. We continue attempts to feed the organism to monkeys and gibbons but usually cannot even recover the organism from the stool. No primate has ever developed any symptoms of diarrhea.

These data appear to demonstrate that V. parahaemolyticus isolated from natural sources does not have the pathogenic properties that isolates obtained from human gastroenteritis specimens have. Results of cultures after intrainestinal inoculation of the organisms suggest that natural isolates may not be as invasive as isolates from diarrheal stools. Additional studies into these findings continue. These data raise epidemiological questions regarding the source of human infection that will have to be answered by additional studies.

Table 1.
Observations of Infant Rabbits Inoculated with V. parahaemolyticus Cells

Source of Organism	Animals Tested	Observation			
		Death*	Diarrhea	Fluid in Large Int.	Fluid in Small Int.
Human isolate (20)	66	14	20	55	24
Natural isolate (9)	33	6	5	16	6

* within 24 hours

Table 2.
Results of Postmortem Culture of Infant Rabbits Inoculated with V. parahaemolyticus Cells

Source of Organism	Animals Tested	Positive Cultures From:		
		Heart blood	Small intestine	Large intestine
Human isolates (20)	66	16	59	64
Natural isolates (9)	33	6	14	24

Table 3.
 Observations of Infant Rabbits Inoculated with Cell-free Concentrate of V. parahaemolyticus Cultures

Source of Organism	Animals Tested	Observation			
		Death*	Diarrhea	Fluid in Large Int.	Fluid in Small Int.
Human isolate (6)	35	1	17	28	4
Natural isolate (2)	6	0	3	4	0
Broth control	15	0	0	4	0

* within 24 hours