

The Effect of Enteroviruses on Ligated Loops of Rabbit Small Intestine

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BACKGROUND: Certain Echovirus serotypes have been implicated as the causative agents of diarrheal outbreaks. However the pathogenic role of most enteroviruses recovered from diarrheal stools is unclear. We do not know whether the virus in stool is a latent or newly introduced virus made to replicate by the diarrheal process, or whether the virus itself is the causative agent of diarrhea. Impeding investigations on diarrhea has been the unavailability of a laboratory assay to measure the effect of enteroviruses on the gastrointestinal tract. Currently, one can only determine the virulence of any enterovirus in human volunteers.

The purpose of this study was to attempt to develop a satisfactory laboratory assay for detecting potential diarrhea-producing viruses. Prompting this study was the availability of gastrointestinal aspirates and stools obtained from troops stationed in Vietnam who were hospitalized in 1970 with diarrhea.

Many investigators have shown that accepted enteropathogenic bacteria isolated from patients with diarrhea produce dilatation and histopathological changes in isolated loops of rabbit small intestine. Nonpathogenic bacteria do not cause these changes. We tested whether diarrheal serotypes of enteroviruses can also produce dilatation of isolated rabbit gut loops. ECHO viruses 9 and 11 were chosen for the pilot study of this technique.

METHOD: Albino rabbits of both sexes weighing 2 kg were fasted for 24 hours before operation. They were anesthetized with methophane and 5 loops of small intestine, 3 cm per loop, were ligated. Test viruses were injected into every other loop, Shigella sonnei served as a positive control and uninfected tissue culture media and viruses not clinically associated with diarrhea served as negative control. High titers of tissue culture grown virus (10^5 – 10^8 TCID₅₀) were used. Rabbits were sacrificed at 30 hours and loop dilatation was graded 0 to 4+, with 3 and 4+ considered "positive" and 1+ and 2+ considered "suspected positive". Results are summarized in Table 1.

Ligated small intestine loops injected with sterile tissue culture media showed dilatation in 9 of 22 attempts, while only 1 of 2 tests with the control positive agent, Shigella sonnei, were clearly positive. All but one of the negative control viruses (16 tests) produced negative tests. However only 16 of 32 tests with ECHO 11 and none of 3 tests with ECHO 9 were positive or suspected positive.

In an attempt to provide more reproducible results, we lengthened and shortened the length of ligated loops, extended the incubation time beyond 30 hours, and used higher titered challenge virus. These changes in methodology did not provide more reproducible results. We conclude from this extensive pilot study that the rabbit gut loop assay, as performed, was irreproducible and unreliable for detecting virulent enteropathogenic viruses.

Table 1. Result of Isolated Rabbit Gut Loop Challenge

<u>Challenge Agent</u>	<u>No. of Tests</u>	<u>No. positive(1)</u>	<u>No. suspected Positive(1)</u>	<u>No. negative(2)</u>
Echovirus 11	32	16	1	15
Echovirus 9	3			3
Shigella sonnei	2	1	1	
Influenza virus A2/K11/68	9			9
Chikungunya virus	2			2
Rhinovirus Cv36	2			2
Denguevirus 2	3	1		2
Sterile media	22	9		13

(1) Dilatation of gut loop 30 hours after challenge (see text)

(2) No dilatation of gut loop 30 hours after challenge.