

3. At7 LF-25

AEC

RESEARCH REPORTS

LF-25

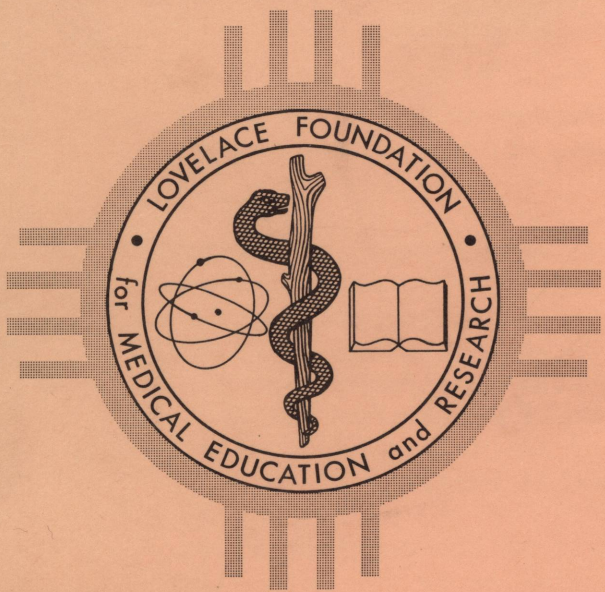
LF-25

# LOVELACE FOUNDATION

for Medical Education and Research

AEC RESEARCH AND  
DEVELOPMENT REPORT

UNCLASSIFIED



## EXPERIMENTAL INFECTION OF BEAGLES WITH ECHO VIRUS TYPE 6

Albuquerque, New Mexico

by

F. F. PINDAK  
AND  
W. E. CLAPPER

May 1965

UNIVERSITY OF  
ARIZONA LIBRARY  
Documents Collection  
Jul 13 1965

ATOMIC ENERGY COMMISSION -  
LOVELACE FOUNDATION  
FISSION PRODUCT INHALATION PROJECT

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

- A. Makes any warranty or representation, express, or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission to the extent that such employee or contractor prepares, handles or distributes, or provides access to, any information pursuant to his employment or contract with the Commission.

Printed in USA. Price \$0.50. Available from the Office of  
Technical Services, Department of Commerce,  
Washington 25, D.C.

LF-25

Biology and Medicine

TID-4500(40th Ed.)

UNCLASSIFIED

EXPERIMENTAL INFECTION OF BEAGLES WITH ECHO VIRUS TYPE 6

by  
F. F. Pindak and W. E. Clapper

Submitted as a  
Technical Progress Report  
to  
The Division of Biology and Medicine  
United States Atomic Energy Commission  
on  
Contract No. AT(29-2)-1013  
May 1965

From the Department of Microbiology  
Lovelace Foundation for Medical Education and Research  
Albuquerque, New Mexico

UNCLASSIFIED

## ABSTRACT

Live Echo 6 virus was administered orally to six beagles previously shown by culture to be free of this agent. Some symptoms suggesting enteric disease were observed in most of the dogs. The virus was isolated from the stools of four and the blood of one, but no neutralizing antibodies could be demonstrated. Repeated intramuscular injection of live virus in six dogs produced titres as high as 1:512 and a four-fold or greater rise in all. No signs of illness were observed in those given parenteral injections.

## ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. C. S. White for helpful suggestions in the preparation of the manuscript. They also wish to thank Dr. J. H. Sherrod, Mr. M. R. Wilson, and other members of the Department of Veterinary Medicine for help with the animals, without which it would have been impossible to carry out the experimental procedures. Our thanks are extended also to Mrs. Beth Bowen for preparation of the manuscript.

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT.....	i
ACKNOWLEDGMENTS.....	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES.....	iii
INTRODUCTION.....	1
METHODS.....	1
1. Oral Administration of Virus.....	1
2. Parenteral Administration of Virus.....	2
RESULTS.....	3
1. Oral Administration of the Virus.....	3
a. Clinical Evaluation.....	3
b. Isolation of Virus and Correlation with Symptoms...	4
c. Antibody Titres.....	4
2. Parenteral Administration of the Virus.....	6
a. Clinical Observations.....	6
b. Antibody Titres.....	6
DISCUSSION.....	6
REFERENCES.....	10

LIST OF TABLES

Table I.	Virus Isolations and Clinical Observations on Dogs Fed ECHO 6 Virus.....	5
Table II.	Serum Neutralization Titres of Dogs Inoculated IM with ECHO Type 6 Virus.....	7



EXPERIMENTAL INFECTION  
OF BEAGLES WITH ECHO VIRUS TYPE 6

by

F. F. Pindak and W. E. Clapper

INTRODUCTION

In a previous report, the authors described the isolation of cytopathic agents from rectal swabs in 21 of 29 healthy beagles<sup>1</sup>. Antiserum produced against one of these agents in a guinea pig neutralized this virus and the prototype ECHO 6 virus to the same high titre. All but one of the other isolates were also neutralized by this antiserum. No reports have been found on the isolation of human enteric viruses from dogs, except the reovirus isolated by Lou and Wenner<sup>2</sup>. Gelfand<sup>3</sup> reported that a few sera from dogs neutralized ECHO 6 and certain other enteric viruses in dilutions up to 1:8. In the present study, antibodies to ECHO 6 virus were found to be absent or present only in very low titres in beagles. Therefore, it seemed desirable to determine whether ECHO 6 virus could cause an actual infection in the beagles or whether this was a transient inhabitant of the intestine which in no way affected the health of the animal. This paper reports the results of oral and parenteral administration of this virus. Infectivity was measured by clinical symptoms, virus isolation, and circulating antibody production.

METHODS

1. Oral Administration of Virus

Live ECHO 6 virus was administered orally to six healthy dogs nine months of age. The virus had been grown in monkey kidney tissue cultures and the infected fluid frozen in gelatin capsules. Three dogs were given  $10^5$  tissue culture doses, and three were given one-half of this

amount. Five hours before feeding the virus, 7 ml of blood were collected from each dog for antibody determinations. Before selecting the six experimental animals, rectal swabs were taken from each of a larger number at weekly intervals for three weeks. The swabs were placed in a small amount of Hanks' basic salt solution (BSS) containing antibiotics and incubated at 37°C for one hour. After centrifugation, the supernatant fluid was inoculated into monkey kidney tissue cultures. Only dogs which had all three cultures negative were used for the experiment.

Blood and fecal samples were taken daily for the first five days and on days 7 and 9. After this, four additional rectal swabs were taken at weekly intervals. On the 28th and 58th days, additional blood samples were collected for serological studies. In total, seven blood and 12 fecal specimens from each dog were cultured in monkey kidney tissue culture following the feeding of the virus.

All the dogs had been vaccinated against rabies, infectious canine hepatitis, distemper, and leptospirosis. This prevented them from having these diseases, some of the symptoms of which might have been mistakenly attributed to the feeding of the ECHO 6 virus. To prevent accidental contamination from the outside or from one another, the following precautions were taken. Each dog was kept in an outdoor 10 x 12 foot wire enclosure which was 10 feet away from pens of other dogs. The floors consisted of 4 to 6 inches of crushed rock. Within each pen was a small wooden shelter, one dish for water and one for food. The approaches to and the space between the pens were also covered with crushed stone, and the entire installation was circled with a fence. Only authorized personnel had access to this compound. Entrance was made only to collect specimens and to feed and water the animals. The attendant wore plastic covers over his shoes which were changed before entering the next pen.

## 2. Parenteral Administration of Virus

Upon completion of the feeding experiment, three of the beagles which had received the virus orally and three new animals were given



intramuscular injections of live ECHO 6 virus at 0, 26, 35, and 56 days. Tissue culture fluid containing  $10^6$  tissue culture doses was injected each time. Blood was drawn for antibody determinations at 0, 8, 14, 48, 70, and 98 days. Antibody titres were determined by neutralization of 100 tissue culture doses in monkey kidney tissue culture.

## RESULTS

### 1. Oral Administration of the Virus

#### a. Clinical Evaluation

First day after administration. The dogs had a generally healthy appearance. Number 6 did not eat his usual amount of food, but no other symptoms were observed.

Second day. Dog No. 1 had a loose stool with some fresh blood. Dogs 2, 3, and 5 appeared normal. Dog No. 6 was anorectic and its stool also had fresh blood.

Third day. The fecal sample of Dog No. 1 again contained blood, as did the sample of No. 6. The latter still refused to take food. No. 4 ate very little and had a mucoid stool.

Fourth day. Dog No. 2, which thus far had been symptomless, had loose stools with some blood.

Fifth day. No. 1 had a diminished appetite and No. 5, to this point appearing normal, now showed blood in the stool.

Seventh day. The blood reappeared in the stool of No. 1 and was found for the first time in No. 3 which had, thus far, shown no other symptoms.

Ninth day. The dogs appeared in generally good condition. No. 5, however, had a rather loose stool.

Fourteenth day. A mucoid stool was noted in Dog No. 2. Blood reappeared in the feces of No. 5, as observed again on the 21st day.

The events taking place after ingestion of the live virus can be summarized as follows:

During the first 24 hours, little change was observed. Two days after the virus was administered, some definite effects were seen. They were manifested in refusal to take food, in the production of loose stools or diarrhea, and in some internal hemorrhaging which was most likely in the lower segment of the colon, since the blood had a rather fresh appearance. Thus, at this time, damage to the intestinal mucosa must have taken place. Some of these symptoms occurred in part of the group later and recurred in one dog 21 days after administration of the virus.

b. Isolation of Virus and Correlation with Symptoms

The virus was isolated from the feces on the first day from Dogs No. 5 and No. 6. At this time all dogs appeared normal. No. 6 had a diminished appetite, which may or may not have been significant. On the second day, the virus was again found in the stool of Dog No. 6, which at this time was anorectic and had some blood in the feces. Dog No. 1 did not yield the virus, although blood was observed in the stool. ECHO 6 virus was found in the blood of Dog No. 2, but no clinical symptoms were observed. This was the only positive blood culture obtained and, also, the only time that the virus was isolated from this dog. Dog No. 3 had acquired the habit of defecating in his water supply. For the first five days, he showed no adverse effect of the ingestion of the virus. However, if the agent was being passed in amounts too small to be detected by our method of testing, reinfection was most likely taking place. On the 7th day, this dog exhibited the same symptoms as the rest, and on the 35th day the virus was isolated from his stool sample. Dog No. 5, with a positive stool culture on the first day, yielded virus again on days 28 and 35. Dog No. 6 had positive stool cultures only the first two days after inoculation. These findings are presented in Table I.

c. Antibody Titres

Neutralization tests were carried out on sera collected just before

Table 1  
 Virus Isolations and Clinical Observations on Dogs Fed ECHO 6 Virus

Days after feeding	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
1					ECHO 6 in stool	Diminished appetite ECHO 6 in stool
2	Loose stool with blood	ECHO 6 in blood		Anorexia Diarrhea		Anorexia Blood and ECHO 6 in stool
3	Stool with blood			Diminished appetite Mucoid stool		Anorexia Stool with blood
4		Loose stool with blood				
5	Diminished appetite				Stool with blood	
6	Stool with blood		Loose stool with blood			
9					Loose stool	
14		Mucoid stool			Stool with blood	
21					Stool with blood	
28					ECHO 6 in stool	
35			ECHO 6 in stool		ECHO 6 in stool	

feeding the virus and on the 28th and 58th days thereafter. No measurable amount of ECHO 6 antibody was detected.

## 2. Parenteral Administration of the Virus

### a. Clinical Observations

None of the six dogs given intramuscular injections of live virus showed signs of illness.

### b. Antibody Titres

The results are summarized in Table II. Before giving the injection, the serum neutralization titres were less than 1:4 in all six animals. Numbers 1, 2, and 3 were dogs to which a single dose of virus had been administered orally some months previously. Numbers 5, 6, and 7 had no known previous contact with the virus. Eight days after the inoculations, a four-fold or greater increase in titre was observed in all except No. 5. Titres increased eight-fold in two dogs (No. 2 and No. 3) that had had previous contact (oral) with the organism. No further increase in titre was seen at 14 days. However, 13 days after the second of two booster inoculations were given, titres were increased at least four-fold over the initial value in all six dogs and values of 1:128 or higher were found in three. The final (third) booster dose was given at 56 days. Fourteen days later, little increase was found in any of the dogs. Thirty-four days after this inoculation, no further changes were noted except in Dog No. 3 which now showed a titre of 1:512.

## DISCUSSION

All of the animals were kept in isolation and observed closely for three weeks before beginning the experiment. At this time, none of the clinical findings observed after the oral administration of virus were seen. It seems apparent, therefore, that the virus did produce some degree of intestinal disturbance, although the extent of involvement could not be fully assessed. Furthermore, the virus inoculum inside the gut was diluted considerably from its initial concentration and yet the small portion of fecal contents actually inoculated into the tissue culture tubes was

Table II  
 Serum Neutralization Titres of Dogs  
 Inoculated IM with ECHO Type 6 Virus

Dog number	Reciprocal Titre on Days After First Dose								
	0	8	14	26	35	48	56	70	90
1	<4	8	8	2nd inoculation of virus	3rd inoculation of virus	32	4th inoculation of virus	32	32
2	<4	16	16	2nd inoculation of virus	3rd inoculation of virus	128	4th inoculation of virus	128	128
3	<4	16	16	2nd inoculation of virus	3rd inoculation of virus	128	4th inoculation of virus	256	512
4	<4	8	8	2nd inoculation of virus	3rd inoculation of virus	256	4th inoculation of virus	256	256
5	<4	4	4	2nd inoculation of virus	3rd inoculation of virus	16	4th inoculation of virus	16	16
6	<4	8	8	2nd inoculation of virus	3rd inoculation of virus	32	4th inoculation of virus	64	64

Dogs 1, 2, and 3 previously had been given the virus orally.

sufficient to produce cell destruction. Therefore, it is highly probable that multiplication of the virus occurred in the intestine. This conclusion is further supported by the fact that, in at least three instances, isolations were made one month or later after the administration of the virus.

Two dogs were negative for the virus at all times tested. Perhaps if larger samples of fecal matter had been inoculated into tissue culture tubes, they, too, would have been found positive. It is also possible that multiplication of virus in the alimentary tract of these animals was too low for detection. One showed the same signs of illness as those from which virus was isolated and one had milder symptoms. They may also have been infected.

The absence of antibodies after infection from oral doses was somewhat surprising, particularly since titrations were carried out at 28 and 59 days, and adequate time for their formation. Lou and Wenner<sup>2</sup> found no antibodies to reovirus type 1 in eight-week old puppies that were inoculated intranasally. They felt that, since the animals were sacrificed on the 13th day, sufficient time for antibody production may not have been allowed. However, appreciable titres were found in some monkeys and rabbits after a similar period of time. Neutralizing antibodies to reovirus were found in 43 of 126 normal dogs, but, of these, only four had titres of 1:64 or more. Gelfand<sup>3</sup> found antibody titres up to 1:8 in a few of 158 dogs tested for ECHO 6 and found only low titres in some for other ECHO, Coxsackie, and polioviruses. These findings suggest the possibility that the dog does not produce antibodies in high titre against infections of the intestinal tract cause by enteroviruses. Antibodies may form in the lining of the gut and not be found in the circulating blood in intestinal disease.

Serum antibodies to ECHO 6 virus can be formed in dogs, however, if the virus is introduced parenterally, as we have shown. Response to the initial dose was comparable to that reported by Melnick<sup>4</sup>, who found chimpanzees produce titres from 1:16 to 1:64 after a single inoculation of this virus.

Viruses apparently identical with those found in humans have been isolated from animals by other investigators. These include adenoviruses

from cattle<sup>5,6</sup>, ECHO 8 from pigs<sup>7</sup>, reoviruses from calves<sup>8,9,10</sup>, dogs<sup>2</sup>, and mice<sup>11</sup>, influenza viruses from pigs and sheep<sup>12</sup>, and a poliovirus from a calf<sup>13</sup>. Antibodies to human disease-producing viruses have also been found in animals. ECHO 6 virus antibodies were observed in a chimpanzee by Itoh and Melnick<sup>14</sup>. Antibodies to poliovirus<sup>12,15</sup>, parainfluenza<sup>16</sup>, reoviruses<sup>17</sup>, and adenoviruses<sup>5</sup> have been found in cattle. In dogs, Gelfand<sup>3</sup> found neutralizing antibodies to poliovirus types 1 and 3, Coxsackie A-9 and B-2, and ECHO viruses types 6, 7, 8, 9, and 12 in low titres. Carmichael and Barnes<sup>18</sup> found complement fixing antibodies to adenovirus and Lou and Wenner<sup>2</sup>, antibodies to reovirus type 1 in dogs. This is ample evidence that non-primates can carry and be infected with many of the viruses causing human disease and yet not necessarily show signs of illness.

The results, reported here and in a previous paper<sup>1</sup>, and some of those quoted above, suggest that the dog may carry the ECHO 6 virus at least intermittently in the digestive tract. If the infective dose is great enough, clinical illness may be observed, but often symptoms may not accompany the carrier state. Antibodies were produced from intestinal infection with this virus either in such low titres that they were not detected or were not produced at all. However, they were found when the live virus was introduced parenterally.

Humans may be the original source of infection of the dog with ECHO 6 virus. Whatever the source, it is known that this animal can become infected, can be a carrier, and, therefore, may be a source of further infection to humans.

It will be of interest to determine the effect of radioactive products on the invasiveness of this and other viruses of a similar nature, particularly since radiation has been reported to increase the susceptibility of some animals to both bacteria and viruses<sup>19,20</sup>. The beagle might prove to be a highly useful experimental animal for the study of possible effects and control of viruses causing human disease in the host exposed to radioactive aerosols.



## REFERENCES

1. Pindak, F. F. and W. E. Clapper, "Isolation of Enteric Cytopathogenic Human Orphan Virus Type 6 from Dogs," *Amer. J. Vet. Res.* 25: 52-54, 1964.
2. Lou, T. Y. and H. A. Wenner, "Natural and Experimental Infection of Dogs with Reovirus Type 1: Pathogenicity of the Strain for Other Animals," *Amer. J. Hyg.* 77: 293-304, 1963.
3. Gelfand, H. M. , "The Occurrence in Nature of the Coxsackie and ECHO Viruses," *Progr. Med. Virol.* 3: 193-244, 1961.
4. Melnick, J. L. , "ECHO Viruses," *Special Publications, N. Y. Acad. Sci.* 5: 365-381, 1957.
5. Klein, M. , E. Earley, and J. Zellat, "Isolation from Cattle of a Virus Related to Human Adenovirus," *Proc. Soc. Exp. Biol. Med.* 102: 1-4, 1959.
6. Klein, M. , J. Zellat, and T. C. Michaelson, "A New Bovine Adenovirus Related to Human Adenovirus," *Proc. Soc. Exp. Biol. Med.* 105: 340-342, 1960.
7. Moscovici, C. , A. Ginevri, and V. Mazzarachio, "Isolamento su Colura di Tessuti di Mi Virus da Enterite Suina," *Zooprofilassi*, July 7, 1956. Quoted from Melnick, J. L. , "ECHO Viruses," *Special Publications, N. Y. Acad. Sci.* 5: 365-381, 1957.
8. Sabin, A. B. , "Reoviruses": Properties of a New Group of Respiratory Enteric Virus Formerly Classified as ECHO 10," *Science* 130: 1387-1389, 1959.
9. Moscovici, C. , M. La Placa, J. Maisel, and C. H. Kempe, "Studies of Bovine Enteroviruses," *Amer. J. Vet. Res.* 22: 852-863, 1961.
10. Abinanti, F. R. , "Respiratory Enteric Viruses in Man and Animals," *Pub. Health Rep'ts.* 76: 897-902, 1961.
11. Hartley, J. W. , W. P. Rowe, and R. J. Huebner, "Recovery of Reoviruses from Wild and Laboratory Mice," *Proc. Soc. Exp. Biol. Med.* 108: 390-395, 1961.
12. Rovary, J. , G. Takatsy, K. Barb, and E. Farkas, "Isolation of Influenza Virus Strains from Animals," *Nature* 193: 907-908, 1962.
13. Koprowski, H. , "Counterparts of Human Viral Disease in Animals," *Ann. N. Y. Acad. Sci.* 70: 369-382, 1958.

14. Itoh, H. and J. L. Melnick, "The Infection of Chimpanzees with ECHO Viruses," *J. Exp. Med.* 106: 677-688, 1957.
15. Kunin, C. M. and E. Minuse, "The Isolation in Tissue Culture, Chick Embryo, and Suckling Mice of Filterable Agents from Healthy Dairy Cattle," *J. Immunol.* 80: 1-11, 1958.
16. Abinanti, F. R., A. B. Horlein, R. L. Watson, and R. J. Huebner, "Serologic Studies of Myxovirus Parainfluenza 3 in Cattle and the Prevalence of Antibodies in Bovines," *J. Immunol.* 86: 505-511, 1961.
17. Rosen, L. and F. R. Abinanti, "Natural and Experimental Infection of Cattle with Human Types of Reoviruses," *Amer. J. Hyg.* 71: 250-257, 1960.
18. Carmichael, L. E. and F. D. Barnes, "Human Adenovirus Complement Fixing Antibodies in New York Family Dogs," *Proc. Soc. Exp. Biol. Med.* 110: 756-758, 1962.
19. Boeke, R. D., "Radiation and Susceptibility to Infection," (A Literature Survey), U. S. Air Force Radiation Laboratory, University of Chicago, Chicago, Illinois, AD No. 108849.
20. Petrov, R. V., "Immunology of Acute Radiation," (A Complete Translation of a Russian Language Book by Petrov, Moscow, 1962) Washington OTS: 63-21545, JPRS: 18,620, 9 April 1963, Chaps. III and IV.

