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ANIMAL VIRUSES IN FARM EFFLUENT
PHASE II and III. 1974 - 1976

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for

THE MICROBIOLOGY LABORATORIES
APPLIED RESEARCH DIVISION

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ANIMAL VIRUSES IN FARM EFFLUENT (PHASE II)

by

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CANADA CENTRE FOR INLAND WATERS

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ABSTRACT

Samples of liquid pig manure were collected at weekly intervals from an aeration tank which received weekly additions of raw liquid pig manure, which was also sampled at the same times. Each sample yielded a swine enterovirus after concentration by PE-60 adsorption, but in three comparative titrations, the infectivity titre in concentrates of the raw manure was a thousand times greater than in the aerated waste. In a laboratory model system, a swine enterovirus retained its infectivity for 67 days after seeding into untreated liquid pig manure, but only for 14 days in aerated liquid manure.

Twenty six samples of surface run-off were collected from sites at which liquid pig manure was routinely spread on agricultural land. A swine enterovirus was isolated from two of these samples after concentration by membrane filter adsorption. A swine enterovirus was isolated from surface soil samples collected from hay stubble, 1, 2 and 8 days after aerated pig waste was spread at the rate of 15 tons per acre. The soil samples were suspended in distilled water and concentrated by PE-60 adsorption. Soil samples collected 15 and 22 days after spreading did not yield virus. Thirty three samples of surface water and 36 samples of ground water collected in areas in which liquid pig manure was routinely spread on the land were examined for the presence of viruses after concentration by talc-celite adsorption. A swine enterovirus was isolated from one surface water sample.

Bovine adenovirus type 3 survived in liquid cattle manure for 1, 3 and 28 days at 37°C, 22°C and 4°C respectively. At these temperatures, the same virus survived in water from 1, 3 and 5 days. Three techniques were tested for the concentration and recovery of adenovirus from water. PE-60 adsorption and talc-

celite adsorption were unsatisfactory for this purpose, but membrane filter adsorption resulted in the recovery of 1-10% of the seeded virus.

The talc-celite adsorption procedure was tested for the concentration and recovery of a swine enterovirus from water. In six replicate experiments, the recovery of the virus from the seeded water was between 10-50%. Further concentration of the eluate from talc-celite adsorption was obtained by hydro-extraction with polyvinyl pyrrolidone.

CONCLUSIONS

1. A swine enterovirus was inactivated more rapidly in aerated liquid pig manure than in non-aerated waste.
2. Swine enteroviruses can survive long enough after liquid pig manure is spread on agricultural land to be detected in soil samples, surface run-off and surface water.
3. A bovine adenovirus can survive long enough in cattle waste and in water to constitute a pollution hazard in water supplies.
4. Animal adenoviruses can be concentrated and recovered from water by membrane filter adsorption.
5. Talc-celite adsorption is a satisfactory method for the concentration and recovery of swine enteroviruses from water.

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INTRODUCTION AND BACKGROUND INFORMATION

Previous studies (Derbyshire, 1974) demonstrated the presence of various animals viruses in liquid pig waste, and showed that certain of these viruses could remain infectious in raw waste for sufficiently long periods to constitute a pollution hazard when contaminated waste is distributed on agricultural land. Primarily because of the offensive odour associated with the spreading of liquid manure on the land, biological treatment of this material has been investigated Lawrence, 1971; Loehr, 1971). Various aeration devices (Report, 1972) have given promising results. Information is not available on the effect of aeration of liquid manure on the infectivity of viruses which it might contain, and one of the main objectives of the present study was to investigate this topic. Studies were conducted both in the field, utilizing an experimental liquid manure aeration tank at the University of Guelph, and in the laboratory, where the stability of a porcine enterovirus seeded into liquid pig manure was compared under conditions of aeration and non-aeration.

Since raw liquid pig manure may contain infectious viruses when it is distributed on the land, it was necessary to determine whether infectious virus could be detected in samples of run-off from sites utilized for manure distribution. Thirteen sites were selected on farms in the Guelph area on which pig manure was routinely spread and on each site a device for the collection of surface run-off was established. Each site was routinely visited at least once per week during a period of five months, with additional visits in relation to the occurrence of heavy rainfall, and the water samples obtained were tested in the laboratory, after concentration, for the presence of viruses. At one of the sites, surface soil samples were collected and tested for virus at intervals after manure had been

spread. Samples of surface water were collected from streams, ponds or rivers on eleven of the same farms, on each of three occasions, and these samples were also tested for viruses, along with a similar number of groundwater samples collected on twelve farms. The purpose of this study was to seek evidence for contamination of water supplies with animal viruses associated with the distribution of liquid manure.

The possible contamination of water with viruses with carcinogenic potential is of particular concern, and attention has been given to this aspect of the pollution problem. The stability of an oncogenic virus, bovine adenovirus type 3 (Darbyshire, 1966), in liquid bovine waste and in samples of surface water, was investigated in the laboratory with seeded waste and water samples held at various temperatures. An attempt was also made to establish effective methods for the concentration and recovery of adenoviruses from large volumes of water. The concentration methods investigated included adsorption with the polyelectrolyte PE-60, talc-celite adsorption, with and without further concentration by hydroextraction with polyvinyl-pyrollidinone, and membrane filter adsorption. Samples of river water seeded with bovine adenovirus type 3 or porcine adenovirus type 2 were utilized in these tests.

The talc-celite layer adsorption technique developed by Dr. Westwood and Dr. Sattar at the University of Ottawa (Westwood and Sattar, 1974) for the concentration and recovery of viruses from large volumes of water was selected for further study by the contractors involved in the Environment Virology programme at the Microbiology Laboratories of the Canada Centre for Inland Waters. Our participation in this study consisted of an investigation of the suitability of talc-celite adsorption for the concentration and recovery of a swine enterovirus from large volumes of water. The possibility of further concentration of the eluate from the talc-celite layer by hydroextraction was also studied.

MATERIALS AND METHODS

Collection and Processing of Raw and Aerated Waste for Virus Isolation

Field studies. Samples of raw pig waste were collected at weekly intervals from a liquid manure tank which received effluent from a swine fattening house. Each sample was collected immediately before the tank was emptied and the contents transferred to an aeration tank for biological treatment. At the same time intervals, composite samples of aerated waste were obtained from different levels of the aeration tank. This sampling procedure was continued for a period of 10 weeks, at the end of which the aeration tank was emptied and the contents were distributed on agricultural land. Each sample of raw or aerated waste consisted of a volume of about 2 litres, which was processed in the laboratory as follows. The waste was centrifuged at 6,000 g for 20 minutes, and the supernatant was passed through a serum treated Millipore AP 20 filter. The pH of 1 litre of each filtrate was adjusted to 4.5, and 1.0 ml of a 10% suspension of the insoluble polyelectrolyte PE-60*, prepared as described by Wallis et al (1969), was added and stirred magnetically for 2 hours. The suspension was then filtered through an untreated AP 20 filter pad, the PE-60 was collected from the pad, suspended in 5 ml of Eagle's minimal essential medium (EMEM), the pH adjusted to 8.0 and the suspension centrifuged at 2,000 rpm for 10 minutes. The supernatant was then filtered through a serum treated 0.45μ Millipore filter, and this filtrate, before and after chloroform treatment, was used for the inoculation of primary pig kidney (PK) cell cultures. The cultures were examined daily for at least 7 days after inoculation at which time negative cultures were passaged once more

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* Monsanto Co., St. Louis, Missouri.

in PK cells. Viruses which were isolated were provisionally identified as swine enteroviruses on the basis of cytopathology and chloroform resistance. In addition, the PE-60 concentrates which were prepared from the first, fifth and ninth pairs of samples of raw and aerated waste were titrated by infectivity assay in PK cells.

Laboratory studies. In order to study further the stability of a porcine enterovirus in liquid waste, a laboratory model was set up in which a volume of 1.4 litres of raw liquid pig manure contained in an Erlenmeyer flask was seeded with the T80 strain of porcine enterovirus (Betts, 1960), a stock of which had been prepared in PK cell cultures, at the rate of 100 median tissue culture infectious doses ($TCID_{50}$) per 0.1 ml. The seeded waste was magnetically stirred and continuously aerated by means of the laboratory compressed air supply at room temperature (22°C). Dow Corning Antifoam A was added as required in order to prevent foaming. A similar volume of seeded waste which was not stirred or aerated was included in the experiment for control purposes. Samples for attempted virus isolation were collected twice weekly for up to ten weeks from the raw and aerated waste. Each sample was chloroform treated by the method of Feldman and Wang (1961), and each supernatant after chloroform treatment was used for the inoculation of PK cell cultures. Negative cultures were further passaged once in PK cells.

Collection and Processing of Surface Run-Off for Virus Isolation

Collection sites were selected on 13 farms in the Guelph area. Each site was established in a location in which liquid pig manure was routinely spread on sloping ground. At the foot of the slope in each instance, a collection device, consisting of plastic sheeting providing drainage into a 4 litre plastic container buried beneath the soil surface, was installed. Each site was visited routinely

at weekly intervals, with additonal visits following heavy rainfall in the area. At each visit, samples of less than 2 litres were discarded, and greater volumes were transported to the laboratory, where they were concentrated by the membrane filter adsorption technique. This procedure was carried out as described below for the attempted concentration of adenovirus from river water, except that pre-filtration through serum-treated Millipore AP 20 filters was necessary before 0.45μ filtration. Each concentrate, with and without chloroform treatment, was passaged twice in PK cell cultures. The presence of cytopathogenic agents was always confirmed by further passages in cell cultures, and by re-isolation of the agent from the original concentrate. Agents which were isolated were identified on the basis of cytopathology, size as determined by membrane filtration (Hsiung, 1965), chloroform sensitivity (Feldman and Wang, 1961) and morphology in negatively stained preparations of cell lysates (McFerran et al, 1971).

Collection and Processing of Soil Samples for Virus Isolation

When the aeration tank on which the field studies were made on aerated waste, as described above, was emptied, the contents were spread on hay stubble at the rate of 15 tons per acre. Following spreading of the aerated waste, samples of surface vegetation and soil to a depth of one inch were collected from the site. Volumes of approximately 1 litre of this material were collected 1,2,3,8,15 and 22 days after spreading. Each sample was suspended in distilled water, and the suspension was centrifuged to remove gross particles. The supernatant was then concentrated by adsorption with PE-60 as described above for liquid manure samples, and each concentrate was inoculated onto PK cell cultures, before and after treatment with chloroform. Each sample was passaged at least one more time in PK cells. Any virus which was isolated was provisionally identified on the

basis of cytopathology, chloroform resistance and morphology in negatively stained preparations examined in the electron microscope. In addition to the soil samples, surface run-off was collected from this site 8,17,23,35 and 52 days after spreading of the aerated waste. The collection and processing of the run-off for attempted virus isolation was the same as that described above.

Collection and Processing of Surface Water and Ground Water for Virus Isolation

Twelve of the farms which were used for the collection of surface run-off were utilized for the collection of surface water or ground water. On eleven of these farms, surface water samples were available from ponds, streams or rivers which received run-off from the sites used for the spreading of liquid pig manure, and on each of the twelve farms, ground water was available from a well which provided the supply of farm water. Three samples of surface water and ground water were collected from each farm. Each sample consisted of a volume of 20 litres, which was concentrated by talc-celite adsorption followed by hydroextraction with polyvinyl pyrolidone as described below for the attempted concentration of adenoviruses from river water so that each 20 litre sample was concentrated by a factor of 4,000. Each final concentrate, before and after chloroform treatment, was passaged twice in PK cell cultures, in the same way as the concentrates obtained from the surface run-off samples.

Stability of a Carcinogenic Virus in Waste and in Water

The WBR 1 strain of bovine adenovirus type 3 was selected for study as a potentially carcinogenic virus (Darbyshire, 1969) which is excreted by cattle. For the investigation of the stability of this virus in farm waste, samples of bovine liquid manure were collected from the field, lightly centrifuged to remove

large particles, and distributed in 10 ml volumes in 20 ml screw-capped vials. Each vial was seeded with approximately 1,000 $TCID_{50}$ of the bovine adenovirus, and the seeded waste was incubated at 4°C, 22°C or 37°C. At intervals of 1,2,3, 5,7,14,21 and 28 days after seeding, one vial from each temperature was frozen at - 20°C. For virus isolation, the contents of each vial were thawed, filtered through a 0.45μ Millipore membrane filter (serum-treated) and inoculated in 0.1 ml volumes onto four embryo bovine kidney (EBK) monolayer cell cultures. The infected cultures were examined daily for two weeks for the appearance of cytopathic effects (CPE). Similar procedures were followed for the determination of the stability of the bovine adenovirus in water, except that river water, which was not centrifuged, was used in place of manure, and seeding with virus was with 100 TCD_{50} instead of 1,000 $TCID_{50}$ used for the liquid manure. For the infectivity tests on the seeded water samples after incubation, embryo bovine lung (EBL) cell cultures were used.

Methods Used for the Concentration and Recovery of Adenoviruses from Water

Attempts were made to concentrate and recover adenoviruses from experimentally seeded water by means of three procedures: PE-60 adsorption, talc-celite adsorption and membrane filter adsorption as follows:

PE-60 adsorption. Volumes of 1 litre of river water were seeded with approximately 1,000 $TCID_{50}$ of bovine adenovirus type 3, and each seeded sample was treated with 1.0 ml of PE-60 suspension as described above. The final concentrate in EMEM was assayed for infectivity in EBK cell cultures.

Membrane filter adsorption. This procedure was based on the technique described by Berg, Dahling and Berman (1971), and was conducted as follows. Samples of 2 litres of river water were seeded with approximately 10^5 $TCID_{50}$ of the 6618

strain of porcine adenovirus type 2 (Clarke, Sharpe and Derbyshire, 1967). The seeded water was buffered with 0.5% dibasic sodium phosphate and the pH adjusted to 7.0 with citric acid. The water was then passed through a 0.45μ Millipore filter, utilizing four or five filters to deal with each sample. The filters were then soaked in 10 ml of 3% beef extract and sonicated for 15 minutes for elution of the virus. The disintegrated filter material was removed by centrifugation, and the supernatant was titrated by infectivity assay in PK cell cultures.

Talc-celite adsorption. This procedure was described by Westwood and Sattar (1974), and demonstrated in the author's laboratory by Dr. Sattar. Samples of 20 litres of river water were seeded with 10^4-10^5 $TCID_{50}$ of porcine adenovirus type 2. The pH of the seeded water was adjusted to 6.0 with HCl, and the water was then passed through a layer of 15 g talc and 5 g celite between Whatman #114 filter paper in a modified Buchner funnel at 4^oC. Then, 50 ml of 10% foetal calf serum in saline was passed through the talc-celite layer. The eluate was collected, and titrated in PK cell cultures. This eluate was further concentrated by hydroextraction at 4^oC with polyvinyl pyrrolidone (Bucca, Casey and Winn, 1960), to a volume of 5 ml, and this concentrate was also titrated by infectivity assay in PK cell cultures.

Concentration and Recovery of a Swine Enterovirus from Water by Talc-Celite Adsorption.

The talc-celite adsorption procedure was tested in six replicates for its ability to concentrate a swine enterovirus from seeded samples of river water. Each sample, of 4-10 litres was seeded with 10^5 - 10^6 $TCID_{50}$ of the T80 strain of porcine enterovirus type 2 (Betts, 1960). The seeded samples were processed as described above, and each 50 ml eluate in 10% foetal calf serum which was

obtained was titrated, together with the seed virus, in PK cell cultures. In addition, the eluates obtained from three of the above replicates were further concentrated by hydroextraction with polyvinyl pyrrolidone K-13 (average molecular weight 40,000). The 50 ml eluate was placed in dialysis tubing, which was covered with a layer of polyvinyl pyrrolidone at 4°C. After dialysis for several hours at 4°C, the volume was reduced to rather less than 5 ml, at which time it was adjusted to 5 ml with distilled water. Samples of the original 50 ml eluate and the final 5 ml concentrate were titrated by infectivity assay in PK cells.

EXPERIMENTAL PHASE AND DISCUSSION

Stability of Animal Viruses During Biological Treatment of Farm Waste

All the samples collected, of both raw and aerated waste, yielded viruses upon concentration by the PE-60 adsorption procedure, and in each instance the virus which was isolated was identified as a porcine enterovirus on the basis of the cytopathology in PK cell cultures, and the chloroform resistance of the virus. Cytopathic effects (CPE)usually appeared earlier in the cultures inoculated with the concentrates from the raw waste. The aerated waste was usually less cytotoxic than the raw waste samples. The results obtained when the concentrates of the raw and aerated waste collected in the first, fifth and ninth weeks of the experiment were titrated in PK cells are given in Table 1. It will be seen that the titre of enterovirus in the raw waste was always at least one thousandfold greater than in the aerated waste. This suggests a significant degree of viral inactivation in the aeration tank, in spite of the addition of fresh, raw waste at weekly intervals. Whether the inactivation which occurred was specifically associated with the aeration procedure, or whether it represented thermal inactivation on storage could not be determined under field conditions, and this problem was investigated in the laboratory model described below.

Some technical problems were encountered in the development of a satisfactory laboratory model for the study of the aeration of liquid manure. In the early experiments, aeration by means of a small aquarium pump was inadequate, as determined by Eh measurements. When the aeration was improved by the use of a compressed air supply, foaming of the aerated waste was encountered, and this was overcome by the use of Antifoam A, and in the model system described under Materials

TABLE 1. TITRATION OF CONCENTRATES OF RAW AND AERATED WASTE SAMPLES

Sample week #	Infectivity titres (Log $TCID_{50}$ per ml)	
	Raw waste concentrate	Aerated waste concentrate
1	4.25	1.0
5	4.0	1.0
9	4.75	1.0

and Methods, satisfactory, continuous aeration for a period of 71 days was obtained. Throughout this period, samples collected from the seeded aerated and control waste were tested for infectivity in PK cells. T80 virus was isolated on first passage in PK cells from the samples collected from the aerated manure four and seven days after seeding, and on second passage from the samples from the aerated manure collected 11 and 14 days after seeding. Thereafter, no virus isolations from the aerated waste were made. The control seeded waste was more highly cytotoxic than the aerated waste, and virus isolation on first passage in PK cells was only possible from the control waste sample collected four days after seeding. However, T80 virus was isolated on second passage from the samples collected twice weekly for 67 days after seeding. The final sample collected from the non-aerated waste 71 days after seeding did not yield virus on second passage in PK cells.

It appears from these findings that T80 virus is inactivated more rapidly in aerated waste than in untreated liquid manure. The mechanism of inactivation is not known, and further studies with additional manure samples and other viruses are required. Knowledge of the mechanisms involved in viral inactivation in aerated waste would also be desirable.

Isolation of Viruses from Surface Run-off

A total of 26 samples of surface run-off were obtained from agricultural land on which liquid pig manure was routinely spread during the summer and fall of 1974. Four samples were collected in July, two in August, ten in September, seven in October and three in November from the 13 collection sites which were established. The collection of a satisfactory sample was dependent upon the occurrence of local rainfall during the sampling period. Each of six of the collection sites yielded only a single sample of greater than 2 litres, three

sites yielded two samples each, three samples were obtained from each of three sites and five samples were obtained from one site. Two confirmed isolations of swine enterovirus were obtained from the surface run-off samples, each from a different location, and both collected at the end of July. During the processing of an additional two samples, from two different sites, cytopathic effects were seen in the second passage of the concentrates in PK cells, but these were not confirmed when attempts were made to reisolate a virus from the concentrate of the original sample, and these latter isolation are regarded as equivocal.

Additional observations, involving the collection of surface soil samples were made at one of the above run-off sites following the spreading of aerated liquid pig manure which had been shown to contain a porcine enterovirus. The soil samples collected 1,2 and 8 days after spreading each yielded a swine enterovirus after concentration with PE-60, while the soil samples obtained 3,15 and 22 days after spreading were negative. Surface run-off was collected at this site 8,17,23,35 and 55 days after the manure was spread, each sampling after the occurrence of heavy rain in the area, but no virus was isolated from these run-off samples.

The above findings indicate that a porcine enterovirus can remain viable in surface soil for a period of at least 8 days after deposition in contaminated liquid manure. The virus can also be eluted from the soil surface by rain water, and may be present in a viable form in concentrations of at least 1 $TCID_{50}$ per 20 ml of surface run-off. This concentration of infectious virus in the run-off was calculated for the observations made on the two positive samples assuming 100% recovery of virus by the membrane filter adsorption technique and no inactivation of the virus in the collection vessel before concentration. Since

some viral infectivity was probably lost at both of these stages, the actual concentration of virus in fresh run-off may well have been at least ten times greater than this.

Isolation of Viruses from Surface Water and Ground Water

Thirty three samples of surface water and 36 samples of ground water were tested between November 1974 and January 1975. The only virus isolated from this material was a swine enterovirus from a surface water sample collected in January. The titre of virus in this water was calculated to be about 1 $TCID_{50}$ per 40 ml, assuming 10% recovery of virus by the talc-celite adsorption hydro-extraction concentration procedure which was used. This finding suggests that, on occasion, surface run-off agricultural land on which raw waste is spread may result in the presence of detectable amounts of infectious animal viruses in surface water.

Stability of Bovine Adenovirus Type 3 in Waste and in Water

The results obtained in these experiments are given in Table 2. The seeding level of adenovirus type 3 is higher in liquid manure than in water and should be considered in the examination of these data.

In liquid manure held at $4^{o}C$, infectivity was detected 28 days after seeding, and this period of survival was longer than that recorded in earlier studies for a porcine adenovirus in pig waste (Derbyshire, 1974). The virus survived for a shorter period in water at $4^{o}C$ than in liquid manure, while survival at $22^{o}C$ and $37^{o}C$ was similar in both water and liquid manure. Further studies of the stability of this and other viruses in water and waste under more closely simulated field conditions are required.

Concentration and Recovery of Adenoviruses from Water

Duplicate experiments were conducted on seeded water with each of the three concentration methods, and the results obtained are given in Table 3. PE-60 adsorption and talc-celite adsorption appeared to be unsatisfactory for the concentration of these adenoviruses from water. In previous studies (Derbyshire, 1974), PE-60

TABLE 2. STABILITY OF BOVINE ADENOVIRUS TYPE 3 IN LIQUID MANURE AND IN WATER

Sample	Seeding level ($TCID_{50}$/ml)	Duration of infectivity (days) at various temperatures		
		4°C	22°C	37°C
Liquid manure	100	> 28	3	1
Water	10	5	3	1

TABLE 3. CONCENTRATION AND RECOVERY OF ADENOVIRUSES FROM WATER

Concentration method	Replicate #	% Recovery of virus
PE-60 adsorption	1	nil
	2	1%
Membrane filter adsorption	1	1%
	2	10%
Talc-celite adsorption	1	nil
	2	1%

adsorption was found to be unsatisfactory for the concentration of bovine and porcine adenoviruses from liquid cattle or pig waste, because of the highly variable results obtained, but the use of the talc-celite adsorption procedure for the concentration of these viruses has not previously been described. Membrane filter adsorption gave recoveries of adenovirus of 1% or 10%, and this procedure holds some promise for successful application under field conditions. England (1972) reported the use of protamine sulfate for the concentration of human adenoviruses from sewage and effluents. We did not attempt to use this procedure in our studies because of the very high costs which would be involved for the treatment of large volumes of water by this technique.

Concentration and Recovery of a Swine Enterovirus by Talc-Celite Adsorption

The results obtained in the six replicate trials of this procedure are given in Table 4. The recoveries of virus shown are computed from the results obtained in parallel infectivity titrations of the seed virus stock and the 50 ml eluates from the talc-celite layers. The recoveries obtained, of between 10 and 50%, indicate that talc-celite adsorption is a satisfactory procedure for the recovery of a swine enterovirus from water, and justify the use of the technique for the concentration of viruses from the surface and ground water samples reported above. Three of the 50 ml eluates were further concentrated by hydroextraction, as described under Materials and Methods. When these final concentrates were titrated in PK cells, no loss of infectivity was detected, and the concentrates were not significantly cytotoxic. Hydroextraction in this way thus provides for a further tenfold concentration of the original sample, and appears to be a worthwhile procedure for routine use.

TABLE 4. CONCENTRATION AND RECOVERY OF A SWINE ENTEROVIRUS FROM WATER BY TALC-CELITE ADSORPTION

Replicate #	Volume of water (litres)	Infectivity titre of virus seed (log $TCID_{50}$)	% recovery of virus
1	4	5.0	10
2	7.5	6.0	10
3	10	5.0	10
4	10	5.0	10
5	10	5.0	16
6	8	5.0	50

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ANIMAL VIRUSES IN FARM EFFLUENT (PHASE III)

by

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for

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ABSTRACT

Inactivation of a porcine enterovirus in water occurred more rapidly under simulated field conditions than in the laboratory. The virus was more stable in sterile distilled water than in river water. It was inactivated more rapidly in water than in liquid pig manure. Inactivation of a porcine adenovirus also occurred more rapidly in water than in liquid pig manure, particularly under simulated field conditions. A bovine enterovirus was consistently more stable under simulated field conditions than in the laboratory, in both water and liquid cattle manure.

A porcine enterovirus, a porcine adenovirus and a bovine enterovirus were all inactivated more rapidly in aerated liquid manure than in non-aerated manure, under laboratory conditions. The difference in inactivation rate was least marked for the bovine enterovirus. Inactivation of a porcine enterovirus in aerated sterile distilled water and in aerated, autoclaved liquid pig manure proceeded at a similar rate as in the same materials which were not aerated.

A porcine enterovirus and a bovine adenovirus which were seeded into liquid pig manure, and a bovine enterovirus seeded into liquid cattle manure, were inactivated by treatment of the manure with calcium hydroxide at pH 11.5. The rate of inactivation was highest for the bovine enterovirus and slowest for the swine adenovirus.

Five porcine enteroviruses which had been isolated from liquid pig manure, surface soil, surface run-off and surface water samples were passed three times in HeLa, BS-C, WI-38 and primary monkey kidney cell cultures. Cytopathic effects were seen only in BS-C and monkey kidney cell cultures inoculated with the virus

isolated from liquid pig manure, and only this virus replicated in the same cultures. No replication occurred in the human cells.

Thirteen samples of sewage and the same number of sewage effluents were collected from a municipal sewage treatment plant which served an area containing six abattoirs. Each sample of 20 litres was concentrated by talc-celite adsorption followed by hydroextraction. Each concentrate was passaged twice through bovine kidney and porcine kidney cell cultures for the attempted isolation of bovine and porcine viruses, but no cytopathic effects were seen.

No virus neutralizing antibodies were detected in serum samples from the two technicians engaged in this project when they were tested against the five porcine enteroviruses which had been isolated from liquid manure, soil or water.

CONCLUSIONS

1. Certain viruses excreted by swine and cattle were sufficiently stable in liquid manure and in water under field conditions to constitute a viral pollution hazard in the environment arising from the disposal of liquid manure on farm land.
2. Aeration of liquid animal manure showed promise as a means of reducing the infectivity of viruses which it might contain.
3. Enteroviruses and an adenovirus were rapidly inactivated by calcium hydroxide treatment of liquid manure under laboratory conditions.
4. An enterovirus isolated from liquid pig manure replicated in monkey kidney cell cultures, but neither this nor four other enteroviruses replicated in human cells.
5. Viruses were not isolated on bovine or porcine cell cultures from samples of sewage or sewage effluent which were collected at a municipal sewage plant.
6. No serological evidence of infection with representative viruses isolated in these studies was found in the personnel involved in the project.

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INTRODUCTION AND BACKGROUND INFORMATION

Previous studies (Derbyshire, 1974, 1975) indicated that various viruses could survive in liquid manure and in water under laboratory conditions for sufficiently long periods to constitute a potential hazard when contaminated liquid manure is distributed on agricultural land. One of the main objectives of the current research was to obtain information on the stability of viruses in liquid manure and in water under conditions which more closely simulated those encountered in the field. Samples of liquid manure and river water were seeded with various viruses, placed into dialysis tubes and submerged in liquid manure tanks or in a stream. The infectivity of the viruses was assayed at intervals. The viruses studied were a porcine enterovirus, a porcine adenovirus and a bovine enterovirus.

Preliminary evidence had already been obtained (Derbyshire, 1975) that a swine enterovirus was less stable in aerated liquid pig manure than in nonaerated manure. These observations were confirmed for the swine enterovirus in quantitative studies, and extended to include studies with a swine adenovirus in liquid pig manure and a bovine enterovirus in liquid cattle manure. Experiments relating to the mechanism of inactivation produced by aeration suggested that the aerobic bacterial flora of aerated waste, rather than the oxygen tension itself, was involved.

Experiments reported by Sattar and Westwood (1974) with domestic sewage contamined with poliovirus indicated that treatment of the sewage with calcium hydroxide to raise the pH produced highly significant reductions in viral infectivity. This procedure was applied on a laboratory scale, to liquid swine or cattle manure which were seeded with a porcine enterovirus, a porcine adenovirus or a bovine

enterovirus. The survival of each virus, in the supernatant and sludge, was assayed by infectivity titrations on these materials during settling periods of up to 24 hours.

In a previous study, the isolation of swine enteroviruses from liquid pig manure, surface soil from land on which pig manure had been spread, run-off from agricultural land, and surface water samples were described. Primary pig kidney cell cultures were used for the isolation of these viruses. Five representative viruses were selected for the determination of their infectivity for human and other primate cell cultures. One virus had been isolated from liquid pig manure, one from surface soil, two from samples of surface run-off and one from surface water. Two human cell lines, HeLa and WI-38, primary monkey kidney cells and the BS-C monkey kidney cell line were used. Each virus was passaged three times in each culture, which was examined microscopically for the presence of cytopathic effects, and the harvest from each passage was titrated for infectivity in pig kidney cell cultures.

In studies elsewhere (Malherbe et al., 1967) liquid wastes from a large municipal abattoir were examined for the presence of viruses on monkey, calf and lamb cell cultures. Enteroviruses, adenoviruses and reoviruses were isolated, mainly from intestinal washings of cattle and sheep. In the present study, arrangements were made with the Kitchener (Ontario) Water Pollution Control Centre for the collection of samples of raw sewage and sewage effluent for the attempted isolation of bovine or porcine viruses. This sewage plant was selected because it receives sewage from six abattoirs in the Kitchener area where cattle and swine are routinely slaughtered, in order to determine whether such sewage contained detectable viruses of these species. Thirteen samples of raw sewage

and the same number of sewage effluents were collected, concentrated by talc-celite adsorption and passaged twice in pig kidney and bovine kidney cell cultures.

Serum was collected from the two technicians who have assisted in the research during the period 1973-1976, and these samples were tested for virus neutralizing antibodies against representative swine enteroviruses which had been isolated during the course of these studies. The viruses used in these tests were the same as those which were passaged in the human and monkey cell cultures.

MATERIALS AND METHODS

Stability of Viruses in Liquid Manure and in Water under Simulated Field Conditions

Viruses. The viruses used were (a) the T80 strain of porcine enterovirus type 2 (Betts, 1960), (b) the 6618 strain of porcine adenovirus type 3 (Clarke et al., 1967), and (c) a bovine enterovirus isolated by the author from surface run-off from a calf rearing operation in the course of another research project. The swine viruses were cultivated and assayed in primary pig kidney cell cultures, and embryonic bovine kidney cell cultures were used for the bovine enterovirus.

Field sites. Three sites were used for these tests. They were (a) a small stream on the Elora Research Station of the University of Guelph, utilized for the study of the stability of each of the above viruses in surface water, (b) a cattle liquid manure tank at the Elora Research Station, which receives effluent from a dairy operation, used for the study of the stability of the bovine enterovirus in manure, and (c) a swine liquid manure tank, at the Arkell Research Station at the University of Guelph, which receives effluent from a swine fattening house, used for the study of both of the porcine viruses in manure.

Stability test procedure. The method used was based on that described by Herrmann et al. (1974) in their work on the persistence of human enteroviruses in lake water. Water and liquid manure were collected from the sites described above, and seeded with appropriate dilutions of the virus under test. The seeded water or liquid manure was then distributed in aliquots of 10 ml, into a series of dialysis tubes for study under simulated field conditions, and into vials for comparative laboratory tests. The dialysis tubes were attached, for protection, to small animal cages, which were then submerged beneath the surface of the stream or in the appropriate liquid manure tank. The vials were held in the laboratory

at room temperature. Immediately after seeding, and at intervals thereafter, seeded samples of water and liquid manure were collected, chloroform treated by the method of Feldman and Wang (1961) to control bacterial contamination, and then titrated by infectivity assay in the appropriate cell culture system.

Effect of Aeration of Liquid Manure on Viral Infectivity

In these experiments, porcine enterovirus type 2, porcine adenovirus type 3 and bovine enterovirus were used. The porcine viruses were seeded in liquid swine manure from the Arkell Research Station, and the bovine enterovirus was seeded in liquid cattle manure from the Elora Research Station. In one experiment, a porcine enterovirus was studied in aerated distilled water, and in autoclaved liquid pig manure in another experiment. The procedure followed with all samples was as follows. The liquid sample under investigation was seeded with an appropriate dilution of the virus under test, and then divided into two aliquots of 1.4 litres which were placed in Ehrlenmeyer flasks. The contents of one flask were magnetically stirred and continuously aerated by means of the laboratory compressed air supply, at room temperature, while the second flask was neither stirred nor aerated. Dow Corning Antifoam A was added as required in order to prevent foaming. Immediately after seeding, and at twice weekly intervals thereafter, the flasks were sampled, and the samples were titrated for viral infectivity in either pig kidney or embryonic bovine kidney cell cultures, after chloroform treatment to control bacterial contamination.

Calcium Hydroxide Treatment of Liquid Manure

The procedures used were based on those described by Sattar and Westwood (1974). Liquid swine manure was seeded with porcine enterovirus type 2 or bovine adenovirus type 3, and liquid cattle manure was seed with bovine enterovirus. One litre volumes of the liquid manure were seeded with a suitable dilution of the appropriate virus,

and immediately after seeding a sample was collected for viral infectivity assay. The seeded manure was continuously stirred while a slurry of lime (calcium hydroxide) was added to increase the pH to 11.5, after which stirring was continued for a further 15 minutes. The manure was then transferred to an Imhoff cone and the sludge allowed to settle. Settling periods of 1, 3 and 24 hours were employed for each virus. At the end of the settling period, a sample of the relatively clear supernatant was collected, the pH adjusted to 7.2 with N.HCl, and retained for infectivity assay. The pH of the sludge was adjusted to 7.2, and the sludge was centrifuged at 1500 g for 30 min. The deposit was resuspended in 20 ml of 10% foetal calf serum in saline and stirred for 15 min. The suspension was again centrifuged, and a sample of the supernatant was collected for infectivity assay. Each of the samples was chloroform treated to destroy bacterial contaminants, and titrated for infectivity in the appropriate cell cultures.

Infectivity of Viruses Isolated from Farm Waste and Water for Human and Other Primate Cell Cultures

Viruses. Five viruses isolated in the course of previous studies (Derbyshire, 1975) from liquid pig manure, soil or water were designated as follows:

PM - isolated from liquid pig manure

S - isolated from surface soil on which liquid pig manure had been spread

RO#1 and RO#2 - isolated from surface run-off from agricultural land on which liquid pig manure had been spread

SW - isolated from a sample of surface water collected on a farm on which liquid pig manure was routinely spread on the land.

All of the above had been previously identified as swine enteroviruses, and a stock of each was prepared and titrated for infectivity in pig kidney cell cultures.

Cell cultures. Four cell types were used. Cultures of WI-38 and primary monkey kidney cells were obtained from Connaught Laboratories Limited, Willowdale, Ontario. The HeLa and BS-C-1 cell lines had been maintained in the author's laboratory for several years. The cells were cultivated in Eagle's minimal essential medium supplemented with foetal calf serum.

Procedure. Each stock swine enterovirus was inoculated in 0.1 ml volumes into 4 tubes of each of the above cell cultures. The inoculated cultures were examined daily for cytopathic effects (CPE), and frozen at -20°C when CPE was complete, or after incubation for 7 days. After freezing, the contents of the tubes were thawed, titrated for infectivity in pig kidney cells, and subjected to a further passage in four more tubes of the appropriate cell culture. These steps were repeated a second time to give a total of three passages of each virus in each cell culture, with infectivity titrations on each harvest, and daily microscopical examination for CPE throughout each passage.

Examination of Sewage and Sewage Effluents for Bovine and Porcine Viruses

Thirteen samples of raw sewage and 13 samples of sewage effluent were collected from the Kitchener Water Pollution Control Centre at the rate of two samples of each per week. Each sample consisted of a volume of 20 litres, and each was concentrated by the talc-celite adsorption method developed by Westwood and Sattar (1974). The procedure followed was exactly as described previously (Derbyshire, 1975) and included further concentration of the eluate from talc-celite adsorption by hydroextraction at 4°C with polyvinyl pyrrolidone, to a volume of 5 ml. The volume which passed through the talc-celite layer varied from 5 to 10 litres of raw sewage, and from 8 to 20 litres of sewage effluent. Each final concentrate was chloroform treated to control bacterial contamination,

and inoculated onto monolayer cell cultures of primary pig kidney and embryonic bovine kidney cells. After incubation of these cultures for 7 days, a second passage was made in the same cell type. The inoculated cultures were examined daily for the development of CPE.

Virus Neutralizing Antibody Tests on Sera from Technicians Engaged in the Project

Serum samples were collected from the two technicians who assisted in this project, from Mr. Earl Brown in April 1975, and from Miss Patricia Roche in March 1976. The sera were titrated for neutralizing antibodies against the five swine enteroviruses which had been isolated from liquid pig manure, soil or water, and which were described above. The procedure used in the virus neutralization tests was described by Derbyshire and Jessett (1968).

EXPERIMENTAL PHASE AND DISCUSSION

Stability of Viruses in Liquid Manure and in Water under Simulated Field Conditions

Porcine enterovirus type 2. The results obtained with this virus are given in Table 1. Viral infectivity in water was lost more rapidly under simulated field conditions than in the laboratory. The tests on the seeded liquid manure under simulated field conditions had to be discontinued after 10 days due to the disintegration of the dialysis tubes in this experiment and also in a replicate experiment. However, the data obtained up to 10 days indicated more rapid viral inactivation in the laboratory than in the manure tank. It was also apparent that this virus was inactivated more rapidly in water than in liquid manure.

Porcine adenovirus type 3. As shown in Table 2, the observations on this virus were restricted to a period of 9 days after seeding because of the problem of disintegration of the dialysis tubes in liquid pig manure described above. As with the porcine enterovirus, inactivation occurred more rapidly in water than in liquid manure, particularly in the water samples tested under simulated field conditions. In liquid manure there was little difference in the inactivation rate under field or laboratory conditions during the period of the experiment.

Bovine enterovirus. No disintegration of the dialysis tubes occurred in liquid cattle manure, and the results obtained with this virus are given in Table 3. The virus was consistently more stable under simulated field conditions than in the laboratory, in both water and liquid manure. This observation contrasted with the findings reported above for the two swine viruses, and the bovine enterovirus was more stable in the water environment than the latter viruses. This greater stability may relate to the source of the bovine enterovirus, which

TABLE 1. THE INACTIVATION OF PORCINE ENTEROVIRUS TYPE 2 IN WATER AND IN LIQUID MANURE UNDER SIMULATED FIELD CONDITIONS AND IN THE LABORATORY

Sample	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity				
		3	7	10	14	21
		Days After Seeding				
Seeded Water						
Field tests	4.5	99.997	≥99.999*	≥99.999	≥99.999	≥99.999
Laboratory tests	4.5	69.4	96.9	99.997	≥99.999	≥99.999
Seeded Manure						
Field tests	6.25	44.0	96.9	99.0	NT**	NT
Laboratory tests	6.25	68.5	99.0	99.05	≥99.999	≥99.999

* 99.999 - Viral infectivity not detected.

** NT - Not tested.

TABLE 2. THE INACTIVATION OF PORCINE ADENOVIRUS TYPE 3 IN WATER AND IN LIQUID MANURE UNDER SIMULATED FIELD CONDITIONS AND IN THE LABORATORY

Sample	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity				
		1	2	3	6	9
		Days After Seeding				
Seeded Water						
Field tests	3.5	≥99.9*	≥99.9	≥99.9	≥99.9	≥99.9
Laboratory tests	3.5	99.1	99.7	≥99.9	≥99.9	≥99.9
Seeded Manure						
Field tests	4.0	0.0	44.0	87.0	90.0	94.4
Laboratory tests	4.0	0.0	0.0	44.0	68.4	90.0

* 99.9 - Viral infectivity not detected.

TABLE 3. THE INACTIVATION OF A BOVINE ENTEROVIRUS IN WATER AND IN LIQUID MANURE UNDER SIMULATED FIELD CONDITIONS AND IN THE LABORATORY

Sample	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity					
		3	7	10	14	17	22
		Days after Farrowing					
Seeded Water							
Field tests	4.5	0.0	68.4	68.4	43.7	90.0	94.4
Laboratory tests	4.5	68.4	94.4	99.7	99.910	99.97	99.99
Seeded Manure							
Field tests	4.0	44.0	68.4	68.4	90.0	90.0	99.4
Laboratory tests	4.0	90.0	94.4	96.9	99.90	99.95	≥99.99*

* 99.99 - Viral infectivity not detected.

was isolated from a sample of run-off water, while the swine viruses were originally isolated directly from infected animals and had subsequently been passaged a number of times in the laboratory. The bovine enterovirus may represent a selected clone of high environmental resistance.

Effect of Aeration of Liquid Manure on Viral Infectivity

Porcine enterovirus type 2. The results obtained with this virus are given in Table 4. Inactivation in the aerated manure was complete in 10 days, but not until 24 days in the non-aerated manure. The rate of inactivation was clearly greater in the aerated manure. These findings confirmed and extended the qualitative observations reported previously for this virus.

Porcine adenovirus type 3. Inactivation of porcine adenovirus type 3 was much more rapid in aerated than in non-aerated waste (Table 5), and occurred somewhat more rapidly than for the swine enterovirus described above.

Bovine enterovirus. The results obtained for the inactivation of a bovine enterovirus in liquid cattle manure are given in Table 6. Infectivity titres were consistently lower in the aerated manure than in the non-aerated samples, but the differences were relatively small, and the greater inactivation rate in aerated manure was less clearly defined than for the swine viruses in liquid pig manure.

Mechanism of inactivation in aerated samples. This was investigated in two experiments. In the first experiment, porcine enterovirus type 2 was seeded into sterile distilled water in place of liquid pig manure. One seeded sample was aerated as for liquid manure, while the second sample was not aerated. The results are given in Table 7. It will be seen that in contrast to the results

TABLE 4. THE INACTIVATION OF PORCINE ENTEROVIRUS TYPE 2 IN AERATED AND NON-AERATED LIQUID PIG MANURE

Liquid Manure	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity						
		3	7	10	13	17	21	24
		Days After Seeding						
Aerated	3.5	97.9	99.7	≥99.9*	≥99.9	≥99.9	≥99.9	≥99.9
Not aerated	3.0	82.2	68.4	90.0	98.2	99.0	99.5	≥99.9

* 99.9 - Viral infectivity not detected.

TABLE 5. THE INACTIVATION OF PORCINE ADENOVIRUS TYPE 3 IN AERATED AND NON AERATED PIG MANURE

Liquid Manure	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity					
		3	6	10	13	17	20
		Days After Seeding					
Aerated	3.7	99.78	≥99.9*	≥99.9	≥99.9	≥99.9	≥99.9
Not aerated	4.0	68.4	68.4	82.0	82.0	99.8	≥99.99

* 99.9 - Viral infectivity not detected.

TABLE 6. THE INACTIVATION OF A BOVINE ENTEROVIRUS IN AERATED AND NON-AERATED LIQUID CATTLE

Liquid Manure	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity						
		1	3	7	11	15	18	21
		Days After Seeding						
Aerated	3.5	68.4	90.0	99.8	99.0	99.7	≥99.9*	≥99.9
Not aerated	3.5	-56.0**	68.4	94.4	97.9	99.0	99.8	≥99.9

* 99.9 - Viral infectivity not detected.

** - Viral infectivity due to density variations was found to be 156% of original inoculum.

TABLE 7. THE INACTIVATION OF PORCINE ENTEROVIRUS TYPE 2 IN AERATED AND NON-DISTILLED WATER

Liquid Manure	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity							
		1	4	7	11	14	19	21	25
		Days After Seeding							
Aerated	4.25	45.0	45.0	68.9	68.9	82.5	82.5	94.5	98.3
Non aerated	3.75	82.2	90.0	94.4	96.8	94.4	98.3	99.7	99.9

obtained with liquid manure, viral inactivation did not occur more rapidly in the aerated water than in non-aerated water - in fact, inactivation proceeded somewhat more rapidly in the non-aerated sample. The inactivation rate of the porcine enterovirus was lower in distilled water than in river water as determined in earlier experiments described above. This latter finding corresponded to similar observations made by Herrmann et al. (1974) who showed that two human enteroviruses were inactivated more rapidly in a lake than in sterile lake water. In the second experiment, the rate of inactivation of porcine enterovirus type 2 was studied in liquid pig manure which was autoclaved before it was seeded with the virus (Table 8). There was no significant difference between the infectivity titres observed in the aerated and non-aerated manure. Inactivation proceeded more slowly than in raw manure as determined in earlier experiments. These findings suggested that the rapid inactivation of the enterovirus in aerated, raw, pig manure is not due to a direct effect of the increased oxygen tension, but is more likely associated with microbial degradation of the viral capsids by microbial species whose growth is favoured by the aerobic conditions produced by aeration of the manure.

Calcium Hydroxide Treatment of Liquid Manure

Porcine enterovirus type 2. The results obtained are shown in Table 9. No infectivity was detected in any of the supernatants, or in the eluted sludge after settling for 3 or 24 hours.

Porcine adenovirus type 3. As will be seen in Table 10, this virus was inactivated less rapidly by lime treatment, than the swine enterovirus in liquid pig manure, as infectivity was readily detected in both supernatant and eluted

TABLE 8. THE INACTIVATION OF PORCINE ENTEROVIRUS TYPE 2 IN AUTOCLAVED AERATED AND NON-AERATED LIQUID PIG MANURE

Autoclaved Pig Manure	Titre at 0 Day Log $TCID_{50}$/ml	Percent Loss of Viral Infectivity							
		1	4	7	11	14	19	21	25
		Days After Seeding							
Aerated	4.0	44.0	0.0	68.4	90.0	94.4	96.9	99.5	99.9
Not aerated	3.5	0.0	-77.0*	43.1	0.0	68.4	90.0	99.0	99.0

* Viral infectivity due to density variations was found to be 176% of original inoculum.

TABLE 9. THE INACTIVATION OF PORCINE ENTEROVIRUS TYPE 2 by CALCIUM HYDROXIDE TREATMENT OF LIQUID PIG MANURE

Settling Time (Hours)	Infectivity Titres (Log $TCID_{50}$ per ml)		
	Seeded Manure	Supernatant	Eluted Sludge
1	4.25	0*	2.5
3	4.5	0	0
24	4.5	0	0

* 0 - Viral infectivity not detected.

TABLE 10. THE INACTIVATION OF PORCINE ADENOVIRUS TYPE 3 BY CALCIUM HYDROXIDE TREATMENT OF LIQUID PIG MANURE

Settling Time (Hours)	Infectivity Titres (Log $TCID_{50}$ per ml)		
	Seeded Manure	Supernatant	Eluted Sludge
1	4.25	4.0	3.25
3	4.0	3.25	2.75
24	4.25	0*	0

* 0 - Viral infectivity not detected.

sludge after settling for 3 hours, but no virus was detected after a 24 hour settling period.

Bovine enterovirus. This virus when seeded into liquid cattle manure was inactivated very rapidly at high pH, since infectivity was not detected in supernatant or eluted sludge samples after a settling period as short as 1 hour (Table 11).

The results obtained in these experiments suggest that lime treatment of liquid animal manure might prove to be an effective method for the removal and inactivation of viruses, and they correspond with the findings of Sattar and Westwood (1974) with poliovirus in domestic sewage. The application of the procedure to liquid animal manure under field conditions would require attention from the engineering aspect at this stage.

Infectivity of Viruses from Farm Waste and Water for Human and Other Primate Cell Cultures

The results of the infectivity titrations on the passages of each of the five viruses isolated from liquid manure, soil or water, in BS-C-1, HeLa, WI-38 and monkey kidney cell cultures are given in Table 12 and 13. Cytopathic effects were produced only by virus strain PM, isolated from liquid pig manure, in BS-C and monkey kidney cells, and this was the only virus for which there was evidence of replication in any of the four cell types. The multiplication of some swine enteroviruses in monkey kidney cells was recognised by Hancock et al. (1959), Bohl et al. (1960) and by Moscovici et al. (1959). Attempts to cultivate the same viruses in human cells have been uniformly unsuccessful (Moscovici et al., 1959; Bohl et al., 1960; Kalter, 1960), and these findings

TABLE 11. THE INACTIVATION OF A BOVINE ENTEROVIRUS BY CALCIUM HYDROXIDE TREATMENT OF LIQUID CATTLE MANURE

Settline time (Hours)	Infectivity Titres (Log $TCID_{50}$ per ml)		
	Seeded Manure	Supernatant	Eluted Sludge
1	3.0	0*	0
3	3.0	0	0
24	2.75	0	0

* 0 - Viral infectivity not detected.

TABLE 12. PASSAGE OF FIVE PORCINE ENTEROVIRUSES ISOLATED FROM LIQUID MANURE, SOIL OR WATER, IN BS-C-1 AND HeLa CELL CULTURES

	Infectivity Titres in Pig Kidney Cell Cultures (Log $TCID_{50}$ per ml)						
		Passages in BS-C-1 Cells			Passages in HeLa Cells		
Virus Strain	Stock Virus	1	2	3	1	2	3
PM	6.5	7.0*	6.75*	7.0*	3.5	0	0
S	4.5	1.25	0**	0	2.75	0	0
RO#1	8.25	6.0	3.0	1.25	5.75	2.75	0
RO#2	6.0	5.0	2.5	0	4.0	1.5	0
SW	7.75	5.5	2.75	0	5.5	2.5	0

* Cytopathic effects seen in these cells.

** 0 - Viral infectivity not detected.

TABLE 13. PASSAGE OF FIVE PORCINE ENTEROVIRUSES ISOLATED FROM LIQUID MANURE, SOIL OR WATER, IN PRIMARY MONKEY KIDNEY (MK) AND WI-38 CELL CULTURES

	Infectivity Titres in Pig Kidney Cell Cultures (Log $TCID_{50}$ per ml)						
		Passages in MK Cells			Passages in WI-38 Cells		
Virus Strain	Stock Virus	1	2	3	1	2	3
PM	5.5	3.5	6.5*	5.25*	2.25	1.75	0
S	4.5	1.75	0**	0	1.75	0	0
RO#1	7.75	5.5	3.5	3.25	3.75	2.25	1.75
RO#2	5.5	3.5	2.5	0	2.25	1.75	0
SW	7.25	4.5	4.5	2.5	4.5	2.75	1.75

* Cytopathic effects seen in these cells.

** 0 - Viral infectivity not detected.

are confirmed by the present study. It seems unlikely that these particular viruses, which have been identified as environmental pollutants, could set up infections in man.

Examination of Sewage and Sewage Effluents for Bovine and Porcine Viruses

No cytopathic effects were seen in either the bovine or porcine cell cultures in which concentrated sewage and sewage effluents were passaged. This finding suggests that there was at least no high level of contamination of these samples with bovine or porcine viruses of abattoir origin. Two factors probably contribute to this situation. In modern abattoir practice, highly contaminated materials would be sterilized before discharge into the sewage system, and the degree of dilution of such contaminated materials in a large municipal system would be high.

Virus Neutralizing Antibody Tests on Sera from Technicians Engaged in the Project

The virus neutralization tests on each of the two sera against the five porcine enteroviruses which were used all yielded negative results. This finding suggests that exposure of the technicians to these viruses during the course of their duties in the laboratory failed to result in infection, and it corresponds to the observation recorded above that the same viruses failed to replicate in cell cultures of human origin.

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