APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1982, p. 1051-1056
0099-2240/82/051051-06$02.00/0

Vol. 43, No. 5

Inactivation by Gamma Irradiation of Animal Viruses in Simulated Laboratory Effluent

F. C. THOMAS,[1]* T. OUWERKERK,[2] AND P. McKERCHER[3]

Agriculture Canada, Animal Diseases Research Institute, NEPEAN, Nepean, Ontario, Canada K2H 8P9[1]; Atomic Energy of Canada Ltd., Commercial Products, Ottawa, Ontario, Canada K2A 3W3[2]; and U.S. Department of Agriculture, Plum Island Animal Disease Center, Greenport, New York 11944

Received 26 October 1981/Accepted 2 February 1982

Several animal viruses were treated with gamma radiation from a ^{60}Co source under conditions which might be found in effluent from an animal disease laboratory. Swine vesicular disease virus, vesicular stomatitis virus, and bluetongue virus were irradiated in tissues from experimentally infected animals. Pseudorabies virus, fowl plague virus, swine vesicular disease virus, and vesicular stomatitis virus were irradiated in liquid animal feces. All were tested in animals and in vitro. The D_{10} values, that is, the doses required to reduce infectivity by 1 $\log_{10}$, were not apparently different from those expected from predictions based on other data and theoretical considerations. The existence of the viruses in pieces of tissue or in liquid feces made no difference in the efficacy of the gamma radiation for inactivating them. Under the "worst case" conditions (most protective for virus) simulated in this study, no infectious agents would survive 4.0 Mrads.

Gamma irradiation has been used for many years to inactivate microorganisms for a variety of purposes (4). These include medical products and biologicals (7), food products (9), and municipal sewage (1, 5, 10). The killing effect of gamma rays for viruses has been examined under several different physical conditions (2, 8, 12). It was the intent of the present study to examine the sensitivity of selected animal viruses to gamma radiation under the worst conditions (i.e., most favorable to virus survival) that might be encountered in effluent from an animal diseases laboratory. The advantages of a gamma irradiation system have been briefly discussed (11).

The kinds of waste envisaged are liquid effluent from the laboratory itself, animal waste from infected animals, and pieces of infectious tissue which might enter the sewage system. For example, the first type would be concentrated virus suspensions from cell cultures, which has been dealt with in a previous report (11).

The inactivation of human viruses in municipal sewage has been studied by several investigators with respect to protective effects of the environment. Ward (12) demonstrated that poliovirus was not significantly protected from irradiation by solids above a content level of 1 or 2%. Whereas both increasing solids content and low temperatures confer a protective effect from irradiation (as compared with aqueous suspensions at higher temperatures), the viruses appear to be killed at practically attainable dose levels. Groneman et al. (2) showed a radioprotective effect of raw sewage on foot-and-mouth disease virus but not on swine vesicular disease (SVD) virus. Their highest D_{10} value (dose required to reduce infectivity by 1 $\log_{10}$), 0.65 Mrad for SVD virus in sewage at 8°C, suggests that a higher dose of gamma radiation is necessary for inactivation under the most protective conditions.

Other studies have examined the effects of the suspending medium on the radiosensitivity of selected viruses. Sullivan et al. (9) showed protective effects of ground beef or reduced temperature (frozen state) or both, although linearity was maintained under all conditions, suggesting the setting of dose according to environmental circumstances. Massa (6) reported on the irradiation of foot-and-mouth disease virus in animal tissues as well as in liquid and dry states. Foot-and-mouth disease virus in the bone marrow, lymph nodes, and blood of infected animals was inactivated at 2.0 Mrads, the highest initial titer having been $10^{5.74}$ median cytopathogenic doses/cm^3. Several other authors have reported on the gamma radiation treatment of sewage, primarily for the inactivation of human pathogens (1, 5, 8, 10).

Available data suggest that viruses suspended in various media, including sewage, are linearly inactivated at a rate for which the important governing factors are the radiation dose, size of

TABLE 1. Sensitivity of some animal viruses in simulated laboratory effluent to gamma irradiation

Container and virus	Source	State and medium	Vol (ml)	Actual radiation dose (dosimetry) (Mrads)	Test System[a]	Results	Approximate D_{10} (Mrad)
Vials in can							
SVD	Pig epithelial tissue taken during acute phase of infection	Chopped tissue (≃1 mm^3), frozen	20	0	CC (pl.) and pigs	$10^{6.8}$ PFU/ml; 2/2 pigs + signs, 2/2 S +[b]	
			20	0.51	CC (pl.) and pigs	$10^{5.5}$ PFU/ml; 2/2 pigs + signs, 2/2 S +	
			20	1.05	CC (pl.) and pigs	$10^{4.9}$ PFU/ml; 1/2 pigs + signs, 2/2 S +	0.54
			20	2.07	CC (pl.) and pigs	$10^{3.0}$ PFU/ml; 0/2 pigs + signs, 0/2 S +	
			20	4.08	CC (pl.) and pigs	0 PFU/ml; 0/2 pigs + signs	
			20	6.04	CC (pl.)	0 PFU/ml	
Vials in can							
SVD	Pig lymph node tissue taken during acute phase of infection	Chopped tissue (≃1 cm^3), frozen	20	0	CC (pl.) and pigs	$10^{3.7}$ PFU/ml; 2/2 pigs + signs, 2/2 S +	
			20	0.51	CC (pl.) and pigs	$10^{3.3}$ PFU/ml; 2/2 pigs + signs, 2/2 S +	
			20	1.05	CC (pl.) and pigs	$10^{2.2}$ PFU/ml; 2/2 pigs + signs, 2/2 S +	0.48
			20	2.07	CC (pl.) and pigs	0 PFU/ml; 0/2 pigs + signs, 0/2 S +	
			20	4.08	CC (pl.) and pigs	0 PFU/ml; 0/2 pigs + signs	
			20	6.04	CC (pl.)	0 PFU/ml	
Vials							
SVD	Cell culture medium and cellular debris	1 part cell culture fluid plus 4 parts (vol/vol) dried municipal sewage sludge, wet homogenate	20	0	CC (pl.)	$10^{5.2}$ PFU/ml	
			20	0.37	CC (pl.)	$10^{3.7}$ PFU/ml	
			20	0.73	CC (pl.)	$10^{3.3}$ PFU/ml	0.38
			20	1.10	CC (pl.)	$10^{2.7}$ PFU/ml	
			20	1.46	CC (pl.)	$10^{1.3}$ PFU/ml	
			20	2.19	CC (pl.)	0 PFU/ml	
			20	2.92	CC (pl.)	0 PFU/ml	
			20	3.65	CC (pl.)	0 PFU/ml	
			20	4.38	CC (pl.)	0 PFU/ml	
Bottles							
Fowl plague	Egg fluid, $10^{9.4}$ ED_{50}/ml[c]	Liquid chicken feces	500	0	Chickens	6/6 chickens dead in 48 h, virus +	
			500	6	Chickens	0/6 chickens dead, 0/6 S +	
Vials							
			20	0	Eggs	$10^{8.2}$ ED_{50}/ml	0.07
			20	0.11	Eggs	$10^{4.7}$ ED_{50}/ml	
			20	0.32	Eggs	$10^{3.0}$ ED_{50}/ml	
				0.63	Eggs	0 ED_{50}/ml	

Bottles							
Bluetongue	Whole sheep blood taken during viremia	Blood + EDTA, chilled	500	0	Sheep	3/3 sheep virus +, 3/3 S +	
			500	6	Sheep	0/3 sheep virus +, 0/3 S +	
Vials							
			20	0	CC (pl.)	10^3 PFU/ml	0.10
			20	0.1	CC (pl.)	10^2 PFU/ml	
			20	0.3	CC (pl.)	0 PFU	
			20	0.6	CC (pl.)	0 PFU	
Bottles							
Pseudorabies	Cell culture medium and cellular debris		500	0	Pigs[d]	1/3 pigs died, virus +; other 2 S +	
			500	6	Pigs	0/3 pigs died, 0/3 S +	
Vials							
		Liquid pig feces	20	0	CC (pl.)		
			20	0.11	CC (pl.)	No virus recovered	
			20	0.32	CC (pl.)		
			20	0.63	CC (pl.)		
Bottles							
Vesicular stomatitis	Egg fluid, $10^{9.2}$ ED_{50}/ml	Liquid pig feces	500	0	Pigs[e]	0/3 pigs signs, 0/3 S +	
			500	6	Pigs	0/3 pigs signs, 0/ S +	
Vials							
			20	0	CC (pl.)		
			20	0.11	CC (pl.)		
			20	0.32	CC (pl.)	No virus recovered	
			20	0.63	CC (pl.)		
Vials							
Vesicular stomatitis	Pig epithelial tissue	Chopped tissue (≈1 mm^3)	2 g	0	Pigs[e]	3/3 pigs signs and lesions, 3/3 S +	
			2 g	6	Pigs	0/3 pigs signs or lesions, 0/3 S +	0.20
			2 g	0	CC (pl.)	$10^{7.3}$ PFU/ml	
			2 g	0.32	CC (pl.)	$10^{4.8}$ PFU/ml	
			2 g	0.63	CC (pl.)	$10^{1.7}$ PFU/ml	

[a] CC, Cell culture (pl., plaques); pigs = pigs exposed to SVD virus; 2 ml intravenously.
[b] S +, Seroconverted.
[c] ED_{50}, Median effective dose.
[d] Pigs exposed to pseudorabies virus: 2 ml intramuscularly, 2 ml orally, and remainder in feed.
[e] Pigs exposed to vesicular stomatitis virus: 1 ml intramuscularly, 1 ml orally, and 1 ml in snout and footpads.

virus, chemical constitution of the media, and temperature. Under all circumstances, however, the process seems to be both reproducible and dependable.

The work reported here examines the doses of gamma radiation that would have to be used to inactivate selected animal viruses under conditions simulating those in sewage from a laboratory, including animal experimentation facilities.

MATERIALS AND METHODS

Viral preparations. (i) SVD virus. Infected pigs were killed during the acute phase of infection, and coronary and footpad vesicular tissue, lymph nodes, and blood clots were harvested. These were cut into pieces of 1 to 10 mm^3 and sealed in several heavy glass serum vials (20-ml size). Individual vials of each tissue were then sealed in tin cans (8 oz [240 ml]) with other vials of material to be irradiated. For each of several doses of radiation, then, there was a can containing, among other material, three vials of virus in tissue, one tissue type per vial. All of the cans were frozen, packed in coolant, and taken to Atomic Energy of Canada Ltd. (AECL), Commercial Products Division, for irradiation at estimated doses of 0, 0.5, 1.0, 2.0, 4.0, and 6.0 Mrads.

Radiosensitivity of SVD virus in dried municipal sewage sludge was also examined. Crude cell culture fluid was mixed with dried municipal waste sludge at 1 part culture fluid to 4 parts sludge (by volume), and the wet homogenate was sealed in 10-ml glass vials. These were transported to AECL for irradiation at estimated doses of 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 Mrads.

(ii) Fowl plague virus. Infected egg fluid was added at 20% (vol/vol) to a mixture of chicken feces and tap water (1:1). One 500-ml bottle was irradiated at 6 Mrads and one was used for the 0-Mrad control. Four 20-ml vials were used for 0-, 0.1-, 0.3-, and 0.6-Mrad doses.

(iii) Bluetongue virus. Infectious blood was obtained by inoculating two adult sheep with a cell culture suspension of type 18 bluetongue virus and bleeding them during temperature rise. The anticoagulant EDTA was added to the blood and it was held at 4°C. Two 500-ml bottles were irradiated at 0 and 6 Mrads each, and four 20-ml vials were irradiated at 0, 0.1, 0.3, and 0.6 Mrads.

(iv) Pseudorabies virus. Virus in culture fluid was added at 20% (vol/vol) to a mixture of pig feces and tap water (1:1). After the material was mixed, it was placed in two 500-ml bottles for irradiation at 0 and 6 Mrads, and four 20-ml vials were irradiated at 0, 0.1, 0.3, and 0.6 Mrads.

(v) Vesicular stomatitis virus. Virus in culture fluid was added at 20% (vol/vol) to liquid pig feces as for pseudorabies virus and was processed as fowl plague virus.

Vesicular stomatitis virus in pieces of epithelial tissue from infected pigs was irradiated in 2-g quantities in sealed glass vials at estimated doses of 0, 0.3, 0.6, and 6 Mrads.

Irradiation procedure. All material was irradiated with coolant in the chamber of a Gammacell-220 (AECL, Commercial Products) at AECL at ambient temperature. The 0-Mrad controls were placed on the housing of the irradiator during the process. Samples were held at 4°C up to the time of irradiation and immediately after. Accurate gamma radiation dose levels were measured by dosimetry methods.

Virus assays. The irradiated virus suspensions were assayed in vivo or in vitro or both as follows.

For SVD virus in tissue, the 0- to 4-Mrad samples were tested by intravenous inoculation of pigs and 0- to 6-Mrad samples were tested by plaque formation in $IBRS_2$ cells. The latter, a pig kidney cell line, were infected as monolayer cultures in plastic culture dishes and, after 4 days of incubation under a semisolid overlay containing minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) and agar (purified agar; Oxoid Ltd., London, England), were stained with neutral red to demonstrate the plaques.

For SVD virus in municipal sludge, all doses were tested in the $IBRS_2$ plaque system.

For fowl plague virus in feces, 0- and 6-Mrad samples were tested in chickens (intramuscular inoculation and feeding) and 0- to 6-Mrad samples were tested by inoculation of embryonating chicken eggs (allantoic cavity).

For bluetongue virus in blood, 0- and 6-Mrad samples were tested in sheep and 0- to 6-Mrad samples were tested by plaque formation in L-929 cells (3).

For pseudorabies virus in feces, 0- and 6-Mrad samples were tested in pigs (intramuscular inoculation and oral) and 0- to 0.6-Mrad samples were tested by plaque formation in $IBRS_2$ cells (as for SVD virus).

For vesicular stomatitis virus in feces, 0- and 6-Mrad samples were tested in pigs (intramuscular inoculation and feeding) and 0- to 0.6-Mrad samples were tested by plaque formation in L-929 cells (as for bluetongue virus).

For the in vivo assays, animals were observed for clinical signs and tested by neutralization tests for seroconversion and, if necessary, viremia or virus-positive tissues, using the in vitro systems described above.

The D_{10} values for the virus samples assayed in cell culture were graphically estimated (11).

The treatments, conditions, radiation doses, and assay systems, including doses given to test animals, are noted in Table 1 for each virus.

RESULTS

None of the viral preparations was infectious for their appropriate host animal after 6.0 Mrads. The approximate D_{10} values by in vitro assay ranged from 0.07 to 0.54 Mrad. The fecal preparations had to be diluted to 1:10 before cell culture assay, thereby reducing the sensitivity of the tests.

As expected from theoretical considerations, primarily virus size, SVD virus was the most resistant of the viruses studied. Its D_{10} values ranged from 0.38 to 0.54 Mrad, and in none of the three preparations did any virus survive the 4-Mrad dose. The 2-Mrad-treated material was not infectious for pigs, but in the case of the pig epithelial tissues, some residual infectivity at the 2-Mrad level was detected in cell culture (Table 1).

Fowl plague virus was completely inactivated at 0.63 Mrad from a beginning titer of $10^{8.2}$ median effective doses per ml. Bluetongue virus gave a D_{10} value of 0.10 Mrad; none survived the 6-Mrad treatment. The pseudorabies virus did not survive well in the fecal material even without irradiation. The 0-Mrad control fecal material, however, was infectious for pigs, whereas the 6.0-Mrad-treated material was not. The vesicular stomatitis virus was even less tolerant of the fecal material than the pseudorabies virus, none surviving the 0-Mrad control treatment as assayed in cell culture. The vesicular stomatitis virus in pig epithelial tissue did survive well, however, and gave a D_{10} value of approximately 0.20 Mrad. The results are summarized in Table 1.

DISCUSSION

Theoretically, the radiation sensitivity of viruses in "worst case" conditions envisaged in sewage should not change beyond the rather limited effects conferred by increased solids and low temperature. Since the density of the effluent would never be much greater than 1 and the gamma source would be calibrated to overcome, quantitatively, any attenuation effect due to target thickness (a few feet at most), inactivation of classical animal viruses in laboratory effluent should be practically attainable with gamma irradiation.

SVD virus is one of the most resistant animal viruses to physical changes and, because of its small size and single-stranded RNA genome, is also one of the most resistant to gamma radiation. Since it occurs in substantial titers in epithelial and other tissues, up to approximately 10^7 PFU/g in this experiment, and is highly infectious for pigs, it is a useful virus with which to test any effluent treatment system. The D_{10} value range of 0.38 to 0.54 Mrad is in good agreement with previous work (11) and work done on similar viruses (6, 9).

Other viruses of considerable significance to domestic livestock, namely, pseudorabies, fowl plague, bluetongue, and vesicular stomatitis viruses, were similarly inactivated at predictable dose levels.

The dose setting strategy should include estimated bioburden of the most resistant viruses. Given dilution factors created by "clean" effluent and normal degradation of viruses in sewage held at ambient temperatures, it is likely that the SVD virus in pig epithelial tissue used here represents the worst case. Since it was completely inactivated at 4 Mrads, this dose should be considered as reasonable. Massa has recommended a lesser dose (3.0 Mrads) for tissues containing foot-and-mouth disease virus (6). Of course, one of the advantages of gamma irradiation is that dose levels can be increased or decreased simply by changing the amount of radioactive cobalt in the source or changing the residence time in the irradiator. This approach could be facilitated by "upstream" holding tanks which would give the advantages of dilution of small volumes of concentrated viral suspensions and constant (buffered) flow rates in the treatment tank, allowing full (24-h) use of the radioactive source. By using more than one holding tank, this concept could be further developed to allow different treatment times (and therefore doses) for effluent with different bioburden levels.

ACKNOWLEDGMENTS

We acknowledge the skillful and extensive contributions of M. Picard and R. Charron of the Animal Diseases Research Institute, N. Shuot of Plum Island Animal Disease Laboratory, and M. L. McIndoo of AECL.

LITERATURE CITED

1. **Epp, C.** 1975. Experience with a pilot plant for the irradiation of sewage sludge. Experiments on the inactivation of viruses in sewage sludge after radiation treatment. Proceedings of the International Atomic Energy Association Symposium on Radiation for a Clean Environment. Int. Atomic Energy Assoc. (Vienna) STI PUB **402**:485–490.
2. **Groneman, A. F., S. Frenkel, and C. Terpstra.** 1977. Radiation inactivation of animal viruses in culture fluid and sewage. A case study, p. 27–34. *In* Radiation for Pollution Control, Working Group on Waste Irradiation, Uppsala, 31 August to 2 September 1977. INIS-mf-4220. European Society of Nuclear Methods in Agriculture, Wageningen, The Netherlands.
3. **Howell, P. C., D. W. Verwoerd, and R. A. Oellermann.** 1967. Plaque formation by bluetongue virus. Onderetpoort J. Vet. Res. **34**:317–332.
4. **Jordan, R. T., and L. L. Kempe.** 1956. Inactivation of some animal viruses with gamma radiation from cobalt-60. Proc. Soc. Exp. Biol. Med. **91**:212–215.
5. **Lemke, H. S., and A. V. Sinskey.** 1975. Viruses and ionizing radiation in respect to waste water treatment. Proceedings of the International Atomic Energy Association Symposium on Radiation for a Clean Environment. Int. Atomic Energy Assoc. (Vienna) STI PUB **402**:99–120.
6. **Massa, D.** 1966. Radiation inactivation of foot-and-mouth disease virus in the blood, lymphatic glands and bone marrow of the carcasses of infected animals. International Symposium on Food Irradiation. Int. Atomic Energy Assoc. (Vienna) STI PUB **127**:329–345.
7. **Polley, J. R.** 1961. Preparation of non-infective soluble antigens with gamma radiation. Can. J. Microbiol. **7**:135–139.
8. **Simon, J., and G. Tamasi.** 1975. Data on the disinfection of liquid manure by gamma irradiation at Baja, Hungary. Proceedings of the International Atomic Energy Association Symposium on Radiation for a Clean Environment. Int. Atomic Energy Assoc. (Vienna) STI PUB **402**:209–216.
9. **Sullivan, R., P. V. Scarpino, A. C. Fassolitis, E. P. Larkin, and J. T. Peeler.** 1973. Gamma radiation inactivation of coxsackievirus B-2. Appl. Environ. Microbiol. **26**:14–17.
10. **Sztanyik, L. B., D. S. Ballantine, R. P. D. Eymery, F. S. Feates, E. Hess, E. Lund, and A. Suess.** 1975. Panel on the radiation treatment of wastes. Proceedings of the International Atomic Energy Association Symposium on Radiation for a Clean Environment. Int. Atomic Energy Assoc.

(Vienna) STI PUB **402**:645–656.

11. **Thomas, F. C., A. G. Davies, G. C. Dulac, N. G. Willis, G. Papp-Vid, and A. Girard.** 1981. Gamma ray inactivation of some animal viruses. Can. J. Comp. Med. **45**:397–399.
12. **Ward, R. L.** 1977. Inactivation of poliovirus in wastewater sludge with radiation and thermoradiation. Appl. Environ. Microbiol. **33**:1218–1219.