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Wastewater Renovation and Reuse: Virus Removal by Soil Filtration

Abstract. *Secondary sewage effluent and renovated water from four wells at the Flushing Meadows Wastewater Renovation Project near Phoenix, Arizona, in operation since 1967, were assayed approximately every 2 months in 1974 for viruses during flooding periods. Viruses, regularly found in the secondary effluent, were not detected in any renovated water samples. Our results indicated that human viral pathogens do not move through soil into the groundwater, but are apparently absorbed and degraded by the soil and reduced in numbers by a factor of at least 10^4 (99.99 percent removal).*

The fate of viruses in wastewater as they contact the soil is an important consideration in land disposal systems (*1*). We need more information on virus movement and survival in soil, especially under field conditions, to properly assess the pollution hazard to our resources. Once a sound data base is obtained for evaluating virus removal mechanisms, then management can implement practices for renovation and reuse of wastewater.

In 1967 an experimental project, the Flushing Meadows Project, in the Salt River bed west of Phoenix, Arizona, was installed to study renovation of secondary sewage effluent by land disposal. Effluent from an activated sludge-type secondary sewage treatment plant was allowed to infiltrate into six parallel horizontal basins (6 m by 210 m, 6 m apart) consisting of 60 to 90 cm of fine loamy sand underlain by several coarse sand and gravel layers to a depth of 75 m, where a clay layer begins. Observation wells for sampling renovated sewage were installed in line midway across the basin area, except that one well was located 22.5 m from the end of the basin area midway between basins 3 and 4. The wells were cased to the bottom with nonperforated steel pipe (15 cm in diameter), except for one well 76.2 m deep, which was perforated from 3 to 9 m. The other wells ranged in depth from 6.1 to 9.1 m. The infiltration rate was about 100 m of wastewater per year. Each year the wastewater was usually applied intermittently with 14-day flood and dry periods. Bouwer *et al.* (*2*) described the infiltration and hydraulic aspects of the project as well as its water quality improvement and economic aspects. Our research showed that wastewater viruses did not move through the soil infiltration system used for renovated wastewater. A more detailed paper covers other microbial aspects of the Flushing Meadows Project (*3*).

Secondary sewage effluent and renovated water samples for virus analysis were collected every 2 months throughout 1974 during the second week of the 14-day flood periods so that the four wells (*4*) sampled would yield "fresh" renovated water (the underground detention time was 5 to 10 days). Each sampling period was about 1 week. During this time, duplicate samples of 174 to 454 liters from each well were processed through a portable virus concentrator by using methods previously described (*5*), but modified (*6*).

The concentration of naturally occurring enteric viruses in the secondary effluent flowing into the infiltration basins was determined from samples of 4 to 20 liters. Viruses were concentrated from the effluent samples by a method similar to that described by Homma *et al.* (*7*).

Viruses were isolated by using primary baboon kidney cells that were obtained from immature baboons, trypsinized, and grown as described by Melnick and Wenner (*8*). Usually only 30 to 50 percent of the total sample was assayed at a time. The sample was divided into two equal volumes, and one subsample was assayed by bottle culture and the other by the overlay method. In the overlay method, 0.1 to 0.2 ml of inoculum was placed on a monolayer of cells in 30-ml flat glass bottles (with a cell surface area of 12 cm^2) and incubated at 37°C for 1 hour. The bottles were then washed with 5 ml of Eagle's minimal essential medium (MEM) to reduce toxicity, drained of excess fluid, and overlaid with 5 ml of agar. The agar overlay medium consisted of single-strength Eagle's MEM without phenol red; 1.5 percent agar (Difco); 23 m*M* $MgCl_2$; neutral red in a final concentration of 1 part in 54,000; 100 units of penicillin; streptomycin, 100 μg/ml; and 0.4 percent $NaHCO_3$. When plaques appeared, virus was "plucked" from the plaques and passed to fresh cultures maintained under fluid media. Progeny virus harvested from these bottles was identified by antiserum pools (*9*) and by specific antiserums.

The second subsample was placed in bottles (0.2 to 0.5 ml per bottle) with drained cell cultures and incubated for 6 minutes at 37°C. Then 4 to 5 ml of Eagle's MEM was added to each of the bottles, which were examined daily for cytopathic effects. When cytopathic effects were observed, the culture fluid was inoculated in fresh bottle cultures to

Table 1. Numbers and types of viral isolates from the secondary sewage effluent and the renovated wastewater wells. (Numbers are averages for duplicate samples from the sewage effluent and the four well sites combined.)

Sampling dates (1974)	Viruses per 100 liters		Types of viruses in sewage effluent
	Sewage effluent	Renovated water	
7 to 11 January	786	0	Poliovirus 2, echovirus 15
12 to 18 March	2745	0	Poliovirus 2, echovirus 7
5 to 9 May	2378	0	Poliovirus 2 and 3
25 June to 9 July	158	0	Poliovirus 2, coxsackievirus B4
27 August to 12 September	7475	0	Reovirus 1 and 2*
19 November to 11 December†	1142	0	Reovirus (undetermined)*
Range	158–7475	0	

*No plaques were noted until after 14 days under agar overlay. †Data for sewage effluent were obtained in the November flood period and data for renovated water (East Well and Well 7, only) in the December flood period.

determine whether the effects were due to chemical toxicity or to viral infection. Both the bottle cultures and the overlaid cell cultures were observed daily for 14 to 21 days.

The number of viruses detected in the sewage effluents ranged from 158 to 7475 per 100 liters and averaged 2118 per 100 liters during 1974 (Table 1). The largest number of viruses was detected during August and September, and the smallest during June and July. Generally, the greatest enteric virus concentrations in sewage occur during the fall of the year, which agrees with our data. The viruses identified in the sewage effluent included seven types, which varied in occurrence with the time of year.

No viruses were detected in any of the well samples, regardless of sampling date during the 14-day flood period (Table 1). Some eluates had nonspecific cytotoxicity, which usually meant that several blind passages in cell cultures were necessary to confirm the absence of viruses. The absence of any detectable viruses in the well water concentrates indicated that the virus count was reduced by a factor of at least 10^4 (99.99 percent) during percolation of the wastewater through 3 to 9 m of the basin soil.

Our results indicate that human viral pathogens do not move through soil into the groundwater, which is significant, especially since the Flushing Meadows Project had renovated wastewater continually for 7 years at infiltration rates of about 100 m per year.

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6. Modifications of virus concentration methods (*5, 7*): (i) conditions for virus adsorption were *p*H 3.5 with 0.0005*M* $AlCl_3$; (ii) no clarifiers were used; (iii) the virus adsorber in the first stage of the concentration procedure consisted of a fiber glass cartridge filter (model K27R10S, Commercial Filter Division) followed by a series of Cox filters 142 mm in diameter with 5-, 1-, and 0.65-μm pore sizes; and (iv) viruses in the first eluate were reconcentrated on a series of Cox filters 90 mm in diameter with 5-, 1-, and 0.65-μm pore sizes, yielding a final, neutralized eluate volume of about 20 ml. Before freezing, 0.25 ml of fetal calf serum and penicillin and streptomycin were added to the final eluate.
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Bovine Leukemia Virus Genes in the DNA of Leukemic Cattle

Abstract. *Reverse transcripts of the RNA genome of the bovine leukemia virus (BLV) as well as ^{125}I-labeled BLV RNA hybridize to the DNA of tissues from leukemic cattle with the adult form of the disease but not to bovine thymic lymphoma or normal bovine tissues.*

Epidemiological and pedigree studies suggest that leukemia in adult cattle is an infectious disease transmitted from animal to animal (*1*). Viral particles produced by short-term cultures of buffy coat cells from leukemic cattle (*2*) have been implicated, by serological and epidemiological studies (*3, 4*), in the etiology of the adult form of leukemia in this species and have been referred to as the bovine leukemia virus (BLV). Experimental transmission of BLV to sheep has also resulted in the development of leukemia in the recipient species (*5*). BLV is an enveloped virus with many of the distinguishing properties of other mammalian and avian leukemia viruses—maturation by budding from the plasma membrane (*2*, 3), RNA genome (*6*), and viral reverse transcriptase activity (*6, 7*)—and is therefore a member of the family Retraviridae (*8*). In order to test whether the genes of BLV can be directly shown in the DNA of bovine leukemia cells and to de-

Table 1. BLV-related sequences in infected and uninfected tissues.

Source of DNA*	$C_0t_{1/2}$†	Hybrid acceleration factor‡	Relative copy number§
Bat lung cell line CCL 88			
Cell line BLV-TblLu	4×10^{-4}	12.5	1
Cell line TblLu	5×10^{-3}	1.0	<0.1
Bovine tissue and a sheep tumor			
BI434‖, leukemia-free herd, buffy coat cells	5×10^{-3}	1.0	<0.1
BH116‖, leukemia-free herd, buffy coat cells	5×10^{-3}	1.0	<0.1
BI196‖, leukemia-free herd, buffy coat cells	4×10^{-3}	0.8	<0.1
Commercial calf thymus	5×10^{-3}	1.0	<0.1
Thymic lymphoma¶	5×10^{-3}	1.0	<0.1
NBC13**, bovine lymphoid cell line	5×10^{-4}	10.0	0.8
BF239‖, multiple-case herd, buffy coat cells	1.7×10^{-4}	29.5	2.4
27-264, leukemic cow, lymph node#	4×10^{-4}	12.5	1.0
27-252, leukemic cow, lymph node#	1.7×10^{-4}	29.5	2.4
Tumor from sheep experimentally infected with BLV¶	1.7×10^{-4}	29.5	2.4
Other species			
Rat, cell line NRK (*20*)	5×10^{-3}	1.0	<0.1
Mouse, BALB/c liver	4×10^{-3}	0.8	<0.1
Guinea pig liver	5×10^{-3}	1.0	<0.1
Domestic cat liver	5×10^{-3}	1.0	<0.1
Dog thymus, cell line FCf2Th (*21*)	4×10^{-3}	0.8	<0.1
Pig kidney, ATCC (CCL 33)	5×10^{-3}	1.0	<0.1
Sheep liver	5×10^{-3}	1.0	<0.1
Human tumor, cell line A204 (*22*)	5×10^{-3}	1.0	<0.1
Rhesus, cell line DBS-FRhL-1 (*23*)	5×10^{-3}	1.0	<0.1

*DNA was extracted from the indicated source (see legend to Fig. 1). †The $C_0t_{1/2}$ is the C_0t value at which 50 percent of the [^{3}H]DNA probe has reassociated. The conditions for hybridization and S_1 analysis are described in the legend to Fig. 1. ‡The hybridization acceleration factor is the $C_0t_{1/2}$ of BLV [^{3}H]DNA with TblLu DNA/$C_0t_{1/2}$ with the indicated DNA. §The relative copy number refers to the frequency of BLV-related sequences per cellular genome normalized to that found in cell line TblLu infected with BLV. ‖The herd and cow designation of the University of Pennsylvania School of Veterinary Medicine at New Bolton Center. ¶Provided by Dr. M. J. Van Der Maaten, National Animal Disease Center, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa. #Leukemic cattle referred to the University of Pennsylvania Clinic, New Bolton Center, by a veterinary practitioner. **Cell line established from peripheral lymphocytes of a leukemic cow.