NOTES

Detection of Animal Viruses in Coastal Seawater and Sediments

SILVIO DE FLORA,* GIUSEPPE P. DE RENZI, AND GIUSEPPE BADOLATI

Institute of Hygiene, University of Genoa, 16132 Genoa, Italy

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Animal viruses, predominantly enteroviruses, were detected in shallow waters at bottom depths and in clastic marine sediments. Viruses accumulated in sandy and slimy deposits of the sea bottom near the shore and could be easily released into water by means of simple mechanical shaking.

The considerable dilution of infective particles hinders detection and quantitation of animal viruses in seawater and in natural waters. A clue to this problem came as a result of the introduction of the polyelectrolyte method, which is a proper and effective procedure for concentrating small amounts of virus in large volumes of water (5, 8, 9). Laboratory studies showed that use of polyelectrolytes results in high rates of virus recovery also in seawater, and the virological monitoring of coastal waters has made it possible to assess a relationship between bacteriological and virological pollutants in the marine environment (3; F. L. Pettrilli, S. De Flora, and L. Lemori, Abstr. 2nd Int. Congr. Mar. Pollut. and Mar. Waste Disposal, Sanremo, Italy, 1973, Abstr. 35, p. 11). We have now detected animal viruses in nearshore shallow waters and particularly in clastic marine sediments, a topic which had not been explored.

Five-liter samples of surface and bottom seawater were collected at a distance of 3 to 70 m from the shoreline and at depths of 0.5 to 12 m. Water-free samples of the sediment upper layer were collected by filling sterile containers with sterile piston-coring equipment. The sand or slime of the seabed was mixed with equal volumes of sterilized seawater, shaken for 30 min in a mechanical shaker simulating wave motion, and allowed to settle at 4°C. Viruses were concentrated from 5 liters of seawater or sediment eluate, acidified to pH 5.0, by adding 500 mg of insoluble polyelectrolytes in the form of a 100-mesh powder (PE60, Monsanto Co., St. Louis, Mo.). After contact from 1 to 16 h (overnight) at room temperature or at 4°C, the sample was filtered by positive pressure through 142-mm AP20 fiber glass pads in a membrane filter holder (Millipore Corp.). The filtration apparatus was sterilized after each water sample by steam. The recovered polyelectrolyte was suspended in 4 ml of pH 9.0 borate buffer, for a theoretical 125-fold concentration factor, and shaken for 10 min in a mechanical shaker (Standard Labo, Paris). The eluted virus was separated from the polymer by low-speed centrifugation. Tenfold dilutions of polyelectrolyte concentrates were inoculated into tissue cultures (0.2-ml aliquots of each dilution into each of five tubes of primary cultures of African green monkey kidney and five tubes of continuous line KB cells) for identification and enumeration of viruses, according to previously described procedures (3, 6, 7; Pettrilli et al., Abstr. 2nd Int. Congr. Mar. Pollut. and Mar. Waste Disposal, Sanremo, Italy, 1973). The virus titer was expressed as the dose infective for 50% of the cultures inoculated. Viruses were classified into groups according to patterns of cytopathic effect, ether sensitivity, and agglutination ability with erythrocytes of a number of animal species at 4 and 35°C. Hemagglutinating (HA) enteroviruses were typed by the hemagglutination inhibition test. Non-hemagglutinating (NA) enteroviruses were assayed with mixtures of poliovirus antisera by means of the neutralization test, and the character of polioviruses was checked by means of the temperature (T) marker to distinguish wild (T') from vaccine-like (T-) strains. The remaining enteroviruses were provisionally classified as non-polio HA- enteroviruses. The bacteriological indexes of pollution were checked in the same samples according to standard methods (1, 10).

Some examples of results obtained under various environmental conditions are shown in Fig. 1. Virus strains could be recovered from...
virtually all of the water samples examined, either from grossly polluted coastal waters close to the discharge of raw sewage or from moderately polluted waters. With a few exceptions, viruses could be detected also in eluates of marine sediments. Most isolates could be classified as entroviruses, including some strains of vaccine-like polioviruses.

The results obtained clearly indicate that the extent of virological and bacteriological pollution of surface marine water is constantly higher than that of bottom seawater. It is also apparent that the variability of virus titer among samples is consistently lower than that of bacteriological indexes, and their quantitative ratio is inversely related to the extent of fecal pollution. This is in agreement with the results presented in previous reports, where the reasons for such variability were examined and ascribed both to technical factors and to the different fates of bacteria and viruses in the marine environment (3; Pettrill et al., Abstr.)

| SAMPLING POINTS | COLIFORMS | E. COLI | FECAL STREPTOCOCCI | VIRUSES RECOVERED | TCD50 | STRAINS |
|-----------------|-----------|---------|--------------------|-------------------|-------|---------|
| 1 | 790,000 | 490,000 | 65,000 | 1.4 | non polio HA- enteroviruses |
| 2 | 17,000 | 4,900 | 2,500 | 0.4 | non polio HA- enteroviruses |
| 3 | 79,000 | 13,000 | 4,700 | 0.8 | non polio HA- enteroviruses |
| 4 | 1,100 | 170 | 90 | 0.4 | non polio HA- enteroviruses |
| 5 | 24,000,000 | 9,200,000 | 1,800,000 | 16.0 | non polio HA- enteroviruses |
| 6 | 490,000 | 24,000 | 14,000 | 1.0 | non polio HA- enterovirus polio 1 (T-)
| 7 | 24,000,000 | 7,900,000 | 2,300,000 | 1.6 | non polio HA- enteroviruses |
| 8 | 17,000,000 | 4,900,000 | 1,600,000 | 0.2 | non polio HA- enteroviruses |
| 9 | 11,000,000 | 2,400,000 | 950,000 | 40.0 | non polio HA- enteroviruses polio 2 (T-)
| 10 | 49,000 | 1,600 | 910 | 0.8 | non polio HA- enteroviruses |
| 11 | 35,000 | 1,100 | 940 | 0.2 | non polio HA- enteroviruses |
| 12 | 17,000 | 290 | 290 | <0.1 | |
| 13 | 49,000 | 5,600 | 3,600 | 0.6 | non polio HA- enteroviruses |
| 14 | 14,000 | 2,400 | 1,100 | 1.2 | non polio HA- enteroviruses |
| 15 | 5,400 | 1,100 | 1,200 | 4.0 | non polio HA- enteroviruses |
| 16 | 3,500 | 920 | 750 | 0.4 | non polio HA- enteroviruses |
| 17 | 1,700 | 350 | 220 | 0.4 | non polio HA- enteroviruses |

Fig. 1. Coliforms, Escherichia coli (fecal coli), fecal streptococci, and viruses recovered per 100 ml of sediment eluates and seawater samples. Asterisks indicate the points of collection. HA refers to hemagglutinating ability of viruses. TCD50, 50% tissue culture infective dose.
Although data concerning the numbers of viruses and bacteria in sand and slime eluates are only indicative of the actual microbiological content of marine sediments, some findings deserve consideration. (i) The concentration of viruses eluted from clastic marine sediments may attain considerable levels (see, for example, the 40 50% tissue culture infective doses/100 ml of eluate of sample no. 3, Fig. 1); (ii) a marked variability exists, however, in the titer of virus recovered from sediment eluates as compared with that found in surrounding waters; (iii) the quantitative ratio of viruses to bacteriological pollutants is lower in sediment eluates as compared with seawater; (iv) appreciable amounts of virus can be eluted from sediments after simple mechanical shaking.

These findings suggest that, in polluted waters, virus particles do sediment to the sea bottom where they mix with sandy and slimy deposits. Viral accumulation may be favored by a longer survival of viruses in moist clastic sediments than in seawater, which appears to be likely on the basis of results of preliminary laboratory studies (Fig. 2). Type 1 poliovirus (strain LSc2ab) was added to 2 liters of virus-free seawater (5,200 plaque-forming units/ml). One liter of infected water was passed through an equivalent volume of virus-free quartz sand, which was kept in the moist state after removal of filtered water. Just after filtration and at intervals of about 5 days, an aliquot of sand was collected and mixed with an equal volume of virus-free seawater, shaken for 30 min in a mechanical shaker simulating wave motion, and allowed to settle at 4 C. Eluates were frozen at -80 C, together with samples of infected seawater, and then titrated for infectivity at the end of the experiment. The plaque-forming units were determined in monolayers of KB cells by incorporating, after adsorption of virus, anti-polio 1 serum into the liquid nutrient medium of cells (Earle minimal essential medium with 3% fetal calf serum, 100 U of penicillin, and 100 µg of streptomycin per ml), according to the principle of the plaque-antiserum method (4). A titration of the filtrate collected immediately after filtration accounted for 7% of the virus, with a calculated 79% of adsorbed virus failing to elute. The decay of virus infectivity was linear either in seawater or in sand. The velocity constants (k per second) of inactivation, calculated according to the equation \( k = (\ln N_o/N)/t \), where \( N_o \) is the initial infectivity titer and \( N \) is the titer after storage for time \( t \), were equal to 4.12 \( \times 10^{-6} \) in seawater and 0.92 \( \times 10^{-6} \) in sand, respectively. Inactivation was therefore about 4.5-fold faster in seawater than in sand.

Sediments could therefore represent a transient reservoir of viruses, and it is important that infective virus particles can be mechanically returned to seawater, thereby completing a natural cycle in the nearshore environment. Wave motions and bottom currents in shallow waters could be responsible for the release of viruses from sediments under natural conditions.

The public health significance of enteric viruses in marine sediments, as well as in seawater, cannot be evaluated from statistical and epidemiological standpoints. However, since it is generally accepted that "any amount of virus in drinking or recreational water that is detectable in appropriate cell cultures constitutes a hazard to those using the water" (2), the release into nearshore waters of viruses deposited in marine sediments could pose an additional hazard for individuals bathing in those waters. Moreover, contamination of fauna and chiefly of edible filtering organisms which live in marine sediments might result in an indirect hazard to human health.

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