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A Scientific Review of Binding and Inactivation of Viruses on and in Food, with a Focus on the Role of the Matrix

Françoise S. Le Guyader[1] and Robert L. Atmar[2]

[1] Laboratoire de Microbiologie, Institut Français pour la Recherche et l'Exploitation de la Mer (IFREMER), Nantes, France, Phone: + 33 (2) 4037 4052, Fax: + 33 (2) 4037 4073, E-mail: sleguyad@ifremer.fr
[2] Departments of Medicine and Molecular Virology & Microbiology Baylor College of Medicine 1 Baylor Plaza, MS BCM280 Houston, TX, USA 77030, Phone: +1 (713) 798-6849, Fax: +1 (713) 798-6802, E-mail: ratmar@bcm.edu

1. Introduction

Foods play an important role in the transmission of enteric viruses. For example, as much as 40% of norovirus infections are estimated to be caused by the consumption of contaminated foodstuffs (52, 67). Although fewer than 5% of hepatitis A virus (HAV) infections are classified as foodborne, a greater percentage may be transmitted by this route since the source of infection is not identified in up to one-half of cases (3, 19, 53). Other enteric viruses, including rotaviruses, astroviruses and hepatitis E virus, are not important causes of foodborne disease but can occasionally be transmitted by foods (12, 13, 61, 74, 96), and non-enteric viruses can occasionally be transmitted in foods (e.g., tickborne encephalitis, Nipah virus) (44, 63).

Transmission of viruses by foods is dependent on several factors: initial contact of the virus with the food, binding or attachment of the virus to the food, survival and persistence of the virus until the food is consumed, and consumption of the food by a susceptible host. The initial contact of the virus with the food may occur at any time during food production, including before harvest, during processing and at the time of preparation (figure 1). Examples include fecal contamination of shellfish growing waters due to poorly functioning sewage treatment plants, use of nightsoil to fertilize crops, use of fecally contaminated water to wash fruits after harvest, and poor hand hygiene by an infected food handler (8, 12, 52, 84, 95).

Almost any kind of food can be involved in virus transmission, but a limited number of foods are most commonly associated with outbreaks. These include uncooked shellfish, fresh fruits and vegetables, and ready-to-eat foods (such as sandwiches and salads). Ready-to-eat foods are often contaminated by an infected food handler at the time of food preparation, while contamination of shellfish usually occurs prior to harvesting and contamination of fruits and vegetables can occur prior to harvesting or during processing. This chapter will review factors that are known to affect the binding of enteric viruses to different food matrices and that affect the persistence and survival of the virus once it is food-associated, with an emphasis on foods that are contaminated by food handlers prior to the preparation stage.

Virus binding to foods

Physicochemical factors

The adsorption of viruses to solid surfaces has been evaluated for a number of different surface types (sorbents), including soil and aquifer sediments, food preparation surfaces and fomites (2, 32, 42, 87). The size of viruses is similar to that of colloids, so theories developed to describe the binding of colloids to surfaces have been used to model virus binding to different sorbents. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability states that adherence of a colloid to a surface results from the interaction of two opposing forces: attractive van der Waals forces and repulsive electrostatic forces. Factors that affect the electrostatic forces include the pH and ionic strength of the solution, the presence of compounds competing for sorption sites, properties of the virus (e.g., its isoelectric point [pI]), and properties of the sorbent (42). For example, at pH values below the pI, the net charge of the virus particle is positive while at pH values above the pI the net charge is negative. Thus, changes in pH will affect the electrostatic interactions with the sorbent, depending on its net charge. This characteristic has been used to elute viruses from sorbents by increasing the pH of the solution bathing the sorbent to >9.0. Increasing the ionic strength of a solution favors adsorption of a virus to a sorbent by decreasing the effective radius of repulsive electrostatic effects. The converse is also true; lowering the ionic strength of the solution is used to elute virus from a surface (32). Experimental results from studies of poliovirus binding to different metal oxides have agreed with those predicted by the DLVO model (42).

The DLVO theory does not consider the contribution of hydrophobic interactions between virus and sorbent, and at least for some viruses (e.g., bacteriophages) used to model enteric virus interactions, hydrophobic interactions significantly contribute to the adsorption of the viruses to selected sorbents (15). Relatively few studies have evaluated the adsorption of viruses to different foods, but Vega and colleagues (91) studied the binding of echovirus 11 (used to model picornaviruses) and feline calicivirus (used to model human caliciviruses) to lettuce and found that these viruses did not have adsorption patterns that fit those predicted by the DLVO theory. In contrast, there are many studies that have examined factors affecting the elution of viruses from different foods, and the same variables (e.g., pH, ionic strength, cation concentration) found to be important in virus binding to non-food surfaces significantly influence virus elution (10, 27, 60, 96). The performance of elution buffers are not exactly the same on two types of berries (raspberries and strawberries), suggesting different binding characteristics of these two sorbents (10). However, no standard procedure or systematic approach to evaluating the adsorption of enteric viruses to different substrates (including foods) has been developed, making it difficult to draw conclusions about the mechanisms involved in virus sorption (42).

The factors affecting virus adsorption to foods are summarized in Figure 2.

Virus contamination of plants

Enteric viruses can cause surface contamination of fruits and vegetables by adsorption following exposure to fecally contaminated soil or groundwater (43). A study from Costa Rica used a simple wash to demonstrate the presence of rotavirus and HAV on the surface of lettuce bought on the market (36). Such surface contamination conceivably could be amenable to virus removal by elution. Another possible route of contamination is uptake of virus through the root system and subsequent transport of the virus into edible portions of the plant via the conducting tissue (phloem and xylem). This route of virus penetration into plants is known to occur with pathogenic plant viruses, and has also been noted to occur with pathogenic salmonella (35, 43).

A limited number of studies have addressed this question for enteric viruses pathogenic for humans.

An early study demonstrated that a poliovirus type 1 strain was absorbed by tomato plant roots but was not translocated to aerial parts of the plant, while a mouse encephalomyelitis virus (another picornavirus) was also absorbed in significant concentrations and occasionally translocated acropetally (70). Polioviruses were also occasionally demonstrable in the upper parts of plants that had been irrigated with virus-contaminated waters (43, 66). Katzenelson and Mills (43) demonstrated that polioviruses most easily gained access to upper part of pepper plants following damage to the stem, but also occurred after damage to the root system. Interestingly, no translocation of virus to the upper parts of the pepper plant (or to those of cucumbers or lettuce) occurred when the root system of intact plants were exposed to virus.

Because of the recent association of hepatitis A virus infections with green onion consumption, Chancellor and colleagues (14) evaluated the uptake of fluorescent microspheres (1 and 10 microns in size) and attenuated hepatitis A virus (a vaccine strain) into growing green onions. The fluorescent beads could be demonstrated within the onion as early as one day after exposure and increased over time, while HAV was detectable within the onion using RT-PCR methods one week after exposure. While these studies did not address the viability of the virus, they suggest that virus can be taken up into the onion by a process that would prevent its removal by simple cleaning of the onion's surface and may suggest a mechanism for contamination of green onions involved in several hepatitis A virus outbreaks (14, 24, 94).

Many edible fruits and vegetables have complex surfaces that prevent removal of contaminating substances by simple washing. For example, washing does not substantially reduce viral titers of HAV from lettuce, fennel and carrots after the surfaces of these vegetables are exposed to a solution containing HAV (17). Similar results were observed when MS2 bacteriophage was used to contaminate the surfaces of a variety of fresh fruits and vegetables, including cucumbers, tomatoes, peppers, lettuce, strawberries, parsley, spring onions, carrots and cabbage (21). Complex surfaces of these foods may also decrease dessication effects that lead to virus inactivation (81).

Shellfish virus uptake and specific binding to tissues

Shellfish are filter feeders and concentrate enteric viruses from their environment while feeding. The majority of accumulated virus is found in the pancreatic tissue, also called the digestive diverticula (83). Virus accumulation in oysters is affected by factors such as water temperature, mucus production, glycogen content of the connective tissue, and gonadal development (9). Mechanical entrapment and ionic bonding are among the mechanisms that have been suggested to explain observed differences in accumulation of different viruses and among different oyster species (9, 26, 68).

Depuration is a dynamic process whereby shellfish purge themselves of contaminants either in a natural setting or in land based facilities. Relaying is the practice of transferring shellfish harvested from contaminated areas to clean shellfish growing waters. Both depuration and relaying are effective approaches to remove bacterial enteric pathogens from contaminated shellfish (79). If shellfish acted as mere filters or ionic traps to concentrate viruses, a simple depuration process should be sufficient to rid oysters of virus, as has been observed for bacteria (79). However, numerous studies have shown that oysters eliminate viruses much less efficiently than bacteria (79, 83). For example, oysters eliminated only ~7% of Norwalk virus compared to 95% reduction in levels of *E. coli* over a 48 hour depuration period (83). Poor depuration efficiency has also been seen for other viruses, including adenovirus, rotavirus, and HAV (37, 46, 62).

Another potential mechanism for the uptake and concentration of viruses in shellfish has been proposed based upon the observation of specific binding of a genogroup I norovirus to shellfish tissues (57). The genus *Norovirus* is divided into genogroups (I-V) and further subdivided into genotypes (from 1 to 20 genotypes per genogroup) (98). Distinct norovirus strains belonging to both genogroup I and II exhibit various binding patterns with different carbohydrate structures of the histo-blood group family, and lack of expression of these carbohydrates has correlated with resistance to infection in humans (4, 88). *In vitro* expression of the viral structural proteins, VP1 and VP2, leads to the spontaneous formation of virus-like particles (VLPs), and the VLPs are used to model viral interactions. For example, no difference in tissue distribution or binding is seen when Norwalk virus VLPs are compared to Norwalk virus in bioaccumulation experiments and in tissue binding experiments to *Crassostrea gigas* (57).

A HBGA A-like carbohydrate, alpha-linked N-acetyl galactosamine, is a ligand present in shellfish tissues that is involved in the specific binding of Norwalk virus VLPs to these tissues. The specificity of the binding as a HBGA type A-like carbohydrate was demonstrated using HBGA-specific carbohydrate antibodies and lectins to block VLP binding to tissues. HBGAs present in saliva also block VLP binding, and periodate treatment, which removes carbohydrate residues, decreases VLP binding to tissues (57). These observations are similar to those made in VLP binding studies with human intestinal tissues (41, 88). Mutant Norwalk virus VLPs that have point mutations in their capsids which abrogate binding to carbohydrate ligands also do not bind to shellfish tissues (57). Binding of Norwalk virus VLPs to a HBGA A-like carbohydrate has also been reported in a different oyster species, *Crassostrea virginica* (90). *In vitro* studies with additional norovirus genotypes are needed to determine whether binding to shellfish tissues is genotype-specific. With such information, epidemiologic studies of shellfish-associated outbreaks can be designed to address the importance of specific binding of noroviruses to shellfish tissues.

In bioaccumulation studies, some Norwalk viral particles can be detected in oyster hemophagocytes (57, 83). It is unclear whether the viral nucleic acids (83) or immunoreactive material (57) detected in phagocytes corresponds to particles being degraded and digested or whether particles are able to escape digestion. Internalization of intact viral particles into cells in the intestine or into hemophagocytes may provide another explanation for the poor efficiency of depuration in removing viruses from shellfish.

Virus inactivation & persistence

Factors influencing virus inactivation

A number of physical, chemical and biological factors influence the rate of virus inactivation in the environment (figure 1)(7, 81). Exposure to higher temperatures (heat), ultraviolet light, lower relative humidity, high pressure and radiation are physical factors that all contribute to loss of virus infectivity. Chemical factors that increase viral inactivation include exposure to extreme pH values (very acidic or alkaline), high salinity, certain enzymes (e.g., proteases and nucleases), ammonia and certain other ions, and a variety of compounds. The presence of bacteria and protozoa are external biological factors that can increase virus inactivation (7). Virus aggregation and the presence of organic matter may protect viruses from inactivation. While the contribution of many of these factors has been studied in a variety of circumstances, there are relatively few studies examining their role on viruses associated with foods.

Temperature effects on virus inactivation in foods

Temperature is one of the physical factors best recognized to play a role in virus stability in food. Storage of foods at refrigerated temperatures (4^{o}C) is a common approach to prolong the consumability of a food, but storage at these temperatures also prolongs virus survival relative to storage at room temperature. For example, Bidawid et al (5) found approximately a two-fold reduction in HAV titer on lettuce stored at 4^{o}C for 12 days compared to a 10,000-fold reduction when the lettuce was stored at room temperature. Virus survival can be prolonged on foods stored at 4^{o}C, as indicated by the detection of infectious poliovirus on celery that had been irrigated with virus-seeded wastewater for more than 2 months (93). The level of persistence varies with the vegetable matrix. No decline in infectious poliovirus titer was observed after two weeks at 4°C on green onion or fresh raspberries, but a ten-fold reduction was observed after 11.6 days on lettuce, 14.2 days on white cabbage and 8.4 days on frozen strawberries (54).
Higher temperatures, such as those achieved in cooking or pasteurization, increase the rate of virus inactivation. However, certain constituents or additives in foods may stabilize the virus, protecting it from inactivation. For example, heat inactivation of HAV is less efficient in dairy products containing a greater fractional content of fat (e.g., cream) compared to products with less fat (e.g., skim milk) (4), and a higher fat content in ground beef decreases the thermal inactivation of poliovirus (29). Higher sucrose concentrations, such as those used as stabilizers in some fruit-based products, increase HAV resistance to heat inactivation in strawberries (23). On the other hand, higher acidity can increase viral susceptibility to heat inactivation (23).

Effects of compounds in foods on viral growth

Compounds in foods may inhibit the growth of or inactivate viruses contaminating those foods. Konowalchuk and Speirs (49, 50) conducted a series of experiments examining the effects of a variety of fruit and fruit products on virus growth. Strawberry extracts inhibited poliovirus growth, and the inhibition was attributed to the presence of phenolic compounds in the fruit (50). Phenolic compounds were also thought to be responsible for the inhibitory activity of grape juice, red wines and white wines against several viruses (coxsackievirus, echovirus, poliovirus, reovirus and herpes simplex virus) (49). The skin and seeds of grapes, which contain the greatest concentration of phenolic compounds, also had the greatest inhibitory activity. This may also explain the observation that red wines had greater inhibitory activity than white wines, which are produced only from fermentation of the juice (4). The inhibitory activity in grape juice was reversible, suggesting that the virus is not inactivated. Fresh apple juice irreversibly inhibited poliovirus growth while many other fruit juices (orange, pineapple, grapefruit, tomato) had no measurable antiviral activity (51). The inhibitory activity of fresh apple juice was lost during storage and was postulated to be due to the presence of tannins in the apple. Oxidation of the tannins during storage could explain the loss of potency apple juice over time (51). Rotavirus infectivity was stable over 3 days at 4°C in a commercial fruit punch (76). Additional studies are needed to determine the exact mechanisms of action of the inhibitory compounds in these foods.

Effects of other environmental factors on virus inactivation

Ultraviolet (UV) light in sunlight acts as the principal natural germicide in the environment (64, 86). The ultraviolet light spectrum can be divided into three groups: UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm). The UV-C portion of the spectrum is the most virucidal, but most of the UV-C rays in sunlight are absorbed by the atmosphere before reaching the

earth's surface. Thus, it is estimated that the majority of the virucidal activity in sunlight comes from the UV-B portion of the spectrum (64). Environmental factors, including the presence of clouds, dust, and pollution, can decrease the penetration of UV-B light through the atmosphere. Double stranded DNA viruses (e.g., adenovirus) are more resistant to UV inactivation than are single-stranded RNA viruses (e.g., picornaviruses, caliciviruses), and viruses with larger genomes are more sensitive than those with smaller genomes (33, 85, 86, 89). It is estimated a full day of sun exposure at latitudes below 37^{o} would produce approximately a 1000-fold decrease in infectivity for the most UV sensitive viruses (64). However, limited experimental data are available on the effects of sunlight on survival of enteric viruses in the environment or on foods.

Field experiments are not able to distinguish which environmental factors are responsible for viral inactivation, but they can show the persistence of viral infectivity. Ward et al. (92) demonstrated that poliovirus could still be recovered from cabbage for up to 5 days after spray irrigation with virus-seeded wastewater, despite mean maximal temperatures ranging from 16-22°C. Subsequent experiments showed that poliovirus could be detected in vegetables (celery, spinach, tomatoes) grown and irrigated with poliovirus-seeded wastewater for 4-13 days after exposure (93). If the celery and spinach were collected just after irrigation and stored in a refrigerator, virus infectivity was still identifiable for 55-76 days (93).

Persistence of Viruses in Shellfish

As noted earlier, shellfish will bioaccumulate enteric viruses, and the viruses will persist under depuration conditions that are sufficient for the shellfish to clear enteric bacteria. This has led to outbreaks of viral disease associated with the consumption of shellfish meeting bacteriological sanitary standards (6, 55, 58, 65, 72, 82). An important question is how long shellfish can retain infectious viruses after bioaccumulation. Virus carriage for an extended period of time was suggested as early as 1973 when an outbreak of hepatitis A was linked to shellfish consumption (68). The shellfish were thought to have been contaminated following a flood in the spring, and retained infectious virus for one to two months before the outbreak. Subsequently, laboratory studies confirmed that infectious HAV could be detected for up to 3 weeks in oyster tissues, and RT-PCR methods detected viral RNA for up to 6 weeks (46). In another laboratory experiment, infectious adenovirus was detectable in mussels for approximately 3 weeks and in oysters for up to seven weeks when maintained in seawater at 4^{o}C, (37). Viral infectivity declined more rapidly when the shellfish were maintained at a higher temperature (18°C). As in the earlier study, viral nucleic acids remained detectable for a longer period of time than did infectivity. VLPs have been used as a surrogate for infectious viruses in field studies and could be detected for several weeks under relaying conditions in an estuary (62).

The duration of virus persistence under natural conditions was examined following a norovirus outbreak. Oysters were collected from a harvesting area implicated as the source of shellfish associated with an outbreak, and noroviral RNA could be detected approximately 4 weeks after the outbreak was identified (55). Although the oysters met regulatory standards for the presence of *E. coli*, the continued exposure to viral contaminants cannot be excluded. Norovirus infectivity could not be assessed due to the lack of an *in vitro* culture system.

Treatment of foods to remove or inactivate viruses

A variety of methods are used to try to reduce the risk of foodborne transmission of contaminating pathogens. Washing food by rinsing it with water is a common practice to remove visible dirt and contaminants, but it is unreliable at removing viruses. For instance, Croci et al.

(17) found that HAV persisted on the surface of a variety of fresh produce despite rinsing in tap water for 5 minutes. Use of a disinfectant to inactivate contaminating viruses has also been evaluated. Dawson et al. (21) found that use of chlorinated (100 ppm free chlorine) water as a wash was not effective at removing the enteric virus surrogate, MS2 bacteriophage, from the surface of any of the fresh produce tested. Gulati et al. (34) tested the ability of a variety of commercially available disinfectants to inactivate feline calicivirus (a norovirus surrogate) applied to a food preparation surface or to strawberries and lettuce. Only peroxyacetic acid and a hydrogen peroxide-containing product effectively decontaminated strawberry and lettuce, but only at a fourfold higher concentration than recommended by the manufacturers (34). This approach does not hold much promise for use against noroviruses due to the poor activity of disinfectants against these viruses (28).

Modified atmospheric packaging (MAP) is a method used to extend the shelf-life of fresh produce. With MAP, produce is packaged while being exposed to elevated carbon dioxide or reduced oxygen concentrations, and these altered gas concentrations inhibit the growth of bacteria that may lead to spoilage. Bidawid et al. (5) examined the effect of different MAP conditions on the survival of HAV when it contaminated the surface of lettuce. No reduction in the survival of HAV was noted, and under some MAP conditions using high carbon dioxide concentrations, HAV survival improved.

High hydrostatic pressure processing (HHP) has emerged as a promising technology for virus inactivation. This method exposes the food to hydrostatic pressures of 200-400 megapascal (MPa) for periods of 1-5 minutes. HHP efficiently inactivates enteric viruses in buffer but inactivation rates are affected by treatment temperature and virus strain (16, 45). It has been applied to shellfish that have bioaccumulated HAV or a murine norovirus, and greater than 1000-fold reductions in viral titer was achieved with a treatment of ≤ 400 MPa for 5 min at 5°C (11, 47). Longer treatment times are required to achieve the same level of inactivation on the virus stock when the virus is oyster-associated compared to when it is suspended in buffer (47). A potential disadvantage of this method is that changes in the character of the shellfish have been demonstrated in organoleptic studies, and some consumers prefer to eat live oysters (20).

Some foods are treated by marination, and the effect of this process has been evaluated in mussels contaminated with norovirus or HAV. The commercial marination process is a two-stage procedure including a preliminary heat treatment (immersion in boiling water or steaming for 3 min) and then marination for several weeks. After 4 weeks of marination, the infectious titer for HAV decreased approximately 50-fold, and human norovirus RNA was still detected by real-time RT-PCR (38). These data suggest that marination alone is not sufficient to inactivate enteric viral pathogens in shellfish.

Gamma irradiation of food is being explored as a potential means for inactivating enteric pathogens that contaminate the food (59). However, there is limited information evaluating the efficacy of this technology on viral pathogens. The dosages (400 Gy) needed to inactivate two norovirus surrogates (feline calicivirus and canine calicivirus) at least 1000-fold are well with in the ranges being evaluated in other food irradiation studies (22, 59).

Cooking is the most reliable manner of inactivating viruses in foods, but there are numerous examples where “cooked foods” were still contained infectious virus and were able to transmit disease (Tables 1& 2). Early studies showed that 7 to 13% of poliovirus in oysters survived a variety of different home cooking procedures (steamed, stewed, fried, or baked respectively) (25). Steaming bivalve mollusks for five minutes or less does not achieve a sufficient temperature in the shellfish meat for viral inactivation (48). In mussels, five minutes after the opening of the valves by steaming, HAV and HRV could be detected showing a reduction in the original titer below 3 log units, whereas PV was no longer detectable (1). HAV-contaminated cockles needed to be immersed for at least 1 min in boiling water to achieve HAV inactivation (69), and viable HAV persisted in contaminated mussels after boiling for 37 seconds, although the HAV was completely inactivated after boiling for 3 minutes (39).

Differences observed in virus reduction may be due, in part, to the method of virus inoculation, by bioaccumulation versus by artificial seeding, suggesting that virus localization may play an important protective role. Unfortunately, many of the cooking methods that are sufficient to inactivate contaminating enteric viruses adversely affect the palatability of the shellfish for consumption.

Consequences of Virus Persistence in Foods

The inability to remove or inactivate enteric viruses from contaminated foodstuffs leads inevitably to human disease in some susceptible consumers. There are numerous examples of outbreaks gastroenteritis or hepatitis associated with the consumption of raw or partially cooked foodstuffs (Table 2). Some shellfish-related outbreaks clearly show that the cooking procedures used did not efficiently inactivate contaminating viruses (Table 2). For example, fried oysters have been responsible for illness as have boiled cockles and clams in paella. This resistance to heating is also seen in other foods, such as grilled liver (97). Processed food, such as packaged lettuce, orange juice and raspberry cake, has been implicated in outbreaks. Contamination of food during preparation also may occur. It can be expected that such outbreaks will continue to occur as long as there are opportunities for viral contamination of foodstuffs and these foods are consumed without treatments effective at inactivating the contaminating viruses.

Conclusions/Summary

Enteric viruses bind to food matrices by a variety of mechanisms, including ionic and hydrophobic interactions, van der Waals forces, interaction with specific ligands (e.g., receptors), and uptake into plants. Removal or inactivation of viruses contaminating foods has proven to be difficult and when ineffective can lead to foodborne outbreaks of gastroenteritis or hepatitis.

The role of food matrix on virus persistence is difficult to estimate as few data addressing this issue are available. To collect more information it will be important to work on improving methods of virus detection, as it is still difficult to detect a virus in a complex matrix due to low levels of virus contamination, the presence of compounds toxic in cell culture and the presence of inhibitors of molecular detection assays. Without sensitive and efficient methods, it will be difficult to obtain information on virus persistence and localization, which in turn may provide insights that lead to improved detection assays. For example, the recognition that most of the enteric viruses contaminating shellfish are located in pancreatic tissues led to a more than ten-fold increase in sensitivity of virus detection by eliminating shellfish tissues that contained inhibitors of PCR. The identification of HAV inside green onion tissues demonstrates that simple washing of the surface of a food may be insufficient to identify viruses responsible for outbreaks of disease; instead all plant tissues may need to be analyzed. A better understanding of the interaction between pathogenic enteric viruses and different food matrices should lead to enhanced measures to remove or inactivate these viruses and ultimately to improved food safety.

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Tables

Table 1 – Effects of different physical factors on virus inactivation in shellfish.

Type of treatment	Recipe	Virus	contamination	Persistence of virus	Ref.
Heat	Stewed oysters in milk	poliovirus	Seeded	10% survival after 8 min	25
	Fried oysters/oil 177°C	poliovirus	Seeded	10% survival after 8 min.	25
	Baked oysters/oven 121°C	poliovirus	Seeded	13% survival after 20 min.	25
	Boiling cockles	poliovirus	Bioaccumulated	No survival after 3 min.	69
		HAV		No survival after 2 min.	
	Boiling mussels	HAV	Seeded	No survival after 3 min for HAV	39
		norovirus		100% persistence for NoV RNA	
	Steamed mussels	HAV	Bioaccumulated	0.1% survival after 5 min.	1
		rotavirus		0.1% survival after 5 min.	
	Steamed mussels	HAV	Seeded	No survival after 3 min for HAV	39
		norovirus		100% persistence for NoV RNA	
	Steamed oysters	poliovirus	Bioaccumulated	7% survival after 30 min.	25
	Mussels in hors d'oeuvre	HAV	Bioaccumulated	Infectious viruses detected after 9 min*	18
	Mussels au gratin	HAV	Bioaccumulated	Infectious viruses detected after 5 min *	18

	Mussels in tomato sauce	HAV	Bioaccumulated	No survival after 8 min.	18
Cold	Oyster kept at 5°C	poliovirus	Bioaccumulated	13% survival after 1 month	25
	Frozen oysters (-17.5°C)	poliovirus	Bioaccumulated	10% survival after 12 weeks	25
Marinade	Steamed mussels and marination (pH 3,75)	HAV norovirus	Seeded	3% survival after 4 weeks for HAV 100% persistence for NoV RNA	38
High pressure	oysters	Murine Nrovirus	Bioaccumulated	No survival after 5 min at 5° at 325 MPa	47
	oysters	HAV	Bioaccumulated	0.1% survival after 1 min at 400 MPa	11

*: infectious virus too low for quantification

Table 2- Selected outbreaks linked to prepared foods

A. shellfish

Virus	Shellfish	Cooking/food preparation	Number of cases	Ref.

HAV	Cockles	Steaming 1-2 min and 4 min. boiling	132	75
HAV	Coquina clams	Frozen and paella cooking	183	6
NoV	Oysters	Kilpatrick or mornay	>2000	71
NoV	Oysters	Grilled, steamed, stewed, fried	131	65
NoV	Oysters	Frozen and served thawed	213	72
NoV, AV	Clam, oysters	Clam soup and cooked oyster from frozen stock	26	82
NoV	Mussels	Cooked	89	78

B. Other food.

Virus	Food	Cooking/food preparation	# of cases	Ref.
HEV	Pig liver	Grilled	10	97
HAV	Iceberg lettuce	Washes	202	80
HAV	Rucola lettuce	Ready to eat	80	73

HAV	Strawberries	Incorporated in a shortcake	128	40
HAV	Green onion	Melted cheese sauce, rice	43	24
HAV	Orange juice	Manufacturing process	351	30
NoV	Raspberries	Frozen dressing	106	77
NoV	Raspberries	Cake	300	31
NoV	Raspberries	Cake	30	56

Figures

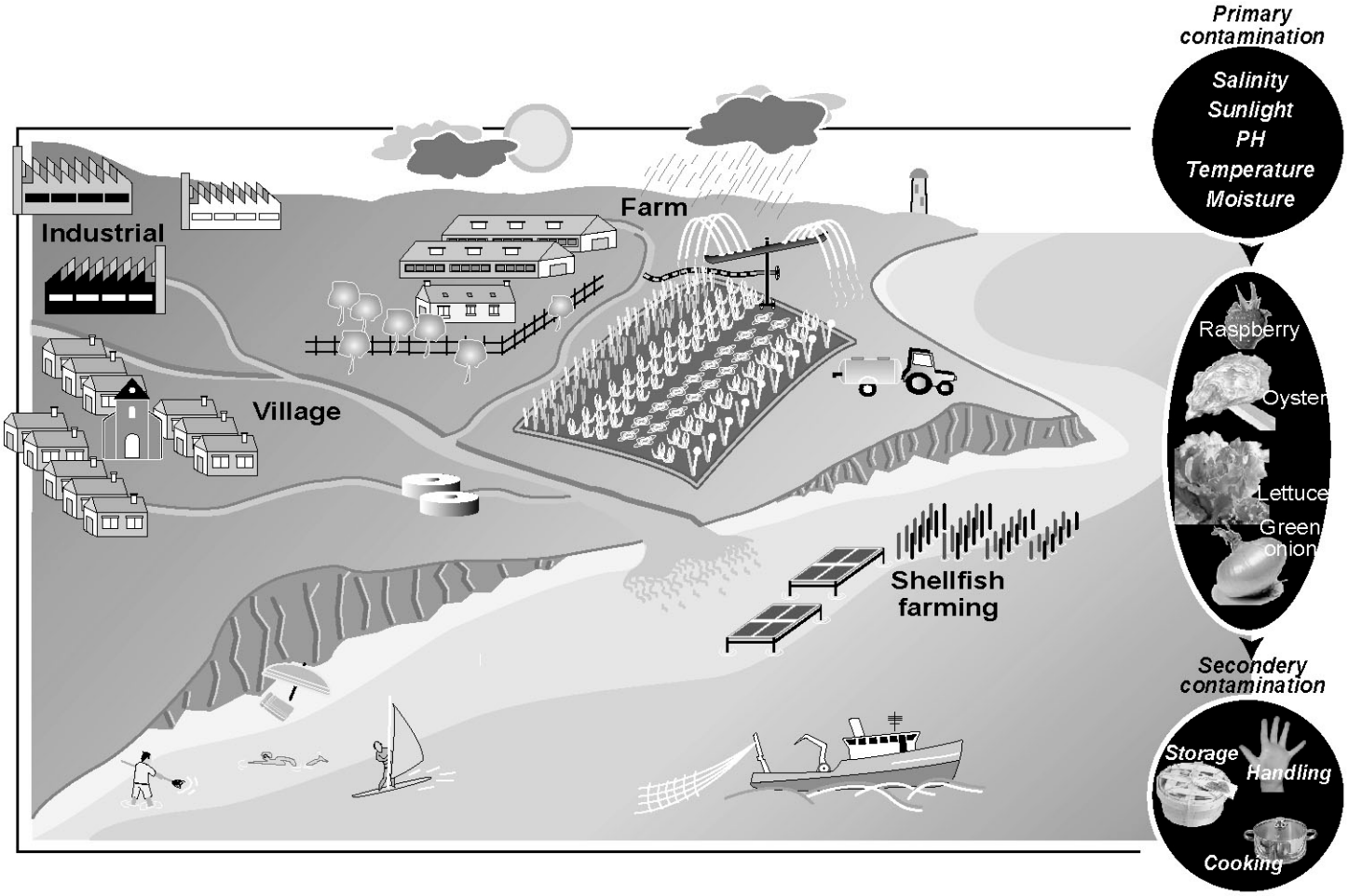

Figure 1 : Environmental sources of contamination for food (vegetables and shellfish)
Food may be contaminated directly by sewage, rivers, or fertilizers. Climate events like rain, sun, temperature may impact virus behavior before harvesting.

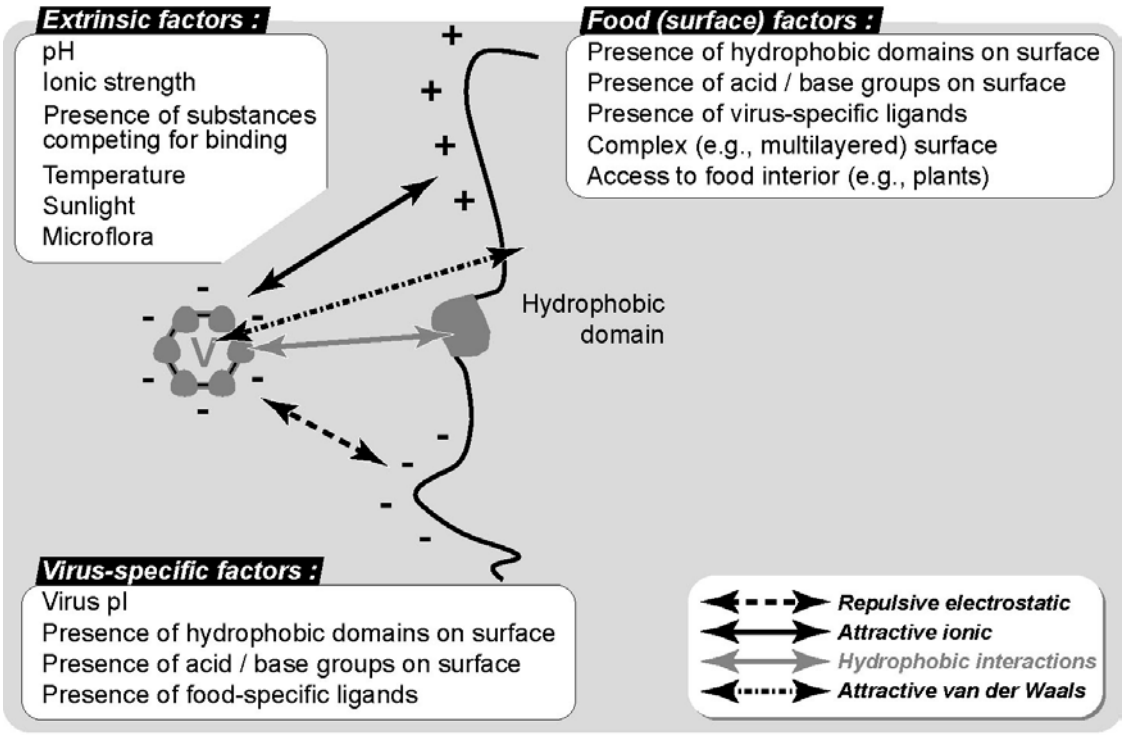

Figure 2: Principal factors affecting virus adsorption and persistence on food surfaces.