Detection of Human Food-Borne and Zoonotic Viruses on Irrigated, Field-Grown Strawberries

Julie Brassard, Marie-Josée Gagné, Mylène Généreux, and Caroline Côté
Agriculture and Agri-Food Canada, Food Research and Development Centre, Saint-Hyacinthe, Québec, Canada, and Research and Development Institute for the Agri-Environment, Saint-Hyacinthe, Québec, Canada

This study evaluated the presence of pathogenic human and zoonotic viruses on irrigated, field-grown strawberries. Norovirus genogroup 1, rotavirus, and swine hepatitis E virus genogroup 3 were detected on strawberries, and irrigation water is suspected as the contamination origin.

Encouragement by public health authorities to adopt a healthy lifestyle has led to increased consumer demand for fresh produce. A wide variety of fresh produce is now available throughout the year in several industrialized countries, mainly because of market globalization. However, vegetables and fruits, particularly berries, are increasingly associated with outbreaks of food-borne illness in several parts of the world (4, 12, 21, 25) despite the perception that these infections are usually related to products of animal origin (32). Produce consumed fresh or after minimal processing is a potential vehicle for enteric pathogen transmission. Some enteric viruses, such as norovirus (NoV), rotavirus, adenovirus, astrovirus, and hepatitis A virus, are responsible for a large proportion of food-borne illness cases (28). In recent years, several studies have focused on optimizing the concentration and detection of these viruses in samples from fresh produce (6, 11, 13). In addition, many laboratory survival assays and epidemiological studies of outbreaks have been conducted for certain pathogenic viruses (9, 10, 33). The resulting data can now be used to provide tools to identify and, eventually, control potential sources of contamination in the field. Potential sources of microbial contamination in the field include irrigation water, soil, organic fertilizers, and human handling (5). There is very little information about the presence of viruses in irrigation water or about their persistence in the production chain and on produce, although these viruses represent a large proportion of food-borne infection agents. The aim of this study was to evaluate the presence of pathogenic human and zoonotic viruses on strawberries at the field scale after irrigation.

A field experiment was conducted in the Laurentides region of the province of Quebec, Canada, in 2009. A split-plot factorial design was set that included the method of irrigation (spray and subsurface drip) as the main plot factor and the mulch (plastic or straw) as the subplot factor. Plots were 7 m long and 8 m wide, and each treatment was repeated four times (see Fig. S1 in the supplemental material). Irrigation was performed on 28 July using water from the Chicot River next to the experimental site. Water samples (500 ml) were taken three times during irrigation at the end of the drip line, at sprinklers, and in the river. Composite samples of 10 strawberries were aseptically collected from each plot before irrigation, 1 h after irrigation, and on 29 July and 3 August. Fruits were cut into 1-g pieces. As sample process controls, murine calicivirus 1 (MNV-1) and feline calicivirus (FCV strain F9) were added to every water and strawberry sample at concentrations of 2.5 × 10⁴ PFU/g for MNV and 2.5 × 10⁵ PFU/g for FCV prior to extraction procedures. This provided an additional quality control check throughout the sample processing. The procedure for virus elution and concentration from the strawberries was adapted from Butot et al. (10). Briefly, 25 g of strawberries was inoculated with MNV and FCV and incubated at room temperature for 30 min. Glycine-NaCl buffer (0.05 M and 0.14 M; pH 7.5) was added to 200 µl of pectinase, and the samples were gently shaken for 30 min. The elution buffer was centrifuged at 5,000 × g for 5 min and filtered through a Whatman GD/X membrane, and the filtrate was concentrated by centrifugation at 5,000 × g for 15 min on an ultrafiltration device (Amicon Ultra-15; Millipore, Billerica, MA). Plant RNA Isolation Aid (Applied Biosystems, Streetsville, Ontario, Canada) (1:1) was added to the concentrate before nucleic acid extraction. For water sample analysis, the OPFLP-04 standard method for the recovery and concentration of viruses present in artificially and naturally contaminated water from Health Canada’s compendium of analytical methods was used (7, 8). Negative controls for each method of concentration of virus obtained from water and fruits (water, fruits, and washing buffer) were included and processed at the same time and tested by the detection systems.

Viral RNA was extracted using an Rneasy viral minikit (Qiagen, Mississauga, Ontario, Canada). In the first step, rotavirus, MNV, FCV, norovirus genogroup I (NoV GI) and NoV GII, and hepatitis E virus (HEV) were detected using conventional reverse transcription-PCR (RT-PCR) and nested RT-PCR assays according to previously described procedures (1, 7, 14, 17, 22). All primers and probes used are shown in Table S1 in the supplemental material. The RT-PCR amplicons were analyzed on 2% (wt/vol) agarose gels stained with ethidium bromide. In the second step, all the positive samples obtained for HEV and rotavirus by conventional RT-PCR detection were directly cloned and sequenced according to the method of Ward et al. (35) and positive samples for NoV were retested for confirmation using real-time RT-PCR systems (18, 23). Nucleotide alignment was performed...
TABLE 1 Molecular detection of food-borne viruses on strawberry samples before and after irrigation

<table>
<thead>
<tr>
<th>Plot</th>
<th>Before irrigation</th>
<th>1 h after irrigation</th>
<th>1 day after irrigation</th>
<th>6 days after irrigation</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>ND</td>
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<tr>
<td>2</td>
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<tr>
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<tr>
<td>4</td>
<td>ND</td>
<td>NoV GI</td>
<td>ns</td>
<td>NoV GI</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>NoV GI</td>
<td>NoV GI, human RV</td>
<td>NoV GI</td>
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<tr>
<td>6</td>
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<td>NoV GI</td>
<td>NoV GI</td>
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<tr>
<td>7</td>
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<tr>
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<td>NoV GI</td>
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<tr>
<td>9</td>
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<td>NoV GI</td>
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<td>11</td>
<td>ND</td>
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<td>NoV GI, human RV</td>
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<tr>
<td>12</td>
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<td>ND</td>
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<tr>
<td>13</td>
<td>ND</td>
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<td>14</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>16</td>
<td>NoV GI</td>
<td>NoV GI</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND: not detected; ns, no sample.

The river used as a source of water is situated in agricultural and residential areas. This could explain the presence of viruses of animal (HEV) and human (rotavirus and NoV) origin on the fruits. Human and swine Torque teno viruses have been found in 5% of fruit samples analyzed (data not shown). Some enteric viruses, such as NoV and rotavirus, are able to withstand certain treatments used for wastewater, and their presence in surface water that contains such effluents is frequently reported around the world (29). As a result, discharge from wastewater treatment plants and septic tanks into environmental water can have an impact on the safety of agricultural products, particularly in horticultural production, where surface water is frequently used for irrigation. NoV GI was detected at various concentrations between $3.0 \times 10^3$ and $5.0 \times 10^4$ particles/g (threshold cycle [$C_T$] values between 31 and 38) in 25% of the strawberry samples analyzed in this study, which is consistent with other studies on fresh produce (leafy green and berries), as NoV, especially GI, was found on between 6.7% and 50% of analyzed samples (2). The authors of that study also suspected irrigation water as a potential source of contamination, because NoV GI is frequently reported to be detected in surface water and appears to be more persistent in the environment than NoV GII (2, 24). NoV GI seems to be also linked to cases of recreational (contact with contaminated water) or sporadic food-borne illness and is less often reported as a cause of food-borne illness outbreaks affecting large numbers of people. NoV GI seems to be transmitted more effectively via food and the environment than by person-to-person contact. Involvement of NoV GI in cases of food-borne gastroenteritis is probably underestimated because of the low contagiousness of the virus, but its persistence in the environment makes it a pathogen that should be monitored, mainly in horticultural and seafood products.

For rotaviruses detected and sequenced in this study, the two isolates showed identity of 98% to 99%, suggesting that they belong to the same virus strain. That strain is a member of rotavirus G1P[8], the most common human rotavirus strain in Canada and the United States (15, 30). Rotavirus is the primary cause of young children’s hospitalization due to gastroenteritis in industrialized countries and is responsible for hidden societal costs (19, 26). Despite the introduction of vaccination against rotavirus, it is important to know which strains are in circulation in the population and the environment because rotavirus is also widespread in wild and domestic animal species, and it has been suggested that zoonotic transmission plays a substantial role in the introduction of novel strains into the human population (3). In addition, it seems that this transmission could be achieved via fresh produce and horticultural products.

Sequencing determined that the HEV detected by nested RT-PCR 1 h after irrigation was of the swine HEV genotype 3 strain. The phylogenetic analysis of the amplified genomic fragment shown in Fig. 1 revealed nucleotide sequence identity of 99% with another swine HEV strain detected on a swine farm in Quebec (35). That study was conducted between May 2003 and January 2004, and the identified strain was still in circulation at the time of the present experiment in the summer of 2009. Discharge from farms into the environment and certain agricultural practices such as manure spreading can also have an impact on the safety of horticultural products (36). Because enteric and hepatic viruses are persistent in the environment, they can be found in surrounding rivers following rain and runoff events and can eventually end up on fresh produce via irrigation (34). The circulation of swine...
478. Hepatitis E virus (HEV) genotype 3 on Quebec farms has already been demonstrated in a previous study. This is the first time that swine HEV, considered a zoonotic virus primarily because of its transmission via contaminated pork (27), has been found on fruits. However, it is not possible to evaluate the survival of the virus or its ability to infect the host, because HEV replication in the laboratory is only moderately successful. The detection of HEV on produce nevertheless paves the way for epidemiological investigations into other possible sources of contamination that could explain the increase in autochthonous cases of hepatitis caused by HEV genotype 3 in countries where the disease is not endemic (20). Recently, in Quebec, a gastroenterology research team demonstrated a high prevalence of HEV in pediatric organ recipients developing chronic hepatitis caused by HEV genogroup 3 genetically related to animal strains. The authors of that study also suggested potential zoonotic transmission of the virus via food in those immunocompromised children (16). It was also reported that individuals with an impaired immune system, including children, the elderly, pregnant women, and people with HIV/AIDS, are more susceptible to such infections even at the low levels of enteric viruses present in the environment or on food (31).

In conclusion, norovirus genogroup I, rotavirus, and swine hepatitis E virus genogroup 3, which are human and zoonotic pathogens responsible for food poisoning and highly resistant in the natural environment, were detected in low proportions on strawberries in the field. In this study, it was not possible to establish a clear link between irrigation water and the contamination origin. The impact of other contamination factors such as agricultural practices and soil should be evaluated in the future.

**Nucleotide sequence accession numbers.** The nucleotide sequences of HEV and rotavirus determined in this study were deposited in GenBank under accession numbers HQ415969 and JF748713 to JF748715.

**ACKNOWLEDGMENTS**

This research was supported by Agriculture and Agri-Food Canada Research Branch Peer Reviewed Research Projects F.1401.EP and by the Programme de Solution à l’Innovation Horticole of the Quebec Ministry of Agriculture and Fisheries and Food.

**REFERENCES**