term to dramatically reduce the high incidence of HPAI in Bangladesh. We have progressively and dramatically increased the scope and benefits of our pilot PVC implementation program, but additional work is needed. To help spread PVC approaches throughout the country, community leaders, imams of local mosques, and school teachers can be trained to implement awareness programs on safe practices for raising poultry and regular cleaning and disinfection of live bird markets. The strengthening of biosecurity measures will help control the spread of HPAI virus and other zoonotic diseases.

Acknowledgments

We acknowledge Zakaria Noyon and Suman Das-Gupta for providing technical assistance in implementation of the project. We thank Paritosh K. Biswas for assisting with data analysis and for reviewing the manuscript.

This project was funded by the United States Agency for International Development.

Shankar P. Mondal, David Tardif-Douglin, Robert Ryan-Silva, and Rich Magnani

Author affiliations: Development Alternatives Inc., Bethesda, Maryland, USA (S.P. Mondal, D. Tardif-Douglin, R. Ryan-Silva, R. Magnani); and University of Minnesota, St. Paul, Minnesota, USA (S.P. Mondal)

DOI: http://dx.doi.org/10.3201/eid1812.120635

References

1. World Health Organization for Animal Health. Update on highly pathogenic avian influenza in animals (type H5 and H7) [cited 2012 Mar 19]. http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2012/
2. World Health Organization. Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO [cited 2012 Mar 19]. http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html
3. Biswas PK, Christensen JP, Ahmed SS, Barua H, Das A, Rahman MH. at al. Avian influenza outbreaks in chickens, Bangladesh. Emerg Infect Dis. 2008;14:1909–12. http://dx.doi.org/10.3201/eid1412.071567
4. Biswas PK, Christensen JP, Ahmed SS, Das A, Rahman MH, Barua H, et al. Risk for infection with highly pathogenic avian influenza virus (H5N1) in backyard chickens, Bangladesh. Emerg Infect Dis. 2009;15:1931–6. http://dx.doi.org/10.3201/eid1512.090643
5. Biswas PK, Rahman MH, Das A, Ahmed SS, Giasuddin M, Christensen JP. Risk for highly pathogenic avian influenza H5N1 virus infection in chickens in small-scale commercial farms, in a high-risk area, Bangladesh, 2008. Transbound Emerg Dis. 2011;58:519–25. http://dx.doi.org/10.1111/j.1865-1682.2011.01235.x

Address for correspondence: Shankar P. Mondal, Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108, USA; email: shankarpm@yahoo.com

Hepatitis E Virus Genotype 3 in Shellfish, United Kingdom

To the Editor: Bivalve mollusks (shellfish), such as mussels and oysters, are filter feeders; they concentrate microorganisms of human and animal origin (up to 100×) from the surrounding environment. Several recent reports have linked the incidence of human infection with hepatitis E virus (HEV) to consumption of undercooked pork, game products, and shellfish (*1*,*2*). Infectious HEV has been found in swine manure and wastewater (*3*); therefore, application of manure to land and subsequent runoff could contaminate coastal water, leading to contamination of shellfish and, subsequently, possible human infection. Because they are filter feeders, bivalve mollusks are biologically relevant sentinels and can indicate potential pathogens that are contaminating the environment. It is essential to ensure that this sustainable resource of coastal areas, where mussels and oysters are farmed or collected wild, is not subjected to environmental contamination that could lead to public health risks.

Risk management for bivalve mollusks, aimed at control of fecal pollution, relies heavily on the use of *Escherichia coli* as an indicator of fecal (sewage) contamination and is enacted under European food regulations (Regulation 854/2004, www.cefas.co.uk/media/455777/extract_reg_no_854_2004.pdf). However, although these regulations probably reduce the number of infections, especially bacterial infections, they are not viewed as adequately controlling the risk for viral infections. Specific risks are posed by the robustness of viruses in the environment and the different behavior of viruses within bivalve mollusks compared with behavior within bacterial fecal indicators.

HEV is deemed to be inactivated during processing procedures used to prepare mussels for consumption; however, HEV is only 50% inactivated at 56°C and 96% at 60°C for 1 hour, it is stable when exposed to trifluorotrichloroethane, and it is resistant to inactivation by acidic and alkaline conditions (*4*). Most shellfish are usually eaten raw, but viable virus can also pose a risk to public health in shellfish that are lightly steamed or preserved by smoking and/or in acetic acid. Indeed, a recent study by the Food Standards Agency, in which >800 oyster samples from 39 growing beds in the United Kingdom were collected and screened during 2009–2011, found norovirus at low levels in at least 76% of oysters (*5*). Other studies identified hepatitis A virus and norovirus in shellfish production areas and in ready-to-eat products in the United Kingdom (*1*,*6*). In fact,

depuration experiments demonstrated no decrease in titers against hepatitis A virus over a 23-hour cleansing period (*7*). In addition, acute HEV infection attributed to consumption of shellfish was diagnosed for 33 passengers who recently returned from a cruise (*2*). However, data have been restricted to questionnaires implicating consumption of shellfish as a source of transmission; no follow-up analyses of the contaminated foodstuff have been conducted. Thus, possible transmission routes for HEV remain poorly studied in the United Kingdom (*2*).

To determine whether HEV is present in mussels collected locally for human consumption, we examined 48 mussels from 5 tidal locations in Scotland. We collected closed mussels from the west coast of Scotland (11 at Lunderston Bay and 28 at Ardrossan) and the east coast of Scotland (9 at Stannergate, Dundee; Ferryden, Montrose; and the Ythan Estuary at Newburgh).

The site at Ardrossan was near a slaughterhouse and a meat preparation purification plant that processes pigs. The plant was considered a potential source of contamination, and mussels were collected in a 10-m^2 area around an outfall (drain/sewage pipe) directly in line with the processing plant.

A total of 36 (92%) of the 39 mussels from the west coast were positive by PCR for HEV, and 5 (55%) of the 9 from the east coast were positive. The mean value of HEV RNA detected in the samples was 4.25 $\log_{10}$ IU/mL (range 3.73–5.2 $\log_{10}$ IU/mL), and the assay was validated by using the current candidate HEV World Health Organization standard (http://whqlibdoc.who.int/hq/2011/WHO_BS_2011.2175_eng.pdf). Phylogenetic analysis showed that most bivalve mollusk sequences clustered with HEV genotype 3 from humans and swine (Figure; online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0924-Techapp.pdf). Also, HEV sequences isolated specifically from a UK human source corresponded with sequences isolated from the bivalve mollusks. The presence of a swine-like HEV genotype 3 in freshwater bivalve mollusks has also been reported in Japan and South Korea (*1*,*9*).

Worldwide, an estimated 40,000 persons die and another 40,000 experience long-term disability as a result of consuming raw or undercooked shellfish (*10*). This study, demonstrating the presence of HEV in mussels collected locally in Scotland for human consumption, raises concern as to whether these shellfish are a potential source of infection, as reported (*2*). The association between environmental contamination with HEV and possible transmission by eating shellfish warrants investigation.

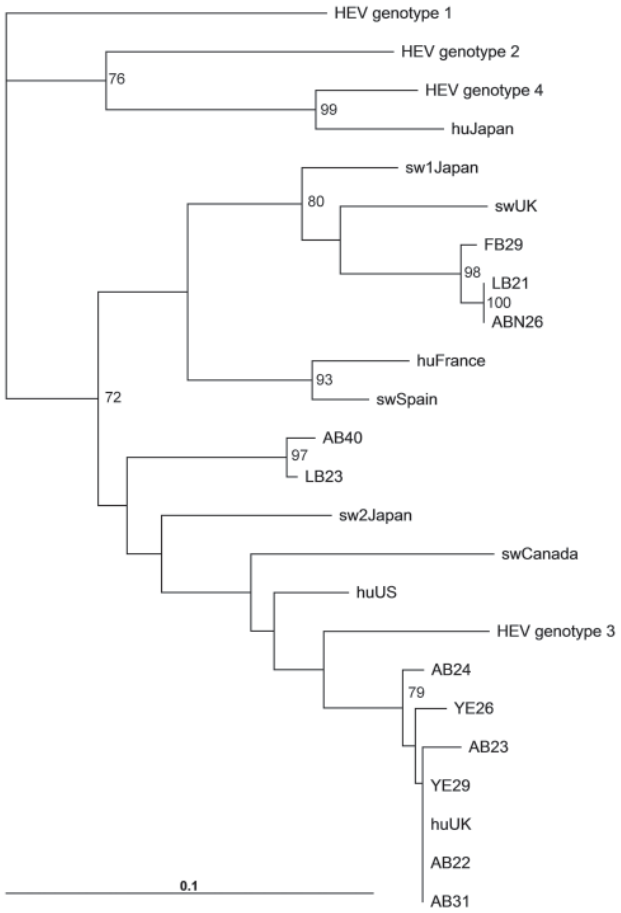

Figure. Phylogenetic analysis of HEV open reading frame 2 sequences isolated from *Mytilus* spp. RNA was isolated from 50–100 mg of digestive gland or gill. Tissue was homogenized in 300 μL phosphate-buffered saline, and viral RNA was isolated by using a viral RNA kit (QIAGEN, Crawley, UK), and PCR was conducted by amplifying nucleotides 6332–6476 as described (*8*). The nucleotide sequences were aligned and bootstrapped, and phylogenetic neighbor-joining trees were constructed by using the ClustalW software (www.ebi.ac.uk/Tools/msa/clustalw2/). Phylogenetic trees were visualized by using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Bootstrap values >70% are indicated. Scale bar indicates nucleotide substitutions per site. Sample site codes: AB, Ardrossan Beach; LB, Lunderston Bay; ABN, Aberdeen; FB, Ferrybridge; YE, Ythan Estuary. Sequences: Sw, swine; hu, human (followed by country of origin). GenBank accession numbers for reference sequences: HEV genotype 1, B73218; HEV genotype 2, M74506; HEV genotype 3, CO31008; HEV genotype 4, C272108; huUK (KernowC1), HQ389543; HuUS, JN837481; swUK AF503512; huFrance, JN906974; swCanada, AY115488; swSpain, JQ522948; sw2Japan AB248521, huJapan AB161719.

This work was supported by European Commission FP6 funded project LSHB-CT-2006-037377 and by the Chief Scientist Office Scotland project reference ETM/32.

Claire Crossan, Paul J. Baker, John Craft, Yasu Takeuchi, Harry R. Dalton, and Linda Scobie

Author affiliations: Glasgow Caledonian University, Glasgow, Scotland, UK (C. Crossan, P.J. Baker, J. Craft, L. Scobie); University College London, London, UK (Y. Takeuchi); and European Centre for Environment and Human Health, University of Exeter Medical School, Truro, UK (H.R. Dalton)

DOI: http://dx.doi.org/10.3201/eid1812.120924

References

1. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, et al. Hepatitis E. Lancet. 2012;379:2477–88. http://dx.doi.org/10.1016/S0140-6736(11)61849-7
2. Said B, Ijaz S, Kafatos G, Booth L, Thomas HL, Walsh A, et al. Hepatitis E outbreak on cruise ship. Emerg Infect Dis. 2009;15:1738–44. http://dx.doi.org/10.3201/eid1511.091094
3. McCreary C, Martelli F, Grierson S, Ostanello F, Nevel A, Banks M. Excretion of hepatitis E virus by pigs of different ages and its presence in slurry stores in the United Kingdom. Vet Rec. 2008;163:261–5. http://dx.doi.org/10.1136/vr.163.9.261
4. Emerson SU, Arankalle VA, Purcell RH. Thermal stability of hepatitis E virus. J Infect Dis. 2005;192:930–3. http://dx.doi.org/10.1086/432488
5. Lowther JA, Gustar NE, Powell AL, Hartnell RE, Lees DN. A two-year systematic study to assess norovirus contamination in oysters from commercial harvesting areas in the United Kingdom. Appl Environ Microbiol. 2012;78:5812–7.
6. Baker K, Morris J, McCarthy N, Saldana L, Lowther J, Collinson A, et al. An outbreak of norovirus infection linked to oyster consumption at a UK restaurant, February 2010. J Public Health (Oxf). 2011;33:205–11.
7. McLeod C, Hay B, Grant C, Greening G, Day D. Inactivation and elimination of human enteric viruses by Pacific oysters. J Appl Microbiol. 2009;107:1809–18. http://dx.doi.org/10.1111/j.1365-2672.2009.04373.x
8. Erker JC, Desai SM, Mushahwar IK. Rapid detection of hepatitis E virus RNA by reverse transcription–polymerase chain reaction using universal oligonucleotide primers. J Virol Methods. 1999;81:109–13. http://dx.doi.org/10.1016/S0166-0934(99)00052-X
9. Li TC, Miyamura T, Takeda N. Detection of hepatitis E virus RNA from the bivalve Yamato-Shijimi (*Corbicula japonica*) in Japan. Am J Trop Med Hyg. 2007;76:170–2.
10. Shuval H. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. J Water Health. 2003;1:53–64.

Address for correspondence: Linda Scobie, Department of Life Sciences, Glasgow Caledonian University, Cowcaddens Rd, Glasgow, G4 0BA, UK; email: linda.scobie@gcu.ac.uk

Autochthonous Gnathostomiasis, Brazil

To the Editor: Gnathostomiasis is an infestation by nematodes of the genus *Gnathostoma*; the main source of infection is raw freshwater fish. In the past, gnathostomiasis was regarded as restricted to certain Asian and Central American countries, but increase of migratory flux and changes in alimentary habits have contributed to importing cases into areas where the disease is not endemic (*1*,*2*). We report a case of autochthonous gnasthostomiasis in Brazil.

A 37-year-old man from Rio de Janeiro sought medical attention in 2005 because of low fever, cough, abdominal tenderness, and pain in the left shoulder. The symptoms started 15 days after a recreational trip to Tocantins, where he practiced sport fishing and ate sashimi-style freshwater raw fish (*Cichla* sp.) that had just been caught. He reported no history of traveling to a gnathostomiasis-endemic area. Initial work-up depicted eosinophilia (43%), and a computed tomographic scan of the chest revealed left pleural effusion. Two weeks later, winding, linear, reddish lesions appeared on his back, which lasted 3 days (Figure, panel A). Serologic testing for *Schistosoma mansoni* was weakly positive. Acute schistosomiasis was diagnosed, and treatment with praziquantel was begun. In 4 weeks, all symptoms faded.

In 2009, the patient took albendazol for helminthic prophylaxis, and 3 weeks later, deep migratory, swelling, reddish nodules occurred on the thorax; each lesion lasted ≈6 days, and new lesions appeared at intervals of 1–5 days in a somewhat linear array (Figure, panel B). By this time, hemograms displayed eosinophilia of 25%, but a computed tomographic scan of the chest showed no abnormalities. Results of a complete ophthalmologic examination were unremarkable, and a fecal examination was negative for parasites. Gnathostomiasis was highly suspected on the basis of the clinical and epidemiologic findings and results of skin biopsies. Histopathologic examination revealed a dense superficial and deep dermal infiltrate of eosinophils and neutrophils but did not show the parasite. Two samples of plasma were sent to Thailand for immunoblot in search of the diagnostic band (24-kDa antigen) of *Gnathostoma spinigerum*, resulting in high titers. Albendazol, 800 mg/day for 21 days, and a single dose of ivermectin, 0.2 mg/kg, were administered and, despite initial improvement, the disease relapsed, requiring a second cycle of the medications. No signs of disease occurred during 2 years of follow-up.

Gnathostomiasis is found mostly in Japan and Thailand. In the Americas, most cases occur in Mexico

Article DOI: http://dx.doi.org/10.3201/eid1812.120924

Hepatitis E Virus Genotype 3 in Shellfish, United Kingdom

Technical Appendix

```
LB21           CGACTGTTAAATTATACACCTCCGTTGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
ABN26          CGACTGTTAAATTATACACCTCCGTTGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
FB29           CGACTGTTAAATTATACACCTCCGTTGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
swUK           CGGCTGTTAAATTATATACTTCCGTCGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
huFrance       CAACTGTTAAGTTATACACCTCTGTCGAGAATGCACAGCAGGATAAGGGTATTGCTATAC 60
AB40           CGACTGTCAAATTATACACATCTGTCGAGAATGCACAGCAGGACAAGGGCATTGCCATAC 60
LB23           CGACTGTCAAATTATACACATCTGTCGAGAATGCGCAGCAGGACAAGGGCATTGCCATAC 60
AB7            CGACAGTAAAGTTATATACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
YE26           CGACAGTAAAGTTATATACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
YE29           CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
huUK           CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB31           CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB22           CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB23           CGACAGTAAAGTTATACACATCCGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB24           CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATTC 60
HEVgenotype3   CAACAGTAAAGTTATACACATCTGTTGAGAATGCGCAGCAAGACAAGGGCATCACCATTC 60
huUS           CAACAGTAAAGTTATATACATCTGTTGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
HEVgenotype1   CGACTGTTAAGTTGTATACATCTGTAGAGAATGCTCAGCAGGATAAGGGTATTGCAATCC 60
HEVgenotype2   CAACCGTGAAGCTCTATACATCAGTGGAGAATGCTCAGCAGGATAAGGGTGTTGCTATCC 60
HEVgenotype4   -GACAGTGAAACTTTACACTTCAGTCGAGAACGCTCAGCAGGACAAGGGTGTAGCTATTC 59
                  * ** **  * ** ** ** ** ***** ** ***** ** *****  *  * ** *

LB21             CTCATGATATAGACTTAGGGGACTCTCGCGTGGTTATC 98
ABN26            CTCATGATATAGACTTAGGGGACTCTCGCGTGGTTATC 98
FB29             CTCACGATATAGACTTAGGGGACTCTCGCGTGGTTATC 98
swUK             CTCACGATATAGACCTAGGGGATTCCCGCGTGGTTATC 98
huFrance         CACACGATATAGACCTAGGGGATTCCCGTGTGGTTGTA 98
AB40             CACATGATATAGATCTGGGAGATTCTCGTGTGGTTATT 98
LB23             CACATGATATAGATCTGGGAGATTCTCGTGTGGTTATT 98
AB7              CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
YE26             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
YE29             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
huUK             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB31             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB22             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB23             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB24             CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
HEVgenotype3     CACACGACATAGATTTAGGTGACTCCCGTGTGGTTATC 98
huUS             CACATGATATAGATCTGGGTGACTCCCGTGTGGTTATC 98
HEVgenotype1     CGCATGACATTGACCTCGGAGAATCTCGTGTGGTTATT 98
HEVgenotype2     CCCACGATATCGATCTTGGTGATTCGCGTGTGGTCATT 98
HEVgenotype4     CACATGATATTGACCTTGGTGAGTCCCGTGTGGTTATT 97
                 * ** ** ** **  * ** ** ** ** *****  *
```

Technical Appendix Figure. ClustalW alignment of sequences used to generate the phylogenetic tree in the Figure (see article text). Sequences were generated from RNA isolated from 50–100 mg of digestive gland or gill. Tissue was homogenized in 300 µL phosphate-buffered saline, and viral RNA was isolated by using a viral RNA kit (QIAGEN, Crawley, UK), and PCR was conducted by amplifying nucleotides

6332–6476 as described (*1*). The nucleotide sequences were aligned and bootstrapped, and phylogenetic neighbor-joining trees were constructed by using the ClustalW software (www.ebi.ac.uk/Tools/msa/clustalw2).

Reference

1. Erker JC, Desai SM, Mushahwar IK. Rapid detection of hepatitis E virus RNA by reverse transcription–polymerase chain reaction using universal oligonucleotide primers. J Virol Methods. 1999;81:109. PubMed http://dx.doi.org/10.1016/S0166-0934(99)00052-X