Expert workshop on practical applications of Whole Genome Sequencing (WGS) data for food safety management, FAO/ WHO**,** 7-8 December 2015

Application in virology

Viruses are among the most common causes of foodborne diseases according to the recent global estimate produced by the WHO FERG initiative, with noroviruses ranking highest in terms of morbidity and hepatitis A in terms of disease severity (Havelaar et al., in press). In addition, the potential for foodborne transmission is a concern with many emerging viral diseases, and a known source of introduction of zoonotic viruses into the human population, for instance for hepatitis E genotypes 3 and 4. Sequence based analysis of clinical samples and food can be used to identify clusters, and this has been used over the past decade to resolve sources of outbreaks. Sofar, the practice has been to use partial genomic sequencing of targets amplified by RT-PCR, leading to practical challenges because the choices of typing targets are not harmonized globally and the resolution of genome-based cluster detection varies depending on the target used (Verhoef et al., 2012). In addition, detection of these viruses in food products has been challenging as they cannot easily be cultured and low levels of contamination suffice to produce clinical disease due to the low infectious dose (estimated to be around 10 particles for noroviruses; Teunis et al., 2008). Viruses do not replicate in foods, and therefore, a huge challenge lies in the sensitivity of molecular detection methods (Knight et al., 2013). Foods most commonly associated with viral contamination are bivalve filter-feeding shellfish and fresh produce (berries, leafy greens, dried tomatoes), but a wide range of products has been associated with food-handler-associated outbreaks.

Noroviruses

Noroviruses are classified into at least 6 genogroups, and further subdivided into at least 38 genetic clusters (genotypes). Human infections are mostly caused by viruses belonging to genogroups I and II (GI and GII), but closely related viruses have been found in different animal species. Zoonotic infection has not been definitively shown or ruled out, and the potential for introduction of genes from animal viruses through recombination after exposure to mixtures of viruses during food contamination events is a concern. Determining the transmission route during an outbreak investigation can be challenging because transmission can occur by multiple routes in a single outbreak: after primary introduction of the virus through food, secondary person-to-person and environmental transmission can rapidly take over, making it hard to trace the disease back to contaminated food. There are distinct differences between genotypes in the rate of secondary transmission and association with food contamination. Analysis of outbreak reports collected through an international collaboration of laboratories across the world sharing sequence data and minimal epidemiological data has laid the foundation for our current understanding of the molecular epidemiology of noroviruses (Koopmans et al., 2003; Vega et al., 2011). An important step has been the agreed nomenclature for sequence typing that can be assigned through an automated web-based typing tool (Kroneman et al., 2011). Foodborne transmission is attributed to 10% (range 9%%-11%) of all genotype GII.4 outbreaks, 27% (25%-30%) of outbreaks caused by all other single genotypes, and 37% (24%%-52%) of outbreaks caused by mixtures of GII.4 and other noroviruses (Verhoef et al., 2015). Next generation sequencing is starting to be explored directly on stool, food, and environmental samples, both by whole community sequencing (metagenomics) and bait based approaches, but substantial validation against the current molecular methods is needed before these can be routinely applied. Biggest hurdles are expected to be the sensitivity for application on food items, and the bioinformatics, although

the latter is a recognized priority in an EU-funded project aiming to develop ICT and bioinformatics infrastructure for use by routine laboratories (www.compare-europe.eu).

Hepatitis A

HAV is a picornavirus which is transmitted mainly by the oral-fecal route. The three genotypes (I, II, III) are divided into A and B subtypes and there is clear genetic clustering, by geographic origin, for countries where HAV is endemic. This is a moving target, as levels of endemicity of hepatitis A have substantially decreased in many countries and regions in the last decade, with improvements in sanitation and hygienic conditions combined with childhood vaccination. In low endemic countries without routine vaccination, the level of immunity is decreasing resulting in increased susceptibility to HAV (Jakobsen, 2010). Vaccination is recommended in these regions for travellers, but of specific and growing concern is the potential for foodborne outbreaks related to imported foods given the globalization of the food market. Many countries producing and exporting fruits, vegetables, and shellfish products are endemic (high-intermediate) for hepatitis A. Strain typing to support outbreak investigations is being used increasingly, and a datasharing platform along the same principles as the global noronet platform (www.noronet.nl) has been developed for hepatitis A (www.havnet.nl; de Sousa, in preparation). The current standard is based on partial genome sequencing, which again is not internationally standardized. Major challenges are the long incubation time of HAV, making epidemiological investigations including food history taking challenging due to recall bias, and the low levels of virus present in food. Other areas that need more work are the geographic representativeness of current reference collections, and the resolution of molecular typing. Nevertheless, molecular typing has been shown to have an added value for public health surveillance, and has resolved some outbreaks that otherwise would not have been detected (Petrignani et al., 2014). Therefore, NGS/WGS is starting to be explored, based on virus detection directly from clinical samples (serum and stool).

Perspective

Unlike the microbiology field, genomic epidemiology has been accessible for routine applications in virology for many years. Nevertheless, the applications have been limited to specialized laboratories, and are not yet part of routine surveillance in most parts of the world. Hurdles have been the cost of sequencing, the sensitivity of methods given the need for detection directly in clinical or food samples, challenges in interpretation of results from food testing in the absence of a cell culture system to confirm viability of viruses found, and low priority of foodborne virus surveillance in international public health organizations. Nevertheless, there is ample evidence of the potential for using pathogen genomics for foodborne disease detection, and increasing interest from international organizations particularly for hepatitis A (EFSA 2014; http://ecdc.europa.eu/en/healthtopics/hepatitis_A/Pages/index.aspx). The potential for using WGS likely will increase resolution, and will be actively pursued by us and others in the coming years.

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