Chapter 9
Genomics and Foodborne Viral Infections

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Background

Foodborne illness or disease remains a major public health problem globally with substantial economic impact. It results from the consumption of contaminated food or water containing pathogenic bacteria, viruses, or parasites as well as chemical or natural toxins. Acute gastroenteritis is the most common clinical manifestation of foodborne disease, and diarrhoea, characterized by frequent loose or liquid bowel movements, is a common cause of death in developing countries and the second most common cause of morbidity and mortality in young infants worldwide with up to 0.8–1.5 million deaths each year [1–7]. High population density, limited access to clean water, frequent flooding and poor sanitation render surface water bodies in developing countries particularly vulnerable to faecal contamination, leading to a high prevalence of diarrhoeal diseases in both children and adults when untreated water is used for food preparation or drinking. In industrialized countries, where sanitation is widely available, access to safe water is high and personal and domestic hygiene is relatively good, diarrhoeal diseases remain a significant cause of morbidity among all age groups. In the majority of cases, symptoms are brief, and patients do not require medical attention. Though typically self-limited, infectious

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diarrhoea episodes result in millions of physician visits annually. A range of pathogens has been associated with foodborne illness, but a handful of organisms cause the majority of acute gastroenteritis cases [8, 9]. It was not until 1972 that viruses were implicated as aetiological agents in diarrhoea; Norwalk virus was identified in the faeces of patients with diarrhoea, followed by rotaviruses in 1973, and enteric adeno- and astroviruses in 1975 [10–13].

Foodborne viral transmission can occur by consumption of food handled by infected food handlers, by contamination of food during the production process (for instance through contaminated water), or by consumption of products of animal origin harbouring a zoonotic virus (Fig. 9.1). Food handler–associated foodborne illness results from the manual preparation of food by an infected food handler shedding viruses, usually resulting in limited outbreaks [14], although their size may be substantial depending on the nature of the contamination. A problem is that food-handlers may transmit viruses before showing symptoms, or have asymptomatic infections [15, 16]. Food contamination can also occur during primary production, as has been observed in particular in fresh produce such as berries and green onions, or bivalve filter-feeding shellfish. Here the nature of contamination is dependent on location of the production area and nature of sewage contamination. In contrast to food handler–associated contamination, production process contamination events may involve multiple pathogens present in sewage, including animal viruses [17–23]. Zoonotic foodborne infection occurs when meat, organs, or other products from an infected animal are consumed. For viruses, this may be the least common mode of transmission, although the potential for such transmission is a cause for concern with every emerging disease outbreak.

Foodborne pathogens share the mode of transmission (fecal-oral) and their ability to infect hosts following oral inoculation. Symptoms may arise from replication and

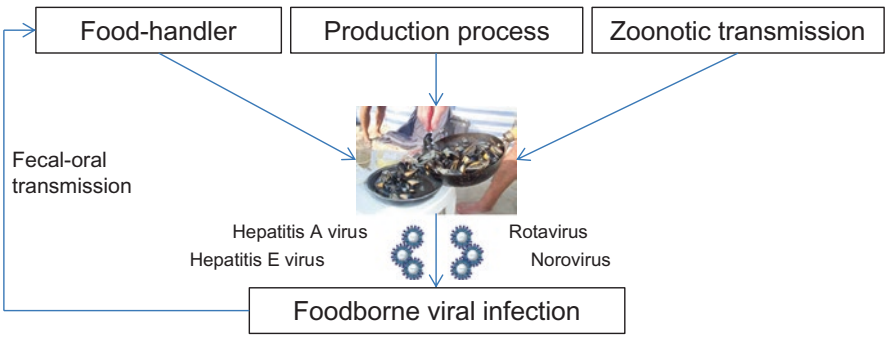

Fig. 9.1 Foodborne viral illness. Foodborne viral transmission can occur by consumption of food handled by infected food handlers, by contamination of food during the production process (for instance through contaminated water), or by consumption of products of animal origin harbouring a zoonotic virus. Foodborne pathogens share the mode of transmission (fecal-oral) and their ability to infect hosts following oral inoculation. Symptoms may arise from replication and the ensuing damage and inflammatory responses in the intestinal tract, but also from generalised infection as observed for instance for orally transmitted hepatitis viruses

the ensuing damage and inflammatory responses in the intestinal tract, but also from generalised infection as observed for instance for orally transmitted hepatitis viruses (hepatitis A and E), or neurotropic enteroviruses. The greatest burden of foodborne viral disease has been attributed to noroviruses and hepatitis A (Fig. 9.1). In addition to these endemic pathogens, the potential for foodborne transmission is a key question in every emerging disease outbreak. In fact, zoonotic emerging infections can be introduced into the population through food preparation or consumption, although these risks are minimal with proper food preparation. Commonly studied in relation to food are viruses from the families Picornaviridae (polio-, entero-, coxsackie-, echo-, and hepatitis A viruses), Reoviridae (rotaviruses), Adenoviridae (adenoviruses 40, 41 primarily), Caliciviridae (noro- and sapoviruses), Hepeviridae (hepatitis E virus), and Astroviridae (Mamastroviruses). They replicate initially in the intestinal tract, are environmentally stable, are shed in high numbers in the faeces of infected individuals with up to 10^{11} virus particles per gram of stool being documented, and are highly infectious with only 10–100 viral particles required for transmission [24, 25]. There is no systematic surveillance for foodborne viral diseases, despite the high burden of disease estimates from some countries [8, 26, 27]. The Food epidemiology reference group (FERG) of the World Health Organisation is currently preparing a global burden of foodborne disease estimate, but the underlying systematic reviews have already signalled large data gaps, particularly from resource limited regions [28].

A combination of factors is responsible for the lack of knowledge of the true incidence of foodborne viral illness. A case of foodborne illness is only identified when a patient falls ill, seeks medical help and undergoes diagnostic testing which leads to identification of the aetiological agent. For some pathogens with long incubation periods (e.g. hepatitis A and E), even when diagnosed, identification of a food source may be extremely difficult due to the long delay between exposure and symptom onset. A third factor compromising the ability to detect foodborne disease is the high rate of asymptomatic infections associated with some pathogens. Therefore, linked cases are difficult to detect. These challenges in diagnosis of foodborne diseases are illustrated by the fact that "unrecognized agents" account for up to 81 % of all U.S. foodborne illnesses and hospitalizations and 64 % of deaths [8, 27, 29]. Rapid population growth and urbanization, deforestation, invasion of previously pristine habitats for agriculture, and increasing demand for animal protein all likely contribute to increased emergence of novel infectious disease threats, while climate change and the increasing global connectedness and mobility facilitate their global spread [30]. Consequently, the pattern of disease outbreaks has changed, from localized clusters of disease in confined populations to dispersed outbreaks with excellent opportunity for further transmission. Similarly, a transition is observed from localized foodborne epidemics to diffuse international foodborne outbreaks due to globalization of the food market [31]. The foodborne nature is often disguised by person-to-person transmission after the initial infection(s) because of the highly infectious nature of most foodborne viral pathogens. Some of these viruses are of major public health concern amongst others because of their food- or waterborne nature, low infectious dose required for infection and serious health-related implications and associated costs.

As in every disease outbreak, including foodborne viral disease outbreaks, the following are some of the most urgent questions to answer: Is the group of ill persons normal for the time of year and/or geographic area or is something extraordinary occurring? If so, which pathogen(s) is causing the disease? Who gets infected? How do people get infected? What is the source of infection? What are transmission routes? How can infection be prevented, treated and/or contained? An integrated multidisciplinary approach utilizing expertise in several areas will be required to understand the dynamics of foodborne viral infection and to mitigate potential effects of future threats. Major challenges regarding recognizing, detecting, characterizing, and effectively responding to foodborne viral threats to health exist, which will be outlined in this chapter, with a focus on how genomics-based tools are a potential candidate to respond to some of these challenges in the field of foodborne viruses.

Foodborne Viruses: What Is Known

Viruses pose a substantial global health burden to humans and the list of human viral infections is ever-changing and continually growing [32]. Mortality in humans from recently emerged viral diseases ranges from a few hundred in the case of severe acute respiratory syndrome (SARS) coronavirus to millions of people from acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV). We are continuously facing novel pathogens, most of which are zoonotic or originated as zoonoses before adapting to humans [33–35], a proportion of which are likely transmitted via food and/or water [30]. Breakthroughs in the field of metagenomics have had far-reaching effects on the identification and characterization of newly emerging viral pathogens and on the recognition that a growing number of diseases that were once attributed to unknown causes are actually directly or indirectly caused by viral agents [32]. Many previously unknown viruses have been characterized in human stool in recent years including sali-, cosa-, bufa-, picobirna-, reco-, anello-, hepatitis E, astro-, and polyomaviruses of which the clinical disease spectrum, route of transmission, and foodborne nature remains to be elucidated [36–43]. For some of the "older" viruses, such as norovirus and hepatitis A virus, the foodborne risk of transmission is clearly recognized, for others such as adeno- and astroviruses the picture is less clear.

Rotavirus

Although rotaviruses are not generally considered primary foodborne pathogens, because person-to-person transmission seems to be the main route of transmission in developed countries, contaminated water sources are considered to be an important source of rotavirus transmission in developing countries [44]. Rotaviruses are

non-enveloped double-stranded segmented RNA viruses from the family *Reoviridae*. The genus Rotavirus contains eight species numbered A–H of which A–C are encountered in humans [45]. Rotavirus A infection is the most common cause of severe gastroenteritis in infants and young children worldwide. Rotavirus B has been found mainly in adults with diarrhoea in China, Bangladesh and India. The viral nucleocapsid outermost layer contains two structural proteins VP4 and VP7 that define the serotype of the virus and are considered critical in vaccine development; more than 40 serotypes have been identified [46]. By the age of five nearly every child has been infected with rotavirus A at least once, the majority of which is anticipated to be symptomatic. The spectrum of rotavirus A disease ranges from mild watery diarrhoea to severe diarrhoea with vomiting and moderate fever. Infection can result in death due to dehydration and electrolyte imbalance that is profuse and life threatening amongst others due to the action of a unique virus encoded enterotoxin NSP4 [47]. The severe impact is primarily observed in young children <2 years of age and can be treated by oral rehydration therapy. Symptoms generally resolve within 3–7 days. Subsequent infections occur from birth to old age but natural immunity renders most of these infections asymptomatic. Rotavirus A is shed in high concentrations in the stool of infected persons and is transmitted via the oral-faecal route with <100 virus particles being sufficient for transmission [45, 48, 49]. Infections occur mainly in late winter or early spring in Europe and colder/drier times of the year in the tropics [50–52]. Rotavirus A vaccines were introduced in 2006, but prior to vaccination policies, rotaviruses caused ~3 million disease episodes per annum in the USA, requiring 500,000 visits to physicians and 60,000 hospitalisations, leading to 20–40 deaths [45, 53–57]. Similar observations were done in Europe [58, 59]. In developing countries rotavirus A infections cause millions of diarrhoea cases, almost two million hospitalizations and an estimated 453,000 infections result in the death of a child younger than 5 years of age annually worldwide [6, 44, 60]. The introduction of proper hygienic measures, clean drinking water, oral rehydration therapy and rotavirus A vaccines reduced disease burden in both developed and developing countries [45].

Norovirus

Noroviruses are positive-stranded RNA viruses belonging to the family *Caliciviridae*. The genus Norovirus is divided into seven genogroups (GI-GVII) that are further subdivided into numerous genotypes [61]. The GI, II, and IV are capable of infecting humans [62], and GII.4 has been associated with the majority of global outbreaks since the mid-1990s. The other genogroups have not been detected in humans, but systematic studies evaluating their role are lacking. Norovirus infections are a leading cause of gastroenteritis outbreaks among all age groups and are transmitted directly from person to person and indirectly via contaminated water and food [63, 64]. They are extremely contagious requiring low viral loads for transmission and are common in closed settings such as healthcare facilities, cruise

ships, and nursing homes [24]. The infection can cause nausea, vomiting, watery diarrhoea and abdominal pain, although asymptomatic infections are common [48]. The disease is usually self-limiting, and severe illness is rare in developed countries. Ahmed and coworkers noted a gradient of decreasing prevalence from community to outpatient to inpatient groups, which supports the notion that norovirus is a more common cause of mild acute gastroenteritis [28], although in the USA norovirus infections result in ~70,000 hospitalizations and 800 deaths yearly [65–67]. In developing countries, noroviruses are estimated to cause more than 200,000 deaths annually among children younger than 5 years of age, and it is predicted that these viruses will become the predominant cause of diarrhoea in all age groups worldwide once rotavirus infection is controlled through vaccination [68–71]. The economic impact of foodborne related norovirus gastroenteritis outbreaks is high with an estimated $2 billion healthcare related costs in the USA alone [72].

Hepatitis A Virus

Hepatitis A is a liver disease caused by hepatitis A virus, a non-enveloped positive-stranded RNA virus belonging to the family *Picornaviridae*. Humans are the only naturally known reservoir for hepatitis A viruses and ~5 % of foodborne viral disease is attributed to hepatitis A virus infection [29]. The virus is spread via the faecal-oral route and the disease is closely associated with inadequate sanitation, poor personal hygiene, and limited access to clean water [73–75]. In developing countries, most children are infected with hepatitis A virus by the age of 10 years and the disease is usually asymptomatic in this age group. Epidemics in these countries are practically non-existent as older children and adults are immune to reinfection. In countries with improved sanitary conditions and transitional economies, the rate of infection in young children is lower, resulting in a higher susceptibility of older children and adults and larger outbreaks of disease. The incubation period is 14–28 days and symptoms range from mild to severe, and can include fever, malaise, loss of appetite, diarrhoea, nausea, abdominal discomfort, dark-coloured urine and jaundice which last for up to 8 weeks. Some 10–15 % of people experience a recurrence of symptoms during the 6 months after the initial infection and fulminant hepatitis and acute liver failure occurs although rarely and is most common in the elderly [4, 76]. In developed countries, hepatitis A infection is uncommon and predominantly associated with high-risk groups, such as people travelling to areas of high endemicity. Hepatitis A viruses are stable in the environment and can resist food-production processes routinely used to inactivate and/or control bacterial pathogens. Seroprevalence data indicate tens of millions infections yearly and an estimated 1.4 million clinical cases occur yearly worldwide which have significant social and economic impact [77]. Improved sanitation, food safety and vaccination are the most effective ways to prevent hepatitis A virus infection [75].

Hepatitis E Virus

Hepatitis E virus is a positive-stranded RNA virus with a genome of ~7.2 kb belonging to the family *Hepeviridae*. Four major genotypes are discerned and novel lineages of hepatitis E viruses have been identified in rabbits, rats, wild boar, ferrets and possibly foxes more recently [78–84]. Different genotypes of hepatitis E virus determine differences in epidemiology; genotype 1 is usually seen in developing countries and causes community-level outbreaks while genotype 3 is usually seen in developed countries and rarely causes outbreaks. Hepatitis E virus is transmitted via the faecal-oral route primarily via faecal contamination of water supplies, shellfish and contaminated animal meat, and possibly through zoonosis from pigs. Human-to-human transmission of the virus is rare. Outbreaks and sporadic cases occur worldwide; the virus is most prevalent in East and South Asia and endemic in Asia, Africa and Mexico [85]. An estimated 20 million hepatitis E infections occur worldwide yearly, which are usually self-limited and resolve within 4–6 weeks. Over three million cases of acute fulminant hepatitis E however occur resulting in over 50,000 deaths [4, 86]. Infection with hepatitis E virus is frequent in children in developing countries, but the disease is mostly asymptomatic or causes a very mild illness without jaundice. It causes acute sporadic and epidemic viral hepatitis most commonly in young adults aged 15–40 years with symptoms including jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea, vomiting, and fever that last for up to 2 weeks. A unique disease profile has been observed in pregnant women, where infections with HEV often result in fulminant liver failure, stillbirth and death in 25% of cases. Treatment and vaccines are unavailable, but currently in development [87].

Enteric Adenovirus

Adenoviruses (Family *Adenoviridae*) are non-enveloped single-stranded DNA viruses with a genome of ~26–48 kb. Adenoviruses infecting humans belong to the genus Mastadenovirus and over 50 serotypes are differentiated based on neutralization assays. Adenoviruses are highly stable in the environment and are thought to spread via respiratory droplets and the faecal-oral route. Adenovirus infections are usually subclinical but certain types are associated with disease which can range from respiratory disease, keratoconjunctivitis, to gastrointestinal disease [88]. Especially human adenoviruses F types 40 and 41 are associated with diarrhoea in young children with acute gastroenteritis and are another major cause of infantile viral diarrhoea in developing countries, following rota- and noroviruses. Symptoms include watery diarrhoea with mucus, fever, dehydration, abdominal pain, and vomiting lasting for 3–11 days [89].

Astrovirus

Astroviruses (Family *Astroviridae*) are non-enveloped positive-stranded RNA viruses with a genome of ~7–8 kb. Classically, eight human serotypes have been described, although since 2008 a large increase in detection of different human astrovirus variants is observed. Human astroviruses spread via the faecal-oral route via contaminated water and/or food and are an important cause of gastroenteritis in young children worldwide. Most astrovirus infections are not severe, self-limited and do not require hospitalization. Disease symptoms can include diarrhoea, followed by nausea, vomiting, fever, malaise and abdominal pain, which last for 3–4 days. The majority of children have acquired astrovirus antibodies by the age of 5 and, looking at the pattern of disease, it suggests that antibodies provide protection through adult life, until the antibody titre begins to decline later in life [90].

Enterovirus Including Poliovirus

Enteroviruses are a genus of positive-stranded RNA viruses in the family *Picornaviridae* with a genome of ~7.5 kb. They are divided in at least 12 species containing over 100 (sero)types. Enteroviruses affect millions of people worldwide each year, are spread through the faecal-oral route, and cause a wide variety of symptoms ranging from mild respiratory illness (common cold), hand, foot and mouth disease, acute hemorrhagic conjunctivitis, aseptic meningitis, myocarditis, severe neonatal sepsis-like disease, and acute flaccid paralysis. Historically, the most prominent member was poliovirus, causing a disabling paralytic illness that has largely been eradicated in most countries through vaccination. Human enterovirus 71 (EV71) epidemics have affected many countries in recent years. Infection commonly causes hand, foot and mouth disease in children, but can result in neurological and cardiorespiratory complications in severe cases. Genotypic changes through inter- and intratypic recombination have been observed, giving rise to serious outbreaks with mortality rate ranging from 10 to 25.7 % [91]. With the emergence of highly pathogenic EV 71 and widespread epidemics, there is great interest in development of an effective EV 71 vaccine and antiviral strategies. In addition, enterovirus 68 has recently emerged as an important cause of severe respiratory disease worldwide [92–96].

As described above, many viruses are able to spread via the faecal-oral route and many more can be detected in human stool in both healthy and diseased adults [36–43, 97–99]. Frequently, the mode of transmission, disease potential and incidence levels of newly recognized viruses detected in stool samples are unknown but potential for food-borne transmission exists. How do we deal with that?

Foodborne Viral Disease Surveillance: Recognition/ Identification

Adequate health crisis management is largely dependent on early detection of potential public health threats. At present, early cluster identification is notoriously difficult as many diseases are not notifiable, diagnostics can be relatively slow and biased for what we know, and clusters are not recognized when patients attend different healthcare facilities. One of the most overlooked but crucial aspects in identifying a potential foodborne related incident is the role that medical practitioners, veterinarians and epidemiologists, in other words the gatekeepers play in recognizing idiopathic disease cases or more than average occurrences of certain disease symptoms [40]. This is not a trivial task as these professionals need to recognize relatively uncommon or completely new infectious diseases, on the basis of changing clinical and epidemiological trends or a "gut-feeling", as syndromic surveillance systems targeting non-respiratory disease are sparse. Integrated networks for syndrome surveillance in combination with routine diagnostic surveillance activities for known pathogens in theory would aid in identification of threats which may otherwise fly under the radar. To date, however, no precise and consistent global baseline syndromic surveillance exists, with the exception of the sentinel surveillance system for influenza. Reliable estimates of the global burden of foodborne viruses are important in order to assess their impact, to advise policy-makers on cost-effective interventions [100], but also to recognize the extraordinary events that signal a potential food-related viral outbreak. The question, however, is how to organise such systems given the ever expanding list of known and potential foodborne viruses.

Classically, many viral pathogens were detected through culture-based and immunological methods, which shifted to molecular detection methods such as polymerase chain reaction (PCR) in more recent years ([101]; Fig. 9.2). The clinical molecular virology field was greatly affected by the development of applications involving viruses that do not proliferate in standard cell cultures and quantitative molecular assays (real time PCRs) that provided medically useful tools in assessing viral load, patient prognosis, treatment response, and antiviral resistance [101]. Currently, the field is moving towards assays that allow detection of multiple viruses. Multiplex PCR assays allow detection of a number of different viruses in a single reaction (e.g. ID-Tag Respiratory Virus Panel Assay identifying influenza A virus [H1, H3, and H5]; influenza B virus; parainfluenza virus types 1, 2, 3, and 4; adenovirus; rhinovirus/enterovirus; RSV A; RSV B; hMPV; and coronavirus [SARS-CoV, NL63, 229E, OC44, and HKU1] [TM Bioscience, Toronto, Canada]). Generic PCR assays are PCR assays specific for a broader taxonomic range than one virus species (e.g. a whole genus or family of viruses), which allows detection of new virus species within already known viral families [41, 102]. These technolo-

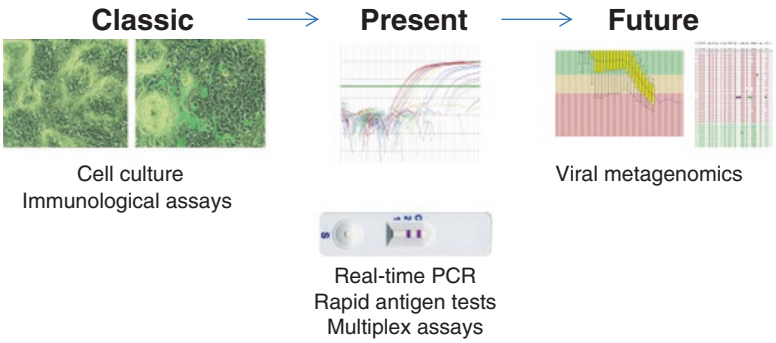

Fig. 9.2 Viral detection methods. Classically, many viral pathogens were detected through culture-based and immunological methods, which shifted to molecular detection methods such as polymerase chain reaction (PCR) in more recent years. Currently, the field is moving towards assays that allow detection of multiple viruses by multiplexing real time PCRs and application of viral metagenomics tools

gies are aiming to decrease time and effort in demonstrating the presence of a known pathogen in clinical samples, although sometimes at the cost of losing some sensitivity [40, 41]. The limitations of the multiplex or generic PCR assays become readily apparent as multiple different viruses or previously unknown viruses can be present in complex biological samples and continuous updating of the assays is required as viruses, especially RNA viruses, are constantly evolving. In addition, in a diagnostic setting discrimination between subtypes or genera of viruses requires additional labour-intensive procedures based on partial genome characterization as is currently done for example for noroviruses, hepatitis A viruses and enteroviruses, for final diagnosis.

With the increasing resolution and use of molecular detection and sequencing, there is great potential for integrated genomic surveillance. The NoroNet network (http://www.rivm.nl/en/Topics/N/NoroNet) in Europe and Asia, and CaliciNet (http://www.cdc.gov/norovirus/reporting/calicinet/index.html) in the US have been developed to aggregate genomic information of noroviruses causing disease outbreaks across the world. In depth bioinformatics analysis of data collected over the course of 10 years has shown the potential merit of genomic surveillance for detection of diffuse foodborne outbreaks [31, 103–105]. Similarly, a regional genomic surveillance database was developed for hepatitis A, enabling cluster analysis as a powerful tool to support outbreak investigations and detect hidden foodborne disease clusters [106]. While these systems target individual pathogens, viral metagenomics tools are a potential candidate to respond to the challenge of obtaining epidemiological estimates on the global disease burden and associated health-related costs of a whole range of (potential) foodborne viruses. Sequence-independent amplification of nucleic acids combined with next-generation sequencing technology and bioinformatics analyses or viral metagenomics is a relatively new promising strategy for rapid identification of pathogens in clinical and public health settings. The detection of viruses using an unselective metagenomics approach has

been hampered by the generally small size of virus genomes compared to bacterial or eukaryotic hosts. The detection is facilitated by enriching for viruses using filtration and nuclease treatments to remove bacterial and human nucleic acids whereas viral nucleic acid is retained through protection by the viral capsids and/or membrane envelopes. In contrast to classical molecular detection techniques that identify a single virus species or virus family, viral metagenomics allows the characterization of numerous known pathogens simultaneously and also novel pathogens that elude conventional testing. This approach has already resulted in the identification of a plethora of previously unknown human and animal viruses, many of which have been found in diarrhoea specimens [36–43, 78, 82, 102, 107]. It may approach sensitivity of routine diagnostic real time PCR assays used classically for virus diagnosis [108–110] with the added value of virus type information becoming available simultaneously. In addition, the cost of next-generation sequencing is dropping steadily and steeply each year outpacing Moore's Law. Although computational resources required for analysis of the vast amount of data are often not included in the calculations, the overall costs will likely be able to compete with conventional viral diagnostic molecular methods in the not so distant future in terms of cost and sensitivity, although not yet in speed.

To obtain insight into the baseline circulation of foodborne viruses and the burden of associated disease, a large and systematic set of enteric samples from around the globe from a large range of different individuals with and without (underlying) disease should be analysed. Human exposure to viral infection and susceptibility to virus-associated disease is dependent on numerous factors, including age, lifestyle, diet, geographic location, climate and season, pre-existing immunity and even host microbiome [107]. Furthermore, the human gut virome is not static and will vary over time due to ongoing zoonotic transmission events from animal reservoirs, increasing globalization, changes in food preference, demographic shifts in human populations, and human intervention strategies [25, 40, 41, 107]. However, with the foreseen further implementation of genomic technologies in routine clinical settings, a huge potential surveillance repository is developing. Its validity will depend on the ability to capture meaningful metadata, but the NoroNet and CaliciNet examples have shown that widespread hidden foodborne outbreaks can be detected with sequence data with minimal associated data. Obviously, the validity of such surveillance programs should be carefully evaluated against the current standards to ensure that they provide the necessary information in a timely, efficient, and cost-effective manner [111].

In conjunction with the amount of surveillance data that is required and the huge amount of data generated by next-generation sequencing, the availability of relatively simple user-friendly bioinformatics tools, curated databases of full and partial viral genome sequences, analysis pipelines, and computational infrastructure are crucial and at present largely under development. One example is COMPARE [A COllaborative Management Platform for detection and Analyses of (Re-)emerging and foodborne outbreaks in Europe] which is a collaboration between founding members of the Global Microbial Identifier (GMI) initiative (http://www.globalmicrobialidentifier.org) and institutions with hands-on experience in outbreak detection

and response. GMI was established in 2011 with the vision to develop the potential of breakthrough sequencing technologies for the field of infectious diseases through a joint research and development agenda, with applications in clinical and public health laboratories across the world. In order to achieve that long-term goal, the GMI group aims to promote development and deployment of novel applications, data sharing and analysis systems across the diversity of pathogens, health domains and sectors. The COMPARE project is set up to put this vision into action in Europe. It aims to improve rapid identification, containment and mitigation of emerging infectious diseases and foodborne outbreaks by developing a cross-sector and cross-pathogen analytical framework with globally linked data and an information sharing platform that integrates methods for collection, processing and analysing clinical samples with associated (clinical and epidemiological) data with state of the art technologies, such as next generation sequencing, for the generation of actionable information for relevant authorities in human and animal health and food safety.

Assuming the major hurdles towards implementation can be overcome, the combination of sustained virus surveillance (both syndrome and diagnostic) with next generation sequencing approaches and a standardized global analytical framework with associated clinical and epidemiological data would provide insight into (1) pathogens or combinations thereof involved in disease burden, (2) as yet unidentified pathogens and zoonotic events, (3) effects of vaccination or other interventions on incidence levels and whether other pathogens fill the niche that vaccination leaves behind, and (4) geographic difference in virus-associated disease burden. This knowledge would in turn guide development and deployment of vaccines and other intervention strategies. Well, everyone has a wish-list and end-goals … what is the practical translation of viral metagenomics in foodborne viral diseases at present?

Use of Genomics-Based Tools for Foodborne Viral Disease Outbreak Detection: Identification/Characterization

Syndrome surveillance has been used for early detection of disease outbreaks, including food-related incidents, to follow the size, spread, and tempo of outbreaks, to monitor disease trends, and to provide reassurance that an outbreak has not occurred. An example is an outbreak of acute norovirus gastroenteritis in a boarding school in Shanghai in 2012, where a diarrhoea syndrome surveillance system covering a dozen sentinel hospitals in Shanghai reported to the Pudong District Center for Disease Control and Prevention (PDCDC) that more than 100 students at a boarding school had developed symptoms of diarrhoea and vomiting within 3 days [112]. A current practical translation of viral metagenomics, which due to its unselective nature allows the characterization of numerous known pathogens simultaneously, is to use it as an identification tool to unravel the causative viral agent. In the cases in Shanghai, an epidemiological study focusing on a number of viruses (and bacteria) with standard molecular assays subsequently implicated norovirus as the etiological agent [112].

At present, viral metagenomics is mostly used in hindsight to obtain whole viral genome sequences for tracking-and-tracing purposes and to obtain epidemiological information after the virus was identified by more standard molecular assays. In epidemiology, identifying pathways of infectious disease transmission allows amongst others quantification of incubation periods, heterogeneity in transmission rates, and duration of infectiousness, which are important parameters to identify potential points of control and predict future spread of viruses. However, foodborne viral outbreaks are notoriously difficult to recognize, and tracking and tracing potential contacts is logistically challenging and often inconclusive. A variety of data sources can be exploited for attempting to uncover the spatio-temporal dynamics and transmission pathways of a pathogen in a population, by combining disease symptoms, data from contact tracing, results of diagnostic tests and, increasingly, pathogen genetic sequences [113, 114]. Identification of related nucleotide sequences of viruses in patients, also referred to as cluster detection, is an important tool in outbreak investigations in modern day public health and clinical laboratories especially in cases that prove difficult to unravel such as diffuse food-borne outbreaks involving several countries [104]. Norovirus genotype profiles have been used for example to estimate the foodborne proportion of norovirus outbreaks, excluding food handlers as a source of contamination [31, 104, 105]. Preferentially, cluster detection-based approaches and epidemiological inferences are done on whole viral genome sequences, as it provides the most detailed view. Next generation sequencing techniques have been used for tracking purposes in hepatitis A virus (HAV) foodborne outbreaks showing that whole HAV genome analysis offers a more complete genetic characterization of HAV strains than short subgenomic regions [115], although for many viruses partial phylogenetic informative genomic regions can be sufficient for answering the basic tracking-and-tracing questions in an outbreak scenario, with the added advantage of being relatively simple allowing local public health laboratories with limited resources to perform the assays [104, 115].

For informing measures for control of foodborne viral diseases, it is critical to understand the epidemiology in more detail and to accurately identify who-infected-whom, which is usually difficult as data about the location and timing of infections can be incomplete, inaccurate, and compatible with a large number of different transmission scenarios. A number of approaches have been developed that combine genetic and epidemiological data to reconstruct most likely transmission patterns and infection dates [113, 114, 116–119]. These tools may allow for epidemiological studies in real time during outbreaks, which can be used to inform intervention strategies and design control policies [120, 121].

The new developments in data generation with new sequencing possibilities in combination with epidemiological data provide a challenge for existing platforms aiming to enlarge the knowledge on geographical and temporal trends in the emergence and spread of (foodborne) virus infections, such as the ECDC Food- and Waterborne Epidemiology Intelligence Platform (FWD-EPIS) [122], The European Surveillance System managed by ECDC (TESSy; http://ecdc.europa.eu/en/activities/surveillance/Pages/index.aspx), The European Commission Early Warning and

Response System (EWRS) [123] and Rapid Alert System for Food and Feeds (RASFF; http://ec.europa.eu/food/safety/rasff/index_en.htm), NoroNet, and WHO networks among others. In addition to these existing systems, there is a multitude of other existing (inter)national databases and networks that have in common that they are widely accepted and used by the scientific and public health community and authorities for exchange of sequence-based data and other relevant structured and semi-structured information of relevance to human health, animal health and/or food safety. None of these is currently capable of handling the complex data from next generation sequencing platforms, but ensuring interoperability of these databases and compatibility of analytical workflows and data information sharing systems will be crucial in order to ensure translation to actionable data.

Viral Metagenomics and Control of Foodborne Viral Illness: Characterization/Containment

For food safety at present, an integrated system for monitoring of specific food safety threats exists in Europe, which involves sampling and pathogen characterization largely through species specific assays for a subset of major pathogens across the food chain, and linking and analysis of these data to study trends, detect diffuse outbreaks, and monitor effects of control measures [124]. Molecular typing plays a crucial role in this system, but relies among others on the willingness of clinicians to refer patients for laboratory diagnostics and of these laboratories to refer isolates to public health laboratories for typing. The changing clinical practice, with rapid transition from culture-based methods to molecular detection, challenges this decade-old model of disease surveillance [125]. In addition, these surveillance systems are less suited to capture the "new generation" of outbreaks, related with the global food market, as illustrated by recent examples of international diffuse foodborne outbreaks showing the vulnerability of the European population and industry for novel food-borne diseases [25, 106, 126, 127]. The currently used microbiological control criteria are not suitable for monitoring of presence or absence of emerging disease risks, and recent studies have shown vast underestimation of levels of contamination for many human pathogens, but also raise questions about the interpretation of molecular detection data in relation to consumer risk [128, 129].

Improvements in the microbiological safety of food have largely been shaped through response to disease outbreaks. Resources for foodborne diseases have been directed mainly to well-known foodborne pathogens and monitoring in the food chain has been implemented based on a farm-to-fork approach [25] by encouraging improvement of hygiene measures and incorporating Hazard Analysis Critical Control Points (HACCP) principles that identify potential contamination hazards and focus on subsequent control and prevention. The latter requires methods for

detection of foodborne pathogens and evidence of their disease association. Most of the microbiological quality control criteria on a global scale rely on standard counts of coliform bacteria as a measure of faecal contamination. Needless to say that these criteria are inadequate for protection against foodborne viruses. Viral metagenomics would theoretically be an option to obtain information regarding viral presence in food. However, microbiological testing of food in general has some limitations as a control option. These are constraints of time, as results are not available until several days after testing as well as difficulties related to sampling as small food samples may not be representative for entire lots, analytical methods and the use of indicator organisms and reference standards. Therefore, it has been argued that there are no practical systems for providing safety or assurance of safety by microbiological end-product testing and viral metagenomics approaches would not change the existing pitfalls.

Concluding Remarks

At present, foodborne pathogen surveillance activities are usually the responsibility of local government departments and are non-existent or at sub-optimal level in both developed and developing countries, are confined to pathogens with known economic impact, and suffer from a lack of integration on a global scale. With the continuing globalization of the food market and changing trends in eating habits [25], it is unsurprising that the general public is faced with an increasing rate of "food safety scares". In order to turn the tide, a huge global effort in virus syndrome and diagnostic surveillance is required, which is justified in the light of global health impact in general, and timely with the development of new metagenomics tools that hold the promise of not only identifying viral pathogens, but possibly the complete microbiome in a single assay. This does not apply to foodborne viral diseases alone. The interrelatedness of animal and human health with global interconnectedness in the twenty-first century is drawing all health related issues together as never before [33]. The combination of sustained pathogen surveillance in animals, humans, plants, environment and food alike with next generation sequencing approaches and a standardized global analytical framework with associated clinical and epidemiological data would provide insight into pathogen incidence, level of co-infections and their correlation to clinical disease instead of focusing on one or a few pathogens as is classically done (Fig. 9.3). This information is crucial in deciding which pathogens provide the most substantial health risk, for evidence-based risk assessments for policy development and to implement preventive measures.

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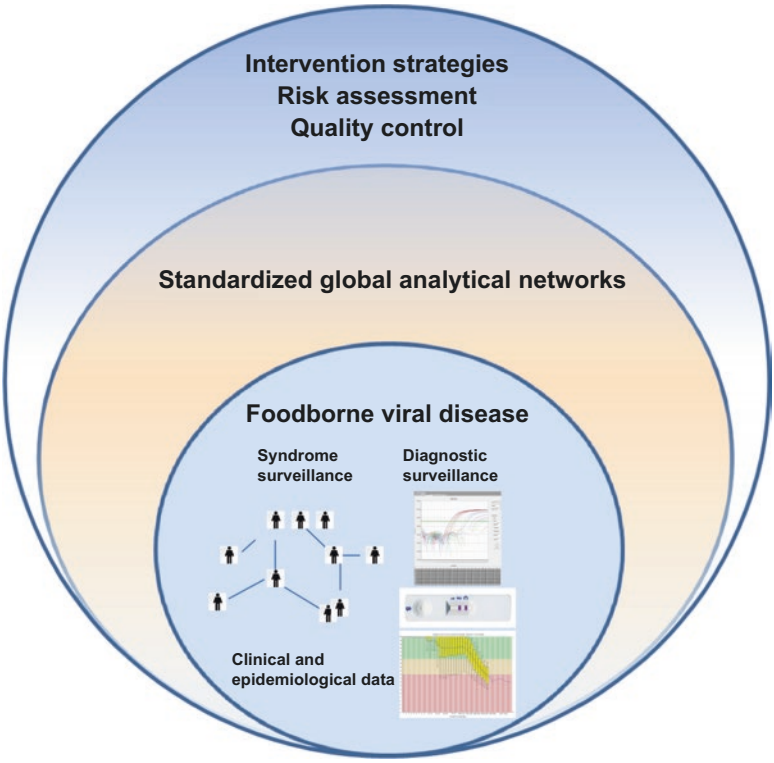

Fig. 9.3 Foodborne viral illness. Schematic overview of the main pillars required for an integrated multidisciplinary approach with a combination of sustained pathogen syndrome and diagnostic surveillance, genomics-based tools, and standardized global analytical networks gathering clinical, epidemiological and genetic data alike would be required to understand the dynamics of foodborne viral infection and to mitigate potential effects of future threats

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