

Wastewater and Environmental Surveillance Summary for Hepatitis A and E viruses

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This document provides information on wastewater and environmental surveillance (WES) for Hepatitis A and E viruses (HAV and HEV). It should be used together with the accompanying *WES Guidance for one or more pathogens*, which includes general and cross-cutting information (available [here](#)).

WES for HAV and HEV at a glance

- HAV and HEV are of intermediate public health significance, and whilst they are endemic in some populations and can cause large outbreaks, they do not have high pandemic potential.
- WES for HAV and HEV is technically and operationally proven. A credible number of independent studies undertaken globally have shown technical and operational success.
- Correlations between clinical and wastewater prevalence, proportions, genotypes, and sequences of HAV and HEV are poor to good, improving with infection prevalence and wastewater concentrations, with WES providing early warning of one or more weeks.
- Results from WES for HAV and HEV can be actionable and are likely to have high acceptability, but there is only one or very few examples of its routine use for this purpose to date.
- Integration of HAV and HEV with other targets, including SARS-CoV-2 and poliovirus, has been proven in multiple pilot studies, using various workflows, including some routine programs.
- For some genotypes of HEV, significant confounding from viral shedding from animal hosts, particularly pigs, can hamper the interpretation of WES results for human prevalence.

Table 1: At a glance assessment of key WES criteria for HAV and HEV (sewered and non-sewered)^{a,b}

Setting	Categorical Assessment (CA)	Public Health Significance	Actionability / Relative value	Technical Feasibility	Operational Feasibility	Acceptability	Optimisation	
	Strength of Evidence (SoE)						Integrated disease response	Multitarget WES
Sewered	CA	Intermediate	Intermediate	High	High	High	Intermediate	High
	SoE	Strong	Moderate	Strong	Strong	Strong	Moderate	Strong
Non-sewered	CA	not separated by sewer category	Low	High	Intermediate	High	Low	High
	SoE		Inadequate evidence	Strong	Moderate	Strong	Inadequate evidence	Moderate

Key:

1. Categorical Assessment (CA) of criteria

Category	Code	Description
High	Green	Criteria is evaluated as met at the highest level
Intermediate	Yellow	Criteria is evaluated as met at an intermediate level (it may be that not all sub-components of the criteria are met)
Low	Orange	Criteria is evaluated as low
Not-supported	Purple	Criteria is evaluated as not supported
Not applicable	Grey	Criteria is not applicable OR cannot be assessed due to inadequate evidence

2. Strength of evidence (SOE)

Evidence level	Code	Description
Strong	Green	High quality consistent evidence, including from multiple relevant studies/settings, at scale, over a prolonged period, with evidence from program settings, not only from research studies or short projects.
Moderate	Yellow	Relevant evidence is available but does not meet criteria for 'Strong' classification. ^c
Inadequate evidence	Grey	Evidence is inadequate and further study/evaluation is needed

^a Further description of the criteria used to assess the applicability of WES for a specific pathogen, as well as the methods used to evaluate them, is included in *WES Guidance for one or more pathogens*. The assessment in Table 1 provides a snapshot at the global level, but country level assessment may differ.

^b Sewered settings refers to closed reticulated sewage systems. Non-sewered settings refers to the diverse settings which are not 'sewered', including open drains and community sampling points. Individual small septic tanks at residential or building level are not viable to sample individually and are not considered here separately. Most WES evidence to date is reported from reticulated sewer settings, often from high-income settings. Yet much of the global population is on heterogeneous non-sewered systems and this has implications for assessment of various WES categories.

^c Evidence classified as 'Moderate' meets one or more of the following criteria: not from numerous settings, for a short period, without program-level evidence, and/or where findings are not consistent or of high quality.

Summary

Key features of WES for HAV and HEV

- HAV and HEV are a moderately significant global public health threat without a significant global pandemic potential.
- Poor WASH coverage is the principal risk factor for both diseases. The viruses are routinely detected globally, and are locally circulating in many low-and-middle income countries. They are detected periodically in high-income countries where infection is often acquired during international travel, or in the case of HEV, from animal sources, such as undercooked meat.
- HAV and HEV are predominantly human-human faecal-oral pathogens, but some genotypes of HEV have a significant zoonotic fraction, particularly in high-income countries.
- Both pathogens are vaccine-preventable, but vaccines are provided only to high-risk groups, or during outbreak situations. Immunity once acquired is prolonged, decades to lifelong, which is a very important distinction relative to other WES targets.
- Global, regional and national agencies have disease monitoring and management programs, based on clinical testing and notification, which WES could support. WES for HAV and HEV should be undertaken in the context of those broader hepatitis surveillance efforts.
- There are decades of experience with sampling and testing of wastewater, and to a lesser extent environmental waters, for both HAV and HEV.
- Most WES studies are pilot or 'proof of concept', with no standard methods having emerged. However, those studies do prove that changes in circulating levels and genetic lineages of the viruses are readily monitored using WES.
- Studies show varying correlations between WES and clinical results, with WES signals typically leading clinical signals by 1-3 weeks, and correlations being improved when clinical prevalence and wastewater virus concentrations are elevated. Environmental waters in non-sewered areas are less well-studied than sewage samples.
- There are few examples of WES being utilised to inform public health actions, with routine WES programs not commonplace. No universal triggers for public health action have been developed, but studies suggest establishing a baseline and a threshold for public health action.
- There are insufficient examples of routine WES with health-impactful actions in multiple independent settings to support the detailed design of a HAV and HEV program or provide a supporting benefit:cost analysis.
- The target sheet discusses incorporating routine WES for HAV and HEV into existing WES programs in some settings, e.g. with frequent outbreaks or high endemicity. The viruses can be readily monitored at low marginal cost simply by testing the RNA extracts from standard WES workflows, including those for SARS-CoV-2 and poliovirus.
- Key questions to test going forward with future research are:
 - What are the preferred sampling, analysis and bioinformatics workflows and how sensitive and specific are they?
 - What are the demonstrated health-impactful use cases to respond to WES evidence?
 - To what extent does animal excreta, particularly from pigs, hamper interpretation of HEV WES?

Key practical considerations for WES for HAV and HEV

Consideration	Suggestion
Sampling sites	<i>In priority order (from high to low priority):</i> • Existing validated WES sites as routinely used for poliovirus, SARS-CoV-2, or other targets. • Inlets to wastewater treatment works. • Nodes within the sewerage system (factoring in hydraulic residence times, and high-risk inputs such as healthcare facilities, and noting confounding animal inputs for HEV). • Higher risk communities, such as low socioeconomic groups. • Sentinel sites in non-sewered systems (i.e. gathering points, such as markets and places of worship). • Environmental waters heavily influenced by human waste, albeit noting confounding from animal waste for HEV.
Sampling approach	<i>In order of preference (from high to low preference):</i> • Composite sampling methods are preferred ○ using flow weighted automatic sampling ○ compositing of serial grab samples ○ passive 'Moore Swab' style samplers • Grab samples • Settled solids
Transport and storage	Conventional cold chain. Freezing of samples has proven acceptable if required.
Analytical methods	For cost-effectiveness, initially just monitor the pan-HAV and pan-HEV targets as part of routine baseline monitoring. Characterize the genotypes, sub-genotypes, and sequences present as part of an agile response during periods of elevated circulation above baseline.
Utilization of WES evidence	<i>During periods of elevation above baseline, in no particular order:</i> • Alert healthcare workers responsible for requesting clinical tests, and the diagnostic laboratories, to test for the viruses that are elevated in the WES samples. • Promote active case-finding in response to elevated WES signals. • Promote vaccination of at-risk groups in areas with elevated WES signals. • Promote WASH in areas with elevated WES signals. • Monitor spatial and temporal trends to evaluate interventions.

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1 General information

1.1 The viruses, associated diseases, and risk factors

Hepatitis A virus (HAV) and Hepatitis E virus (HEV) are genetically unrelated viruses, but they have several common features. Both viruses:

- Are non-enveloped viruses with singled-stranded positive-sense RNA genomes.
- Have multiple genotypes including:
 - Six HAV genotypes, of which three (HAV 1 to 3) cause most human disease, primarily HAV 1.
 - Eight HEV genotypes, of which four (HEV 1 to 4) cause most human disease.
- Show genotypic heterogeneity, with the dominant genotypes and subtypes differing regionally (e.g. the predominant HEV genotypes are 1 and 2 in Asia and Africa, 3 in Europe and America, and 4 in Asia).
- Are typically transmitted via the faecal-oral route.
- Can cause no symptoms, particularly in children, but may cause acute hepatitis (liver inflammation), which can be fatal, leading to a case fatality rate of approximately 1% of those with hepatitis symptoms, primarily among persons with compromised immunity, impaired liver function, or pregnancy.
- Have effective vaccines, but these are not universally offered, and are typically only targeted to groups at high risk of exposure or serious illness and in response to outbreaks.
- Typically trigger long term or lifelong immunity after natural infection or vaccination.

There are some differences between the two viruses:

- Unlike the largely faecal-oral human-infectious HAV genotypes and HEV genotypes 1 and 2, HEV genotypes 3 and 4 are typically zoonotic and are often associated with consumption of inadequately heated meat rather than faecal-oral transmission.
- Vaccines are well-established for HAV, but only recently introduced for HEV.
- HEV is particularly hazardous for pregnant women among whom case fatality rates can reach 25%.

Most people (approximately 90%) in LMICs are thought to be infected during childhood with HAV, experiencing typically mild symptoms, followed by immunity. In HICs, HAV is less common, with less people having immunity, leading to fewer infections, but often more serious illness due to sporadic outbreaks resulting from infections in previously unexposed non-immune elderly persons that typically (approximately 70% of cases) develop hepatitis.

1.2 Global burden and geographic distribution

Infection can occur as outbreaks or as sporadic cases. WHO reported that globally, HEV was estimated to have caused 3,450 deaths and an estimated 19.47 million cases of acute hepatitis E during 2021, being responsible for 5.4% of global disability-adjusted life years (DALYs) related to acute hepatitis. With

a similar disease burden, globally, HAV was estimated to have caused 7,134 deaths during 2016, being responsible for 0.5% of the mortality due to viral hepatitis.

Both viruses are in circulation and causing disease in humans globally. Both viruses are more prevalent in low-and middle-income countries, and in high-risk groups in high-income countries, where WASH coverage is poor. In high-income countries the viruses typically occur sporadically or as part of small outbreaks, rather than as large, widespread outbreaks. Based on serological assessments, approximately 25% of the global population are estimated to become infected with HEV at some point during their lifetime, with prevalence inversely proportional to country per capita income or level of development.¹

1.3 Routes of transmission

HAV

In high-income countries, HAV is typically associated with travellers that acquired the infection in countries in which the virus is locally circulating, or in imported foods.

HEV

The dominant HEV genotypes in circulation differ between regions. Genotypes 1 and 2 are more prevalent in low-income regions with poor WASH coverage where they circulate primarily between humans. Genotypes 3 and 4 more prevalent in high-income countries where they typically occur as sporadic outbreaks in humans due to transmission from meat.

Genotype 1 and 2, the most prevalent HEV genotypes in Africa and parts of Asia, are mainly faecal-orally transmitted. Contamination of drinking water often leads to large-scale outbreaks, affecting several hundred to several thousand people. Some of these outbreaks have occurred in areas of conflict and humanitarian emergencies, such as war zones and camps for refugees or internally displaced populations, where sanitation and safe water supply pose special challenges.

1.4 Zoonotic hosts and potential reservoirs

HAV and HEV genotypes 1 and 2 are largely limited to human hosts, whereas zoonotic HEV genotypes 3 and 4 infect a diverse range of animal hosts, primarily pigs, as well as infecting some other hosts, including pigs, cattle, sheep, goats, horses, camelids, camels, macaques, cats, dogs, rats and mice.^{1,2}

1.5 Human pandemic potential

The human pandemic potential is low as the viruses are not rapidly spread via airborne transmission pathways, and the fomite and faecal-oral transmission pathways are relatively slow compared to pathogens with high pandemic potential.

2 HAV and HEV and wastewater and environmental waters

2.1 Potential inputs to wastewater and environmental waters

HAV

HAV is shed primarily via faeces, typically prior to symptom onset, which would be the primary source of viral shedding to wastewater. Lower levels of the virus can be found in serum and saliva. The virus can be shed for many weeks following infection, with symptoms, particularly acute hepatitis, often occurring some weeks after infection.³

HEV

It is possible that HEV may be present in wastewater and environmental waters from animal waste, including wild and domesticated animals, particularly but not limited to, pigs, including both domesticated and wild pigs.⁴

HAV and HEV

HAV and HEV are readily detected in samples of wastewater and environmental water. For instance, systematic reviews of global studies reported HAV⁵ and HEV⁶ prevalence, respectively, at 31% and 16% in untreated wastewater, and 15% and 7% in surface waters. Concentrations reported were up to extreme peaks of 10 log₁₀ and 7 log₁₀ genome copies per L in untreated wastewater and surface water, respectively.

2.2 Target persistence, degradation and risk of infectious virus

HAV and/or HEV have been routinely detectable in wastewater samples collected in many countries, including high and low income countries in Asia,⁷ Africa,^{8–10} Europe,^{11,12} United States,^{13–15} Uruguay,¹⁶ and Argentina.^{17–19} Based on these and their cited studies, HAV and HEV degradation and persistence in the environment is well characterized: there is little degradation under the conditions and transit time periods associated with diverse sanitation systems across different climate zones. This, combined with the elevated levels of HAV and HEV in fecal shedding and available analytic methods make these viruses highly feasible targets for WES from a technical perspective.

2.3 HAV and HEV WES experience

Several HAV and/or HEV WES studies have been reported, undertaken as pilot or experimental WES studies, although not as part of a routine, broader, integrated public health surveillance programs. For instance:

- A seven-month HAV and HEV WES study was undertaken in sewage treatment plant and environmental water samples from six Asian countries, of varying levels of development, from high to low, for both HAV and HEV, to help provide information on prevalence, to evaluate the use of WES as a tool to identify high-risk countries to help focus allocation of resources.⁷ HAV and HEV detection varied markedly between the six countries tested. Results were not compared to clinical data.

- A 13-month HAV WES study was undertaken in environmental water samples in São Tomé and Príncipe to gain an understanding of prevalence in an area with limited clinical testing.⁸ The virus was routinely detected in water samples, but results were not compared to clinical data.
- A 30-month study in South Africa found a weak correlation between clinical and untreated sewage treatment plant samples for HAV.⁹ WES did not detect HAV in approximately half of the samples from which positive results might have been expected based on the results of clinical samples. The authors recommended further studies to understand why there were discrepancies.
- A 12-month HEV WES study was undertaken in untreated sewage samples in Cameroon to gain an understanding of the prevalence and genotypes in an area with limited clinical testing.¹⁰ The main finding of the study was that the genotypes found were not typically considered human pathogens, and were similar to Orthohepevirus genotype C1, which is only rarely reported in immunocompromised individuals. It wasn't clear whether these were from animal shedding into the wastewater, and/or from infections in humans.
- A 15-month study in two French cities found moderate to good correlations between HEV genotypes, sub-types, and concentrations, in samples from sewage treatment plants and plasma HEV RNA from blood donors, with the wastewater peak reported one to three weeks ahead of those in blood donors.¹¹
- A nine-year study in 20 Italian regions found HEV in 5% of samples from 48 sewage treatment plants and determined their genotypes, sub-types, sequences, and concentrations, with genetic similarity being reported between clinical and WES samples.¹²
- A four-month study in a US city sampled wastewater (combined sewage and stormwater) and tested for HAV following a large outbreak, with high concentrations up to 8 log₁₀ genome copies per L being detected, with a relationship being established between levels reported in wastewater and clinical cases one week later.¹³ HEV was detected in the same study, albeit at much lower levels, and not associated with known clinical cases.
- A 10-month study of 191 wastewater treatment plants in 40 US states tested for HAV, which found a weak but perceptible relationship between levels reported in wastewater and clinical cases one week later.¹⁵ A strong relationship was found between clinical and WES data during an outbreak that occurred in one state during the study, with the WES data leading clinical by approximately one week.
- A 17-month study of two wastewater treatment plants in Argentina tested for HAV and HEV RNA and found the viruses in 39% and 23% of samples, respectively.¹⁷ No relationship was established between levels reported in wastewater and clinical serum and stool sample, but there were very few clinical cases reported (7 HAV and 10 HEV), hampering statistical analysis. A longer and broader Argentinian study had similar challenges testing for relationships between WES and clinical data for HEV due to limited clinical results,¹⁸ whilst stronger relationships were observed for HAV.¹⁹

There was one report of routine WES being undertaken as part of integrated public health surveillance.¹⁴ Seven months after initiating the program, results from samples from two wastewater treatment works in the US demonstrated a clear increase in HAV concentrations during a period of elevated HAV cases in the community. The clinical cases were detected as part of routine public health surveillance, with the elevated WES signal supporting an assumption that cases were above baseline. This collective evidence triggered enhanced case-finding, awareness-raising, vaccination promotion, public health alerts, and

environmental health inspections of places where at risk populations were residing. The evidence from both clinical and WES results assisted with assessing the effectiveness of these interventions.

Some important unpublished (at the time of writing) studies are underway, including in Uganda and Pakistan, that are testing for multiple genotypes 1 or 2, and including phylogenetic comparisons with sequences from clinical samples.

Special considerations for HEV

As with many zoonotic pathogens, one potential challenge with interpreting WES results for HEV comes from zoonotic genotypes and subtypes that circulate between animals and humans, particularly pigs. Wastewater samples have confirmed the presence of HEV subtypes that were found in samples from both humans and pigs in the same area, but it is not possible to discriminate WES signals as being from humans and/or pigs,^{10,16} and in general the relationships between clinical and WES samples are worse for HEV¹⁸ than HAV,¹⁹ possibly due to the zoonotic confounding factor, and/or the lower level of clinical HEV than HAV cases. Therefore, wastewater and environmental water catchments that capture both human and animal waste, particularly waste from pigs, may yield detectable HEV, but interpreting the extent to which this is related to HEV circulating in the human rather than the animal population is not simple and may not be possible in some situations.

Summary of WES experience with HAV and HEV

In summary, a small number of studies have compared the prevalence and genetic lineage of HAV and/or HEV in wastewater and environmental samples to clinical samples in the relevant populations. Whilst few in number, mostly short in duration and small in scale, the results were consistent with WES providing a potentially useful complementary surveillance tool. During outbreaks, WES results demonstrated a lead time of 1-3 weeks relative to clinical reports.^{11,13,15} Prevalence, burden, and genetic information, showed varying levels of correlation between WES and clinical surveillance results, with correlations being strongest during periods of elevated infection, i.e. outbreaks. One routine program demonstrated operational value to health authorities, with WES helping to identify the extent of the outbreak, and the effectiveness of interventions.¹⁴ For HEV genotypes 3 and 4, the presence of animal reservoirs, particularly pigs, can limit the ability of WES to identify human versus animal inputs to the wastewater or environmental water catchments.¹⁶

3 HAV and HEV surveillance

3.1 Overall HAV and HEV surveillance and response

Public health surveillance for HAV and HEV depends on the testing of patients presenting with hepatitis symptoms, such as jaundice, or other evidence of poor liver function. Based on these symptoms, testing for antibodies to the virus in serum, or potentially viral RNA in stools or blood, are used to identify infection. If suspected, e.g. during an outbreak situation, stools of patients with gastrointestinal symptoms can be tested for the viruses, and these can yield positive results earlier than testing for serological responses.

3.2 Viral hepatitis-related surveillance systems and data sources

In most settings, positive clinical samples are notified to the regional and national surveillance systems. However, there is a lack of standardization in analytical laboratory methods for detecting and enumerating the viruses, whether in clinical, veterinary, food, or environmental samples.¹ In addition, in areas where the infections are less common, tests for the viruses are less likely to be requested. This leads to challenges in comparing results where different analytical laboratory methods have been used, and likely significant under-ascertainment and reporting.

4 WES objectives and related public health actions

4.1 Routine WES for HAV and HEV

In high-income countries, and other regions where the viruses are not locally circulating, there are typically few cases detected locally. In such settings, most cases detected are suspected as having been acquired in other countries. In these settings, the principal use case for routine WES is the detection of sporadic cases going on to seed outbreaks at levels that might be missed or their detection delayed by clinical testing. These cases may be missed due to asymptomatic or mild illness, particularly in situations where there is only limited clinical testing for these pathogens. The benefit of WES in this context is to alert primary healthcare workers to promote clinical testing for the pathogens, and to assess moving to agile WES surveillance to follow trends and locations. Routine WES for HAV and HEV in high-income countries may also be helpful in regions that border LMICs with high HAV and HEV prevalence. There is sufficient evidence to conclude that routine WES would be expected to detect increases above baseline approximately one or more weeks ahead of clinical cases becoming evident in such scenarios, even from just a small number of cases in a large sewer catchment.

In low-to-middle-income countries, and other regions where the viruses are locally circulating, and where cases are routinely detected locally, most cases detected are suspected as having been acquired locally. The principal use case for routine WES in such settings is to follow trends and locations to help assess the effectiveness of interventions. Some studies have demonstrated that WES has the potential to be used for the detection of novel genotypes emerging, e.g. in response to selective pressure from vaccination or the use of antivirals.^{10,20} Whilst there may only be an intent to use WES for HAV and HEV in an agile or reactive context, in practice it is essential to include both viruses in a routine program in order to establish a normal baseline concentration of both viruses.¹³ Failure to maintain a routine program loses the ability to establish baseline levels and prevents setting actionable concentration thresholds.

4.2 Agile (or responsive) WES for HAV and HEV

There is evidence that the correlation between WES and clinical results is improved when virus levels are increased during outbreak or hyperendemic scenarios. An agile response use case is to step up WES during suspected outbreaks as part of targeting interventions, measuring their benefits, and providing confidence relating to when interventions can be relaxed.

4.3 Potential public health actions arising from the addition of WES for HAV and HEV

Based on the current state of the science, WES has a potential, but not a proven, role in identifying elevated HAV or HEV in the population, which could inform public health actions, such as encouraging:

- clinical testing for HAV and HEV among persons with hepatitis and possibly gastroenteritis;
- active case-finding;
- vaccination promotion;
- WASH promotion; and
- inspection of premises suspected as transmission hotspots to identify and stem exposure.

5 WES additional methodological considerations for HAV and HEV

This section should be read in conjunction with general methodological consideration in Section 5 of *Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration* (available [here](#)). At the time of writing there are no standard methods for WES for HAV and HEV. Therefore, this section does not provide examples of, or recommendations for, specific methodological protocols or procedures. Rather, this section summarises the key considerations that are specific to undertaking WES for measles that are worthy of consideration when designing and selecting methods.

5.1 Sampling methods

A range of successful sampling methods have been demonstrated, including simple grab samples and composite samples from inlets to wastewater treatment plants, grab samples from septic tanks and open drains, and passive samplers placed in sewerage systems.^{7-12,15-17,21}

One limitation of such short (months rather than years) studies is that they may miss waves of HAV or HEV infection that may rise and fall over time.⁷ Prolonged studies, over many years, would be beneficial, to understand geographical and seasonal patterns of community infection.

5.2 Laboratory methods and interpretation

A wide range of successful virus concentration methods have been demonstrated, including simple centrifugation, polyethylene glycol precipitation and centrifugation (including using the standard poliovirus concentration method), electropositive filters (following the USEPA standard wastewater virus testing methods), and commercial kits; with viral RNA being extracted using commercial kits, from both cold-chain transported fresh or frozen samples; followed by a wide range of detection and quantification methods using RT-qPCR and RT-dPCR; and sequencing, including whole genome sequencing, for typing.^{7-13,15,17} However, these various methods have not been systematically compared, and they may perform differently, highlighting the need for research to compare and select the most suitable methods.⁷ Primer and probe sequences have been successfully described for both HAV²²⁻²⁵ and HEV,^{26,27} but once again these have not been systematically compared in multiple settings. Where reported, detection sensitivities, expressed as limits of detection or quantification, are typically approximately 4 log₁₀ viral genome copies per L.^{7,15}

5.3 Reporting and communication

The principal user of HAV and HEV WES results is the healthcare sector, to help provide information on whether the viruses are circulating above baseline levels, which in turn can guide requests for clinical tests, and decisions on active case-finding and vaccination. Communication to the public can include WASH promotion if levels are elevated and a hyperendemic or outbreak situation is suspected.

5.4 Acceptability of WES for HAV and HEV

HAV and HEV are not diseases that carry stigma, or that have significant sociopolitical impacts. They are both circulating at some level globally, with all countries finding some cases. They are not diseases that have high pandemic potential, or that may lead to impacts on business or tourism if found. Therefore, the acceptability of HAV and HEV WES is rated as high.

DRAFT FOR PILOT

6 Integrated surveillance and multitarget WES considerations

6.1 Integration of HAV and HEV WES into existing surveillance and response

Routine, operational use of WES for HAV and HEV as part of integrated public health surveillance has only been demonstrated in one context.¹⁴ However, this example was consistent with what has been proposed, and what would be reasonably inferred from pilot studies, i.e. that WES has potential for early identification of hyperendemic periods and outbreaks, and for monitoring trends in prevalence and circulating variants, which could be integrated with, and would be complementary to, and add value to, public health surveillance.⁹

6.2 Integration of multi-target WES together with HAV and HEV

There are no established routine operational HAV and HEV WES programs demonstrating integration of HAV and/or HEV integration as part of multi-target WES, or within existing multi-modal public health surveillance programs linked to public health action. However, successful proof of concept pilot studies have demonstrated the technical feasibility of the monitoring component of such an approach. This includes monitoring both HAV and HEV together, and along with other viruses.

HAV and/or HEV have been tested either alone or together in samples that were also tested for other respiratory viruses, (including one or more of SARS-CoV-2, respiratory syncytial virus, influenza A and B virus); enteroviruses (including poliovirus); norovirus, sapovirus, and rotavirus; along with human polyomavirus 2 or pepper mild mottle virus as normalizing agents; and spiked phage or coronaviruses viruses as assay recovery controls; demonstrating the ability to test HAV and HEV alongside other pathogenic viruses, normalizing agents, and assay recovery controls, in the same samples.^{7–9,13,15–17}

Importantly, the work flows used for other viruses, such as poliovirus and SARS-CoV-2, can be used to select sample points, sampling methods, sample transport, virus concentration, and extraction of viral RNA. As such, HAV and HEV can be readily integrated with other common targets of WES with a need only to include the additional primer and probe combinations, and/or to include them as targets in the cDNA sequence analysis.

7 Key knowledge gaps and applied research priorities

Both clinical and WES analytical methods have their own biases¹. This creates challenges in comparing within each discipline (i.e. comparing clinical studies with one another; or WES studies with one another) and even more so when undertaking integrated WES across disciplines (i.e. comparing clinical with WES studies).

There are no agreed standard methods for any components of the WES (or indeed clinical) workflows, and the most appropriate methods to use may differ between contexts. Variables include what other targets HAV and HEV are to be tested alongside, and the organizational, technical and financial capacity of the analytical parties involved in delivering the WES program.

In the short term, the priority is to develop good practice guidance on selecting the most suitable workflows and methods, and these should be evaluated prior to committing to any largescale study.

In the longer term, standardization of WES (and clinical) methods will improve comparability between WES and clinical results within, and between, studies.

Further studies, particularly over longer timeframes, and in multiple contexts, are necessary to provide more quantitative evidence to inform benefit:cost analysis. Whilst only one of the studies cited in this document was routine and coupled to public health action,¹⁴ collectively the studies published to date have demonstrated the technical feasibility of HAV and/or HEV WES, alone, or in combination with one another, and/or with other targets (as discussed in section 6.2). The next step is to implement more routine programs to demonstrate the delivery of actionable results that inform beneficial public health interventions and to refine methods and set triggers for action.

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