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# Detection of hepatitis A virus (HAV) in fresh fruits, vegetable, wastewater and manure from irrigated farms in Ouagadougou, Burkina Faso

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## ABSTRACT

*Objectives*: Hepatitis A virus (HAV) has been detected as causal agent in several epidemics associated with fruit and vegetables consumption. The aim of the present study was to assess the presence of HAV in fruit and vegetables, manure and irrigation wastewater used in the urban and peri-urban irrigated plots of Ouagadougou.

*Methodology and Results*: A total of 288 samples including 30 lettuces, 42 tomatoes, 32 carrots, 30 strawberries, 80 wastewaters and 74 manures were collected from 4 market garden sites in and around Ouagadougou, and processed for HAV detection. RT-nested PCR was performed with specific primers to detect RNA of HAV. From all the samples, a HAV detection rate was 20.8% (60/288) [CI95, 16.1 - 25.5%]. Indeed, 7/30 (23.33%) of lettuces, 12/42 (28.57%) of tomatoes, 4/32 (12.5%) of carrots, 10/30 (33.3%) of strawberries, 20/80 (25%) of irrigation water (wastewater) and 7/74 (9.5%) of manures were positive for HAV RNA detection.

*Conclusions and application of findings*: These results testify to the existence of HAV in the environment, which can come from irrigation water and/or untreated manure, from farmers and/or people infected with viruses, and thus contaminate fruit and vegetables during production. Results also indicate that contaminated fresh vegetables, when consumed raw, are potential passive vectors for the transmission of food-borne viral diseases. These results underline the need for scrupulous compliance with good agricultural and hygienic practices on

farms (composting, aeration or anaerobic digestion of soil improvers, and the use of treated wastewater for irrigation and vegetable washing), in order to reduce the pathogen load.
**Keywords:** HAV, raw fruit and vegetables, wastewater, manure, RT-PCR

## INTRODUCTION

Fresh fruit and vegetables are an important part of a healthy, balanced diet, providing fiber, minerals and vitamins. They are widely recommended and increasingly used in the human diet for their health benefits. Today, fruit and vegetables are a highly appreciated source of nutrients. However, fruits and vegetables eaten raw are recognized as sources of enteric virus transmission, and many foodborne illnesses (Goyal, 2006; Batz *et al.*, 2013; Painter *et al.*, 2013; Fuzawa *et al.*, 2020). Hepatitis A virus (HAV) is the most widespread foodborne enteric virus, causing viral hepatitis quite exclusively on acute form and not chronic one. In 2019, WHO has estimated 159 million the number of acute HAV infections, with 39,000 deaths and 2.3 million disability-adjusted life years worldwide (WHO, 2022). Hepatitis A virus (HAV) belongs to the genus Hepatovirus, within the Picornaviridae family, of which it is the only species (Hepatitis A Virus) (Knowles *et al.*, 2012; Smith and Simmonds, 2018). Members of the Hepatitis A Virus species contain viruses found in human and non-human primates (Costa-Mattioli *et al.*, 2003). In human population 5 groups are phylogenetically distinguishable (HAV-1A, 1B, IIA, IIB, IIA and IIB) and might be related to geographical distribution and risk group but all Hepatitis A viruses belong to a single serotype and are highly conserved in their antigenic properties (Robertson *et al.*, 1992; Cristina and Costa-Mattioli, 2007; Smith and Simmonds, 2018). The HAV genome is a single-stranded, positive-sense RNA molecule approximately 7500 nucleotides (nt), with a long 5'UTR (734 nt), a single ORF (6 684-93 nt) and a final short 3'-UTR 53-59 nt (McKnight and Lemon, 2018). HAV infects epithelial cells of the small intestine and hepatocytes of primates; it is non-cytopathogenic and released from the cell within membranous structure (eHAV) that are probably removed when travelling the biliary tract up to the intestine (Feng *et al.*, 2013; Lemon *et al.*, 2017). If the virus is predominantly replicated within the liver, it is excreted via the bile and present in feces in very high titer. Viral shedding is at its highest before the onset of clinical signs of hepatitis (jaundice) and as the non-enveloped virion (Wang *et al.*, 2015), is very stable, resistant to acid pH and elevated temperatures (up to 60°C for 10 hours) it main remains in the aqueous medium for month (Croci *et al.*, 1999; Nainan *et al.*, 2006). The virus is thus largely present in environment in densely populated area where sanitation and prevention procedure are poorly followed (Tjon *et al.*, 2006). In developing countries, foodborne diseases linked to contaminated fruit and vegetables are common and, in some regions, cause a high proportion of illnesses. However, due to the lack of investigation and surveillance of foodborne diseases system in most of these countries, a very high proportion of epidemics go undetected, or very few are mentioned in scientific reports (Toe *et al.*, 2017). In Burkina Faso, agriculture is the population's main source of income and the mainstay of the country's food security. Increasing food needs, socio-economic intermingling and the development of processing technologies have led in recent years to a diversity of foods sold (fruit, vegetables, and salads) in various catering environments. The fruit and vegetables used in salads come mainly from urban and peri-urban agriculture (Robert *et al.*, 2018). They are exposed to environmental conditions or factors that can introduce all kinds of

pathogenic microorganisms (bacteria, parasites, viruses) (Adamu *et al.*, 2012; Holvoet *et al.*, 2014; Aw *et al.*, 2016). These sources of contamination are classified as pre-harvest (irrigation water, soil, poorly composted manure, domestic and wild animals.) and post-harvest (human handling, equipment, containers, washing and rinsing water, flying insects...) (Amoah *et al.*, 2007). This underlines the fact that raw fruit and vegetables can be contaminated with HAV. Early detection, control and prevention of contamination factors in fresh vegetables throughout the supply chain are paramount. Thus, the aim of the present study was to assess the presence of HAV in fresh products in the urban and peri-urban perimeters of Ouagadougou, and to establish whether the wastewater used for irrigation and manure are potential sources of fresh produce contamination.

## MATERIAL AND METHODS

**Study period, sites and samples :** A prospective study was conducted from September 2021 to July 2022 at four (4) market garden production sites (Boulmiougou, Loumbila, Silmandé, Tanghin) (Fig 1). These sites were chosen on the basis of fruit and vegetable availability, market garden production intensity and geographical location.

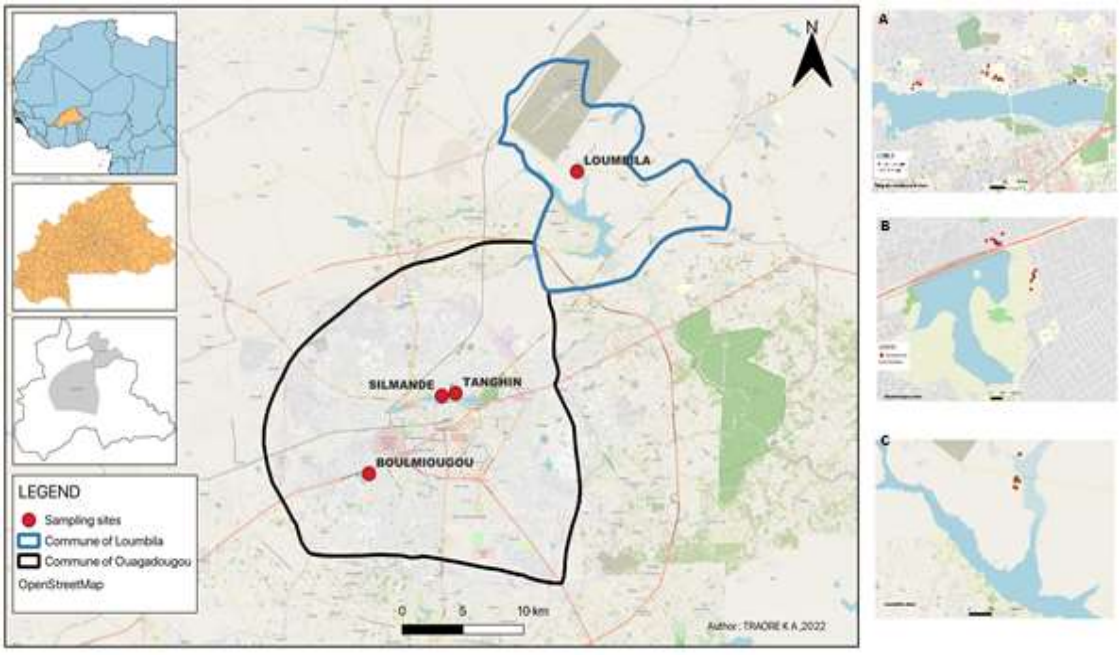


**Fig 1.** Geographical distribution of sampled market gardening sites

Legend: A: Market gardening sites in Tanghin and Silmandé; B: Market gardening sites in Boulmiougou; C: Market gardening sites in Loumbila

**Sampling strategy:** Only ripe vegetables ready for sale or consumption were sampled. Tomatoes (*Lycopersicon esculentum*), and strawberries (*Fragaria* sp), with visible damage and/or cracks were not sampled. Each lettuce (*Lactuca sativa*) sample consisted of three plants. Similarly, a sample of tomatoes, strawberries or carrots (*Daucus carota*) was made up of three tomatoes, three strawberries and three carrots respectively, taken from different parts of the same field. A total of 74 samples (3 g per sample) of

manure used for spreading, 40 samples (300 ml per sample) of surface water and 84 fresh produce products; tomato strawberry carrot and lettuce from irrigated plots were collected in a sterile plastic for laboratory analysis.

**Water samples processing:** Water samples were processed using the method adapted from Ahmad *et al.* (2010). Each sample were divided into aliquots of 40 ml, and 0.25 volume of 50% (w/v) polyethylene glycol (PEG) 8000/1.5 M NaCl was added (pH adjusted at 7–7.5) to reach a final concentration of 10% (w/v) PEG 8000 with sodium chloride to the final concentration of 0.4 M. This mix was stirred at room temperature for 3 h, and then centrifuged at 10,000×*g* for 90 min. The resulting pellet was suspended in 500 µl of 10 mM phosphate buffer and stored at -20˚C.

**Fresh produce samples processing :** Vegetable samples were processed using the method adapted from Coudray *et al.* (2013).Twenty Five( 25) g of fresh produce in small pieces were mixed with 40 ml of Tris–glycine buffer (100 mM Tris–HCl, 50 mM glycine, and 1% beef extract, pH 9.5) in a sterile plastic bag. After 20 min at room temperature with constant rocking (approximately 70 oscillations/min) to remove the viruses from the surface of the vegetable's matters, the preparation was distributed into clean centrifuge tubes. After centrifugation at 10,000×*g* for 30 min at 4 °C. Vegetable matters were discarded and the supernatant was transferred into clean centrifuge tubes and the pH was adjusted to 7.2 ± 0.3. For precipitation, 0.25 volume of 50% (w/v) PEG 8000/1.5 M NaCl were added to the eluates and stirred for 2 h at room temperature. After additional centrifugation for 30 min at 10,000×*g* at 4 °C, the pellets were dissolved in 500 µl of 10 mM PBS and stored at - 20 °C until use.

**Treatment of feces and manure samples:** Manure samples were processed using the method adapted from Kokkinos *et al.* (2012). A 10% manure suspension in phosphate-buffered saline was prepared, then vortexed for 15 sec and centrifuged at 10,000×*g* for 10 min. The supernatant was collected and stored at -20°C for subsequent analysis.

**Extraction of total RNA:** Fresh produce suspensions and irrigation water samples were first of all treated with chloroform-butanol (1:1). These mixtures were briefly homogenized, then centrifuged at 10000× *g* for 15 min. The upper aqueous phase of each sample served as samples for final nucleic acid extraction. Nucleic acids were extracted using a FavorPrep™ viral nucleic acid extraction kit, according to the manufacturer's instructions. Samples were stored at -20ºC.

**RT-Nested PCR:** For molecular detection of HAV, the specific RT-nested-PCR protocol, targeting the VP1//2A gene (Figure 2), a method adapted from Taffon *et al.* (2011) was carried out. Viral RNA extracted from the samples was reverse transcribed into cDNA using the Solis BioDyne FIREScript RT cDNA Synthesis MIX kit (Solis Biodyne, Estonia), following the supplier's protocol. Thus, 0.1–5 µg of extracted RNA/DNA was mixed with 20 µl of a reaction mixture containing 1× of RT medium with DTT, a 200 µM concentration of each deoxynucleoside triphosphate (dNTP), 5 µM of random primers, 200 U of FIREScript® Reverse Transcriptase, and 22U of RiboGrip® RNase Inhibitor. RT was carried out at 42°C for 1 h, and the tubes were then heated to 70°C for 15 min to inactivate the enzymes. HAV viral cDNA was detected by using Solis BioDyne's 5x FIREPol®Master Mix Ready to Load kit (Solis BioDyne, Tartu, Estonie), according to the manufacturer's instructions. The primer sequences are shown in Table 1. The first PCR was performed using 3.5 µl of cDNA synthesis with FIREPol® DNA polymerase. The thermocycling conditions were 3 min at

95°C, followed by 30 cycles of 30 seconds at 95°C, 1 min at 40°C and 1 min at 72°C. Final extension was 10 min at 72°C. For the nested-PCR, 5 µl of cDNA template obtained from the first PCR were used. The thermocycling conditions were 3 min at 95°C, followed by 30 cycles of 30 seconds at 95°C, 1 min at 48°C, 1 min at 72°C with a final extension 10 min at 72°C. Nuclease-free water was used in all experiments as a negative control. PCR products (5 µl) were analyzed on 2% agarose gel. Fragment sizes were compared using a commercially available size standard 100 bp DNA ladder (Solis Biodyne, Estonia).

**Table 1:** Primers used for HAV amplification

| Target | Name primers | Sequences (5' → 3') | Size | Reference |
|---|---|---|---|---|
| **VP1//2A** | 1852-fw<br>1853-rev | TATTCAGATTGCAAATTAYAAT<br>AAYTTCATYATTTCATGCTCCT | 393 pb | Taffon et al. (2011) |
| | 1854-fw<br>1855-rev | TATTTGTCTGTYACAGAACAATCAG<br>AGGRGGTGGAAGYACTTCATTTGA | 267 pb | |

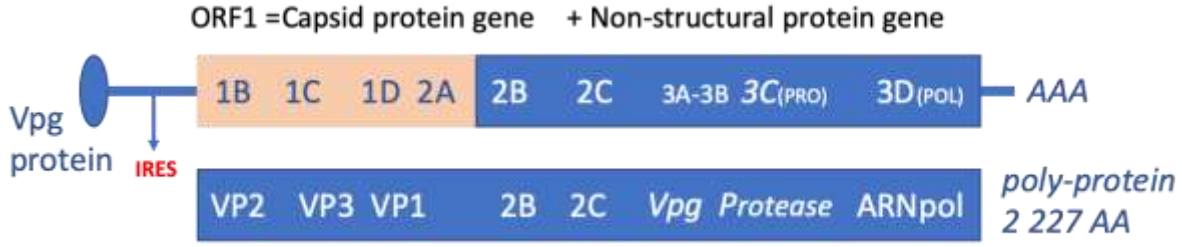


**Fig 2**: Organization of the hepatitis A virus (HAV) RNA genome and polyprotein segmentation (Adapted from Mcknight and Lemon, (McKnight and Lemon, 2018))

## RESULTS

Analysis of the nested RT-PCR revealed bands on the gel at the expected size 267 base pair (bp) within the junction of VP1//2A gene (Fig 2). Of all samples, HAV was detected in 20.8% (60/288) [95% CI, 16.1 - 25.5%] (Table 2). Specifically, 7/30 (23.33%) lettuces, 12/42 (28.57%) tomatoes, 4/32 (12.5%) carrots, 10/30 (33.3%) strawberries, 20/80 (25%) irrigation water and 7/74 (9.5%) manure were positive for HAV.

**Table 2:** Prevalence of HAV in fruits, vegetables, manure and irrigation water

| Product | | Prevalence number and (percentage) in Sampling sites | | | | |
|---|---|---|---|---|---|---|
| | | Boulmiougou | Silmandé | Tanghin | Loumbila | Total |
| Vegetable and fruit | Carrots | 0/0 (0%) | 0/0 (0%) | 4/32 (12.5%) | 0/0 (0%) | 4/32 (12.5%) |
| | Lettuces | 4/15 (26.7%) | 2/5 (40.0%) | 1/10 (10.0%) | 0/0 (0%) | 7/30 (23.3%) |
| | Strawberries | 10/30 (33.3%) | 0/0 (0%) | 0/0 (0%) | 0/0 (0%) | 10/30 (33.3%) |
| | Tomatoes | 3/10 (30.0%) | 0/0 (0%) | 0/0 (0%) | 9/32 (28.1%) | 12/42 (28.6%) |
| Manure | | 0/20 (0%) | 3/20 (15.0%) | 4/20 (20.0%) | 0/14 (0%) | 7/74 (9.5%) |
| Waters | | 6/25 (24.0%) | 9/25 (36.0%) | 0/5 (0%) | 5/25 (20.0%) | 20/80 (25.0%) |

Below is a representative gel picture in which the displayed bands (lanes 1, 4, 6, 8, 9, 10, 11, 17 and 18) indicate detection of HAV from the irrigation water, tomatoes, lettuce, and manure (Fig 3). The marker used is 100 bp and the specific bands are of approximately 267bp


**Fig 3.** Electrophoretic profile of amplified sequences for HAV

Legend: Amplicons with a size of 267 bp (expected size of the VHA target gene) indicated by red arrows in wells labelled 1 and 4 (manure), 6, 8 and 9 (tomato), 10 and 11 (lettuce), 17 and 18 (water). No PCR product was detected in wells CN (negative control on the right of the picture); 16 (water); 15, 2, 3 (manure); 5, 7 and 12 (tomato), 13 and 14 (lettuce).

## DISCUSSION

In Burkina Faso, fruit and vegetables are growing in the open-air environment and consequently subject to environmental contamination. HAV was detected in fruit and vegetable samples with an average prevalence of 24.6% (33/134) in the 4 market garden sites studied. This result can be explained by certain risky practices that contribute significantly to the spread of human pathogens in the field (Okonko *et al.*, 2008), notably the poor hygiene among growers, the use of uncleaned harvesting tools, as well as the use of untreated animal manure and contaminated irrigation water (Hamza *et al.*, 2009; WHO, 2012). These data indicates that fruits and vegetables eaten raw can be source of HAV. Indeed, fruits and vegetables eaten raw are recognized as sources of enteric virus transmission, and many foodborne pathogens (Goyal, 2006; Batz *et al.*, 2013; Painter *et al.*, 2013; Fuzawa *et al.*, 2020). The rate of detection of HAV in fresh produce is close to that it is observed in Mansoura and Giza regions, Egypt which reported presence of HAV in 27% in fresh produce (Shaheen *et al.*, 2022), as the 28.2% found in Mexico (Felix-Valenzuela *et al.*, 2012), or the 33.3% in Brazil (Marti *et al.*, 2017). All these data from different countries indicated the frequent contamination of fruit and vegetables by enteric virus such HAV. To note that detection rates of 2% have been reported in fresh produce in Egypt and Australia (Torok *et al.*, 2019; Abd Al-Daim, 2024). The diversity of prevalence between the different studies can be attributed to the variability of environmental conditions or different molecular methods. The rate of HAV detection in water and manure samples was 25% (20/40) and 9.5% (7/74) respectively. This rate observed in irrigation water is in agreement with that reported by (Takuissu *et al.*, 2023) in untreated wastewater (31.4%) in their systematic

review. This result therefore confirms the idea that irrigation with untreated water and the spreading of untreated manure can lead to HAV contamination of crops (Khan *et al.*, 2014; Garcia Garrido *et al.*, 2015). HAV has also been detected in irrigation water in South Africa 37% to 76% in (Saïd *et al.*, 2014), in Brazil 8.3% (Rigotto *et al.*, 2010) and in Italy 24.2% (La Rosa *et al.*, 2014) with some variation in prevalence that could be explained by the water sources. Plots irrigation water is mostly from dams and rivers, while others came from wastewater. The poor environmental hygiene constitutes a factor allowing rainwater to drain waste and contaminants towards the surrounds of the watercourses used to grow vegetables. The presence of HAV on cultivated fruit and vegetables, in irrigation water and manure, can be considered a potential health risk ignored by consumers of fresh vegetables and by growers who handle water and manure without protection (Adjaye-Gbewonyo, 2008; Heaton and Jones, 2008). Several epidemics of HAV due to consumption of fruit and vegetables have been reported worldwide (Dentinger *et al.*, 2001; Amon *et al.*, 2005; Wheeler *et al.*, 2005; Butot *et al.*, 2007; Bosch *et al.*, 2011). This study is limited by the available few clinical data within the period of sampling. However, previous studies in Burkina Faso have reported an incidence of 1.1% in asymptomatic pregnant women, showing a small but significant circulation of the virus (Hortense *et al.*, 2019). Acute HAV infection can lead to fulminant hepatitis in pregnant women, due to reduced immunity associated with pregnancy. Groom et al. (Groom *et al.*, 2019) have reported cases of obstetric complications and premature deliveries in symptomatic forms of hepatitis A during the 2nd and 3rd trimesters of pregnancy.

## CONCLUSION AND APPLICATION OF RESULTS

To the best of our knowledge, this study provides the first data on HAV contamination of fruit and vegetables, water and manure at market garden sites in Ouagadougou and the surrounding areas. Contamination of fresh produce is the result of poor production practices. The presence of HAV particles on fruit and vegetables, which are generally not treated before consumption, can lead to hepatitis A in the population. The data obtained in this study can therefore be used to assess the public health risk associated with the consumption of fresh produce. They can also serve as scientific support for a larger-scale study in Burkina, focusing on environmental monitoring of enteric viruses.

## AUTHORS' CONTRIBUTIONS

KAT, PR, NB conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, formal analysis and investigation. KAT, SG, MS: drafted the manuscript. KAT, NB: data curation, investigation, writing and editing. KAT: resources, validation, writing and editing. KAT, SG, MS, JBO, LBO, WPBT, PR, NB: Review and editing. All authors contributed to the article and approved the submitted version.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

## REFERENCES

Abd Al-Daim, S., 2024. "Detection of hepatitis a virus in fresh vegetables and irrigation water: An unseen health risk in damanhur, egypt". Biomedical Journal of Scientific & Technical Research, 54. DOI 10.26717/BJSTR.2024.54.008556.

Adamu, N.B., J.Y. Adamu and D. Mohammed, 2012. Prevalence of helminth parasites found on vegetables sold in maiduguri, northeastern nigeria. Food Control, 25(1): 23-26. Available from https://www.sciencedirect.com/science/article/pii/S0956713511004208. DOI https://doi.org/10.1016/j.foodcont.2011.10.016.

Adjaye-Gbewonyo, K., 2008. Farmers' perceptions of benefits and risks from wastewater irrigation in accra, ghana. In: FUAF Foundation. Urban Agriculture Magazine.

Ahmad, T., Y. Waheed, S. Tahir, S.Z. Safi, K. Fatima, M.S. Afzal, Z.U. Farooqi and I. Qadri, 2010. Frequency of hev contamination in sewerage waters in pakistan. J Infect Dev Ctries, 4(12): 842-845. DOI 10.3855/jidc.612.

Amoah, P., P. Drechsel, M. Henseler and R.C. Abaidoo, 2007. Irrigated urban vegetable production in ghana: Microbiological contamination in farms and markets and associated consumer risk groups. J Water Health, 5(3): 455-466. DOI 10.2166/wh.2007.041.

Amon, J.J., R. Devasia, G. Xia, O.V. Nainan, S. Hall, B. Lawson, J.S. Wolthuis, P.D. Macdonald, C.W. Shepard, I.T. Williams, G.L. Armstrong, J.A. Gabel, P. Erwin, L. Sheeler, W. Kuhnert, P. Patel, G. Vaughan, A. Weltman, A.S. Craig, B.P. Bell and A. Fiore, 2005. Molecular epidemiology of foodborne hepatitis a outbreaks in the united states, 2003. J Infect Dis, 192(8): 1323-1330. DOI 10.1086/462425.

Aw, T.G., S. Wengert and J.B. Rose, 2016. Metagenomic analysis of viruses associated with field-grown and retail lettuce identifies human and animal viruses. Int J Food Microbiol, 223: 50-56. DOI 10.1016/j.ijfoodmicro.2016.02.008.

Batz, M.B., E. Henke and B. Kowalcyk, 2013. Long-term consequences of foodborne infections. Infect Dis Clin North Am, 27(3): 599-616. DOI 10.1016/j.idc.2013.05.003.

Bosch, A., S. Bidawid, S. Le Guyader, D. Lees and L.A. Jaykus, 2011. Norovirus and hepatitis a virus in shellfish, soft fruits and water. In: Rapid detection, identification, and quantification of foodborne pathogens.

Butot, S., T. Putallaz and G. Sánchez, 2007. Procedure for rapid concentration and detection of enteric viruses from berries and vegetables. Appl Environ Microbiol, 73(1): 186-192. DOI 10.1128/aem.01248-06.

Costa-Mattioli, M., A.D. Napoli, V. Ferré, S. Billaudel, R. Perez-Bercoff and J. Cristina, 2003. Genetic variability of hepatitis a virus. J Gen Virol, 84(Pt 12): 3191-3201. DOI 10.1099/vir.0.19532-0.

Coudray, C., G. Merle, S. Martin-Latil, L. Guillier and S. Perelle, 2013. Comparison of two extraction methods for the detection of hepatitis a virus in lettuces using the murine norovirus as a process control. J Virol Methods, 193(1): 96-102. DOI 10.1016/j.jviromet.2013.05.003.

Cristina, J. and M. Costa-Mattioli, 2007. Genetic variability and molecular

evolution of hepatitis a virus. Virus Res, 127(2): 151-157. DOI 10.1016/j.virusres.2007.01.005.

Croci, L., M. Ciccozzi, D. De Medici, S. Di Pasquale, A. Fiore, A. Mele and L. Toti, 1999. Inactivation of hepatitis a virus in heat-treated mussels. J Appl Microbiol, 87(6): 884-888. DOI 10.1046/j.1365-2672.1999.00935.x.

Dentinger, C.M., W.A. Bower, O.V. Nainan, S.M. Cotter, G. Myers, L.M. Dubusky, S. Fowler, E.D. Salehi and B.P. Bell, 2001. An outbreak of hepatitis a associated with green onions. J Infect Dis, 183(8): 1273-1276. DOI 10.1086/319688.

Felix-Valenzuela, L., M. Resendiz-Sandoval, A. Burgara-Estrella, J. Hernández and V. Mata-Haro, 2012. Quantitative detection of hepatitis a, rotavirus and genogroup i norovirus by rt-qpcr in fresh produce from packinghouse facilities. Journal of Food Safety, 32. DOI 10.1111/jfs.12009.

Feng, Z., L. Hensley, K.L. McKnight, F. Hu, V. Madden, L. Ping, S.H. Jeong, C. Walker, R.E. Lanford and S.M. Lemon, 2013. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature, 496(7445): 367-371. DOI 10.1038/nature12029.

Fuzawa, M., R.L. Smith, K.M. Ku, J.L. Shisler, H. Feng, J.A. Juvik and T.H. Nguyen, 2020. Roles of vegetable surface properties and sanitizer type on annual disease burden of rotavirus illness by consumption of rotavirus-contaminated fresh vegetables: A quantitative microbial risk assessment. Risk Anal, 40(4): 741-757. DOI 10.1111/risa.13426.

Garcia Garrido, H.M., R.W. Wieten, M.P. Grobusch and A. Goorhuis, 2015. Response to hepatitis a vaccination in immunocompromised travelers. The Journal of Infectious Diseases, 212(3): 378-385. Available from https://doi.org/10.1093/infdis/jiv060 [Accessed 7/3/2024]. DOI 10.1093/infdis/jiv060.

Goyal, S.M., 2006. Methods of virus detection in foods. In: Viruses in foods, S. M. Goyal, (Ed.). Springer US, Boston, MA: pp: 101-119.

Groom, H.C., N. Smith, S.A. Irving, P. Koppolu, G. Vazquez-Benitez, E.O. Kharbanda, M.F. Daley, J.G. Donahue, D. Getahun, L.A. Jackson, N.P. Klein, N.L. McCarthy, J.D. Nordin, L. Panagiotakopoulos and A.L. Naleway, 2019. Uptake and safety of hepatitis a vaccination during pregnancy: A vaccine safety datalink study. Vaccine, 37(44): 6648-6655. DOI 10.1016/j.vaccine.2019.09.043.

Hamza, I.A., L. Jurzik, A. Stang, K. Sure, K. Uberla and M. Wilhelm, 2009. Detection of human viruses in rivers of a densly-populated area in germany using a virus adsorption elution method optimized for pcr analyses. Water Res, 43(10): 2657-2668. DOI 10.1016/j.watres.2009.03.020.

Heaton, J.C. and K. Jones, 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. J Appl Microbiol, 104(3): 613-626. DOI 10.1111/j.1365-2672.2007.03587.x.

Holvoet, K., I. Sampers, M. Seynnaeve and M. Uyttendaele, 2014. Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. Int J Food Microbiol, 171: 21-31. DOI 10.1016/j.ijfoodmicro.2013.11.009.

Hortense, R.P.S., O.J. Bienvenue, T.K. Abdoulaye, O.B. Lalidia, K. Asséta,

R. Pierre and B. Nicolas, 2019. Sero-prevalence and risk factors for hepatitis a virus infection among pregnant women at the samandin medical center, ouagadougou, burkina faso. Journal of Advances in Biology & Biotechnology, 21(3): 1-9. Available from https://journaljabb.com/index.php/JABB/article/view/398 [Accessed 2024/07/03]. DOI 10.9734/jabb/2019/v21i330095.

Khan, W., M. Irshad, G. Rehman and A. Hussain, 2014. Hav in fresh vegetables: A hidden health risk in district mardan, pakistan. Springerplus, 3: 675. DOI 10.1186/2193-1801-3-675.

Knowles, N.J., T. Hovi, T. Hyypiä, A.M.Q. King, A.M. Lindberg, M.A. Pallansch, A.C. Palmenberg, P. Simmonds, T. Skern, G. Stanway, T. Yamashita and R. Zell, 2012. Family - picornaviridae. In: Virus taxonomy : Ninth report of the international committee on taxonomy of viruses, E. L. M. J. A. Andrew M.Q. King and B. C. Eric, (Eds.). Elsevier, San Diego - London: pp: 855-881.

Kokkinos, P., I. Kozyra, S. Lazic, M. Bouwknegt, S. Rutjes, K. Willems, R. Moloney, A.M. de Roda Husman, A. Kaupke, E. Legaki, M. D'Agostino, N. Cook, A. Rzeżutka, T. Petrovic and A. Vantarakis, 2012. Harmonised investigation of the occurrence of human enteric viruses in the leafy green vegetable supply chain in three european countries. Food Environ Virol, 4(4): 179-191. DOI 10.1007/s12560-012-9087-8.

La Rosa, G., S.D. Libera, M. Iaconelli, A.R. Ciccaglione, R. Bruni, S. Taffon, M. Equestre, V. Alfonsi, C. Rizzo, M.E. Tosti, M. Chironna, L. Romanò, A.R. Zanetti and M. Muscillo, 2014. Surveillance of hepatitis a virus in urban sewages and comparison with cases notified in the course of an outbreak, italy 2013. BMC Infect Dis, 14: 419. DOI 10.1186/1471-2334-14-419.

Lemon, S.M., J.J. Ott, P. Van Damme and D. Shouval, 2017. Type a viral hepatitis: A summary and update on the molecular virology, epidemiology, pathogenesis and prevention. J Hepatol. DOI 10.1016/j.jhep.2017.08.034.

Marti, E., M. Ferrary-Américo and C.R.M. Barardi, 2017. Detection of potential infectious enteric viruses in fresh produce by (rt)-qpcr preceded by nuclease treatment. Food Environ Virol, 9(4): 444-452. DOI 10.1007/s12560-017-9300-x.

McKnight, K.L. and S.M. Lemon, 2018. Hepatitis a virus genome organization and replication strategy. Cold Spring Harb Perspect Med, 8(12). DOI 10.1101/cshperspect.a033480.

Nainan, O.V., G. Xia, G. Vaughan and H.S. Margolis, 2006. Diagnosis of hepatitis a virus infection: A molecular approach. Clin Microbiol Rev, 19(1): 63-79. DOI 10.1128/cmr.19.1.63-79.2006.

Okonko, I., Omezuruike, A. Ogunjobi, Adewale, E. Fajobi, Aloysius, B. Onoja, E. Babalola, Temilade and A. Adedeji, 2008. Comparative studies and microbial risk assessment of different ready-to-eat (rte) frozen sea-foods processed in ijora-olopa, lagos state, nigeria.

Painter, J.A., R.M. Hoekstra, T. Ayers, R.V. Tauxe, C.R. Braden, F.J. Angulo and P.M. Griffin, 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, united states, 1998-2008. Emerg Infect Dis, 19(3):

407-415. DOI 10.3201/eid1903.111866.

Rigotto, C., M. Victoria, V. Moresco, C.K. Kolesnikovas, A.A. Corrêa, D.S. Souza, M.P. Miagostovich, C.M. Simões and C.R. Barardi, 2010. Assessment of adenovirus, hepatitis a virus and rotavirus presence in environmental samples in florianopolis, south brazil. J Appl Microbiol, 109(6): 1979-1987. DOI 10.1111/j.1365-2672.2010.04827.x.

Robert, A., J. Louis Yengué, F. Augis, M. Motelica-Heino, E. Hien and S. Alain, 2018. L'agriculture ouagalaise (burkina faso) comme modèle de contribution au métabolisme urbain : Avantages et limites. VertigO : La Revue Électronique en Sciences de l'Environnement(Hors-série 31): https://journals.openedition.org/vertigo/21857. Available from https://shs.hal.science/halshs-02044292. DOI 10.4000/vertigo.21857.

Robertson, B.H., R.W. Jansen, B. Khanna, A. Totsuka, O.V. Nainan, G. Siegl, A. Widell, H.S. Margolis, S. Isomura, K. Ito and et al., 1992. Genetic relatedness of hepatitis a virus strains recovered from different geographical regions. J Gen Virol, 73 ( Pt 6): 1365-1377. DOI 10.1099/0022-1317-73-6-1365.

Saïd, R., M. Wolfaardt and M.B. Taylor, 2014. Molecular characterisation of hepatitis a virus strains from water sources in south africa. Water Sci Technol, 69(5): 923-933. DOI 10.2166/wst.2013.799.

Shaheen, M.N.F., E.M. Elmahdy, L.H.I. Mahmoud, I.A. Hammad and E.R.S. Soliman, 2022. Quantitative rt-pcr detection of human noroviruses and hepatitis a virus in fresh produce and surface water used for irrigation in the mansoura and giza regions, egypt. Environ Sci Pollut Res Int, 29(19): 28139-28148. DOI 10.1007/s11356-021-18412-3.

Smith, D.B. and P. Simmonds, 2018. Classification and genomic diversity of enterically transmitted hepatitis viruses. Cold Spring Harb Perspect Med, 8(9). DOI 10.1101/cshperspect.a031880.

Taffon, S., G. Bidini, F. Vichi, G. Corti, D. Genovese, L.A. Kondili, R. Bindi, F. Armellini, F. Leoncini, A. Bartoloni, F. Mazzotta and M. Rapicetta, 2011. A unique hav strain circulated in patients with acute hav infection with different risk exposures in tuscany, italy. J Clin Virol, 50(2): 142-147. DOI 10.1016/j.jcv.2010.10.011.

Takuissu, G.R., S. Kenmoe, J.T. Ebogo-Belobo, C. Kengne-Ndé, D.S. Mbaga, A. Bowo-Ngandji, J.L. Ndzie Ondigui, R. Kenfack-Momo, S. Tchatchouang, J. Kenfack-Zanguim, R. Lontuo Fogang, E. Zeuko'o Menkem, G.I. Kame-Ngasse, J.N. Magoudjou-Pekam, C. Veneri, P. Mancini, G. Bonanno Ferraro, M. Iaconelli, L. Orlandi, C. Del Giudice, E. Suffredini and G. La Rosa, 2023. Occurrence of hepatitis a virus in water matrices: A systematic review and meta-analysis. Int J Environ Res Public Health, 20(2). DOI 10.3390/ijerph20021054.

Tjon, G.M., R.A. Coutinho, A. van den Hoek, S. Esman, C.J. Wijkmans, C.J. Hoebe, B. Wolters, C. Swaan, R.B. Geskus, N. Dukers and S.M. Bruisten, 2006. High and persistent excretion of hepatitis a virus in immunocompetent patients. J Med Virol, 78(11): 1398-1405. DOI 10.1002/jmv.20711.

Toe, E., A. Dadie, E. Dako and G. Loukou, 2017. Bacteriological quality and risk factors for contamination of raw

mixed vegetable salads served in collective catering in abidjan (ivory coast). Advances in Microbiology, 7: 405-419. DOI 10.4236/aim.2017.76033.

Torok, V.A., K.R. Hodgson, J. Jolley, A. Turnbull and C. McLeod, 2019. Estimating risk associated with human norovirus and hepatitis a virus in fresh australian leafy greens and berries at retail. International Journal of Food Microbiology, 309: 108327. Available from https://www.sciencedirect.com/science/article/pii/S0168160518306974. DOI https://doi.org/10.1016/j.ijfoodmicro.2019.108327.

Wang, X., J. Ren, Q. Gao, Z. Hu, Y. Sun, X. Li, D.J. Rowlands, W. Yin, J. Wang, D.I. Stuart, Z. Rao and E.E. Fry, 2015. Hepatitis a virus and the origins of picornaviruses. Nature, 517(7532): 85-88. DOI 10.1038/nature13806.

Wheeler, C., T.M. Vogt, G.L. Armstrong, G. Vaughan, A. Weltman, O.V. Nainan, V. Dato, G. Xia, K. Waller, J. Amon, T.M. Lee, A. Highbaugh-Battle, C. Hembree, S. Evenson, M.A. Ruta, I.T. Williams, A.E. Fiore and B.P. Bell, 2005. An outbreak of hepatitis a associated with green onions. N Engl J Med, 353(9): 890-897. DOI 10.1056/NEJMoa050855.

WHO, 2012. Cinq clefs pour cultiver des fruits et des légumes plus sûrs : Promouvoir la santé en réduisant la contamination microbienne. World Health Organization Available from https://iris.who.int/bitstream/handle/10665/75197/9789242504002_fre.pdf?sequence=1

WHO, 2022. Who position paper on hepatitis a vaccines. Weekly Epidemiological Record, 97(40): 493–512. Available from https://iris.who.int/bitstream/handle/10665/363396/WER9740-eng-fre.pdf?sequence=1.