

# Detection of Adenovirus in Fresh Fruit, Vegetables, Wastewater and Manure from Irrigated Farms in Ouagadougou, Burkina Faso

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## Abstract

Enteric viral pathogens are responsible for numerous epidemics associated with the consumption of fresh fruit and vegetable, whether raw or minimally processed. The aim of the present study was to assess agricultural practices and the presence of adenovirus (AdV) in fruits and vegetables, manure and irrigation wastewater sampled in the urban and peri-urban perimeters of Ouagadougou. A total of 286 samples including 30 lettuces, 42 tomatoes, 30 carrots, 30 strawberries, 74 manures and 80 wastewater samples were collected from four market garden sites in and around Ouagadougou. Nested PCR was performed with specific primers to detect adenoviruses (AdVs). A face-to-face survey was carried out using a questionnaire on market garden production practices. Overall, adenoviruses prevalence was 5.9% [IC95, 3.2% - 8.7%] in all samples analyzed. It was specifically 7.14% (3/42) from tomatoes, 6.7% (2/30) from lettuces, 20% (6/30) on strawberries and 7.5% (6/80) in irrigation water. The survey showed that irrigation water came from untreated sources (dam, well, canal) and then 52% of farms used untreated manure. No farms have implemented measures to limit access by domestic and wild animals. This work shows the presence of human adenoviruses in surface irrigation water and fresh produce, which is of concern when fresh produce is consumed raw. To reduce the public health risks associated with consuming these foods, it is essential to follow good hygiene and cultivation practices.

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## Keywords

Adenovirus, Raw Fruits and Vegetables, Nested PCR, Wastewater, Manure, Ouagadougou

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## 1. Introduction

Market gardening is a highly specialized form of agriculture [1]. This sector provides fruits and vegetables that are most often produced during the dry season. Fruits and vegetables offer multiple health benefits. They boost the immune system, combat malnutrition and prevent non-communicable diseases [2]. Fruit and vegetable production provides livelihoods and food for millions of people [1]. It contributes to a better quality of life for family farmers and their communities and strengthens resilience [3]. However, fruits and vegetables that are mainly eaten raw can be sources of enteric pathogens [4]. Indeed, the WHO [5] estimates that every year, approximately 600 million illnesses are foodborne, and 420,000 people die worldwide as a result. These diseases are often associated with two main food groups: fruits and vegetables and animal products [2]. Several types of enteric viruses, such as hepatitis A virus, hepatitis E virus, adenovirus, norovirus and rotavirus, have been detected in raw fruits [6]-[9] and vegetables and are potentially implicated in foodborne epidemics [10].

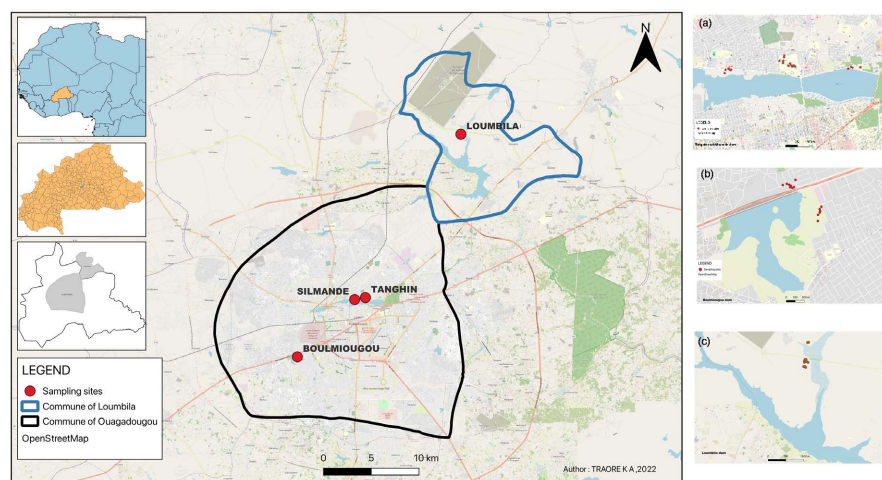
Human adenoviruses (HAdVs) are agents of gastroenteritis and represent the third most common cause of childhood diarrhea. HAdV belongs to the family Adenoviridae and the genus Mastadenovirus [11]. HAdV was first isolated from human adenoidal tissue and is characterized as a non-enveloped, non-segmented, and double-stranded DNA virus with a genome length of 3.4 - 3.6 kb that encodes three major capsid proteins, namely, hexon, penton base and fiber [12] [13]. To date, 113 types of HAdVs have been identified and classified into seven species (A - G) [14], of which species B, C, and E are highly contagious and are responsible for severe respiratory disease. In Burkina Faso, adenovirus types 40/41 (species F) were detected in 5% of diarrhea cases in children under 5 years of age [15]. In 2016, Ouédraogo *et al.* [16] reported a prevalence of 31.2% for adenoviruses, including 10.2% and 5.1% for HAdV types 40 and 41, respectively, in children in the same country. Adenovirus infections occur without seasonality in patients of all ages [17] [18] and susceptible populations, including children, military recruits and immunocompromised patients [19]. Type 40/41 viruses are transmitted via the fecal-oral route and are highly excreted in feces [20]. Studies have reported significantly high concentrations and seasonal variability of these viruses in various types of water worldwide, such as wastewater, surface water, seawater, freshwater, and treated and disinfected drinking water [21]-[24]. HAdV has also been proposed as an indicator of fecal contamination and water purification efficiency [25]-[27], reinforcing its potential as a foodborne virus. Adenovirus contamination of fruits and vegetables can occur mainly through irrigation

water, natural fertilizers, infected food handlers or the use of contaminated equipment at some point in the food production chain. In Burkina Faso, wastewater and manure are widely used at fruit and vegetable production sites. However, very little data are available on the environmental prevalence of adenovirus. This study was designed to investigate the presence of human adenoviruses in the irrigation water-manure-vegetable continuum. Thus, the aim of the present study was to assess agricultural practices and the presence of adenoviruses in fresh products in the urban and peri-urban perimeters of Ouagadougou and establish whether the wastewater used for irrigation and manure is a potential source of fresh produce contamination.

## 2. Materials and Methods

### 2.1. Sites and Samples

This prospective study was conducted from September 2021 to July 2022. Samples were collected at four market garden production sites (Boulmiougou, Loumbila, Silmandé, and Tanghin) (**Figure 1**). These sites were chosen on the basis of fruit and vegetable availability, market garden production intensity and geographical location. A total of 74 samples of manure were used for spreading, 80 samples of surface water and 132 fresh produce products (tomato (*Solanum lycopersicum* L.), strawberry (*Fragaria* sp.), carrot (*Daucus carota*) and lettuce (*Lactuca sativa*)) irrigated with this water were collected in three urban areas and one peri-urban area of Ouagadougou.



**Figure 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.

### 2.2. Sampling Strategy and Surveys

Only ripe vegetables ready for sale or consumption were sampled. Tomatoes and strawberries with visible damage and/or cracks were not sampled. Each lettuce sample consisted of three plants. Similarly, a sample of tomatoes, strawberries or

carrots comprised three tomatoes, three strawberries and three carrots, respectively, taken from different parts of the same field.

A 300 ml sample of irrigation water was taken from the retention basins (wells, dams, canals). Approximately 3 g of manure was collected from the manure sample in a sterile plastic tube.

A questionnaire survey was carried out on market garden farms to assess good agricultural practices (GAPs) and good hygienic practices (GHPs) (**Appendix**). The survey consisted of field observations and face-to-face interviews with 300 randomly selected market gardeners. Particular attention was given to the irrigation water used per site, human and animal activity and manure handling.

### 2.3. Preparation of Water Samples

The samples were processed using a method adapted from Ahmad *et al.* [28]. Water samples were divided into aliquots of 40 ml, and 0.25 ml of 50% (w/v) polyethylene glycol 8000/1.5 M NaCl was added to each sample (pH adjusted at 7 - 7.5) to reach a final concentration of 10% (w/v) PEG 8000 with sodium chloride to a final concentration of 0.4 M. This mixture 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 mL of 0.15 M phosphate buffer and stored at -20°C.

### 2.4. Preparation of Fresh Produce Samples

Vegetable samples were processed using a method adapted from Coudray-Meunier *et al.* [29]. Fresh produce (25 g) in small pieces was 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 material, the preparation was distributed into clean centrifuge tubes. After centrifugation at 10,000×*g* for 30 min at 4°C, then the vegetable matter was discarded, the supernatant was transferred to clean centrifuge tubes, and the pH was adjusted to 7.2 ± 0.3. For PEG precipitation, 0.25 volumes of 50% (w/v) polyethylene glycol 8000/1.5 M NaCl were added to the eluates and stirred for two hours (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.

### 2.5. Treatment of Feces and Manure Samples

Manure samples were processed using a method adapted from Kokkinos *et al.* [30]. A 10% manure suspension in phosphate-buffered saline was prepared, 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.

### 2.6. Nucleic Acid Extraction

Fresh produce suspensions and irrigation water samples were first treated with

chloroform-butanol (1:1). These mixtures were briefly homogenized and then centrifuged at  $10000\times g$  for 15 min. The upper aqueous phase of each sample served as the sample for the final nucleic acid extraction. Nucleic acids were extracted using a FavorPrep™ viral nucleic acid extraction kit according to the manufacturer's instructions. The samples were stored at  $-20^{\circ}\text{C}$ .

## 2.7. Nested PCR

Viral DNA extracted from the samples was detected by a HAdV-specific nested PCR protocol targeting the hexon capsid protein-encoding gene, as previously described [31], using Solis BioDyne's 5× FIREPol® Master Mix Ready to Load kit, according to the manufacturer's instructions. The primer sequences are shown in **Table 1**. The first PCR was performed using 3.5  $\mu\text{l}$  of extracted viral DNA for cDNA synthesis with FIREPol® DNA polymerase. The thermocycling conditions were 3 min at  $95^{\circ}\text{C}$ , followed by 30 cycles of 1 min at  $95^{\circ}\text{C}$ , 1 min at  $51^{\circ}\text{C}$  and 1 min at  $72^{\circ}\text{C}$ . The final extension time was 10 min at  $72^{\circ}\text{C}$ . For nested PCR, 5  $\mu\text{l}$  of cDNA template obtained from the first PCR was used. The thermocycling conditions were 3 min at  $95^{\circ}\text{C}$ , followed by 30 cycles of 1 min at  $95^{\circ}\text{C}$ , 1 min at  $53^{\circ}\text{C}$ , and 1 min at  $72^{\circ}\text{C}$ , with a final extension of 10 min at  $72^{\circ}\text{C}$ . Nuclease-free water was used in all experiments as a negative control. PCR products (5  $\mu\text{l}$ ) were analyzed on 2% agarose gels. Fragment sizes were compared using a commercially available standard 100 bp DNA ladder (Solis Biotdyne, Estonia).

**Table 1.** Primers used for amplification of the adenovirus hexon protein-encoding gene.

Primers	Sequence (5' → 3')	Amplicon size	Reference
AV1b	GCCCCGAGTGGTCTTACATGCACATC	First PCR	[31]
AV2b	CAGCACGCCCGGGATGTCAAAGT	301 pb	
AV3c	GCCACCGAGACGTA CTTCAGCCTG	Nested PCR	
AV4c	TTGTACGAGTACGCGGTATCCTCGCGGTC	143 pb	

## 2.8. Statistical Analysis

Microsoft Excel 2019 and Sphinx V5 software were used for data entry, analysis and some graphics. The lower and upper limits of the 95% confidence interval (CI) for a proportion were also calculated.

## 3. Results

### 3.1. Production Site Characteristics

Market garden sites have been developed around engineered water sources (dams, canals) and wastewater sources (domestic or industrial) (**Figure 2**). Given the distance between these dams and the sites, the market gardeners dug ditches to drain the water from the dam to the market garden perimeters. However, 71% of farmers use wells as their sole source of water for irrigation.

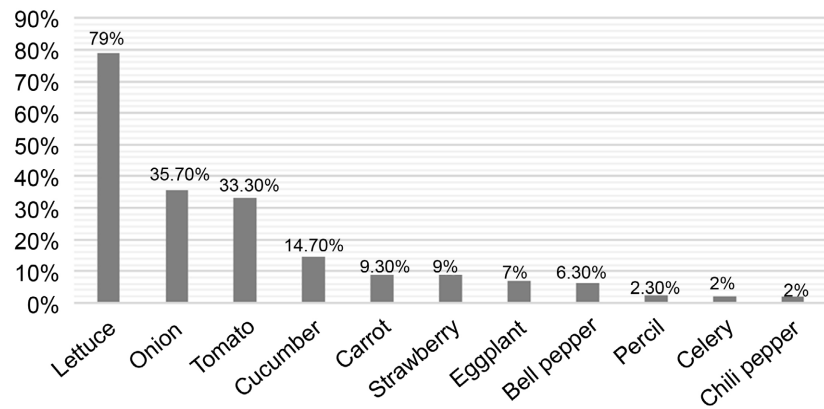


**Figure 2.** Characteristics of the production sites. Legend: (a) Tomato fields; (b) Irrigation water sources (dams near fields); (c) Lettuce fields; (d) Strawberry fields; (e) Carrot fields; (f) Manual irrigation method; (g) Ditches; (h) Manure in lettuce fields; (i) (j) Irrigation water sources (well); (k) Waste in lettuce fields; (l) Manure.

The market garden sites are of the traditional type, laid out in the open air, with no protection against urban wastes, wind, sun or animals (sheep, goats, pigs, rodents, reptiles, birds). Polyculture was the farming technique adopted at the sites (**Figure 3**). The crops found at the sites and eaten raw were leaf and bulb onions (*Allium cepa*), tomatoes (*Solanum lycopersicum*), eggplants (*Solanum melogena*), pepper (*Capsicum frutescens*), lettuce (*Lactiva sativa*), carrots (*Daucus carota*), strawberries (*Fragaria* sp.), cucumbers (*Cucumis sativus*), bell pepper (*Capsicum annuum*), celery (*Apium graveolens* L.) and parsley (*Petroselinum crispum*). Fresh fruit and vegetable production was dominated by lettuce (79%), onion (35.7%) and tomato (33.3%).

### 3.2. Sociodemographic Characteristics of the Producers

The producers interviewed were all from Burkina Faso, mostly men (66.67%), aged between 35 and 45 years (65%). Sixty-nine percent of the producers said



**Figure 3.** Types of fruits and vegetables grown on market garden sites.

they had received no basic education, and 43% had been growing vegetables for more than 20 years (**Table 2**).

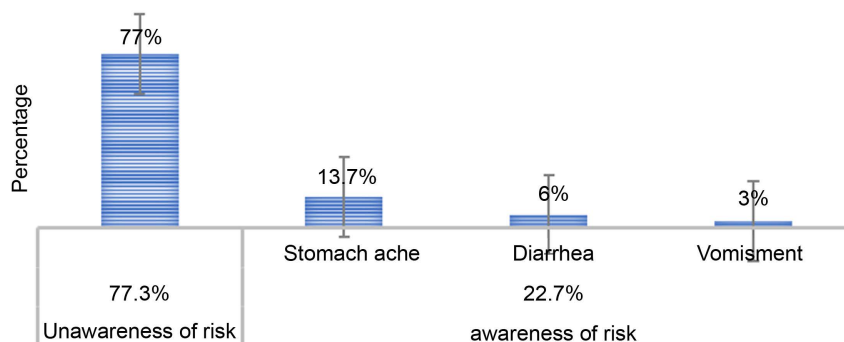
**Table 2.** Sociodemographic characteristics of producers.

Parameters (n = 300)	Proportion
<b>Gender</b>	
– Male	206 (68.67%)
– Female	94 (31.33%)
<b>Nationality</b>	
– Burkinabè	300 (100%)
– Non-Burkinabè	0
<b>Age</b>	
– Under 30 years old	28 (9.33%)
– 30 to 45 years old	195 (65%)
– Over 45 years old	77 (25.67%)
<b>background</b>	
– Out of school	209 (69.67%)
– Primary	62 (20.67%)
– Secondary	28 (9.33%)
– Higher education	1 (0.33%)
<b>Experience in market gardening</b>	
– Less than 5 years old	59 (19.67%)
– 5 to 15 years	49 (16.33%)
– 15 to 20 years	63 (21%)
– More than 20 years old	129 (43%)

### 3.3. Producers' Knowledge of Health Risks

The majority of growers (77.3%) had no knowledge of the risks associated with

using (untreated) wastewater to irrigate market garden plots (**Figure 4**). Those aware of the health risks associated with wastewater use (22.7%) cited stomach upset (13.7%), diarrhea (6%) and vomiting (3%) as symptoms of ingesting irrigation water.



**Figure 4.** Farmers' level of awareness of the risks associated with using (untreated) wastewater for irrigation and symptoms listed by those who are aware of these risks.

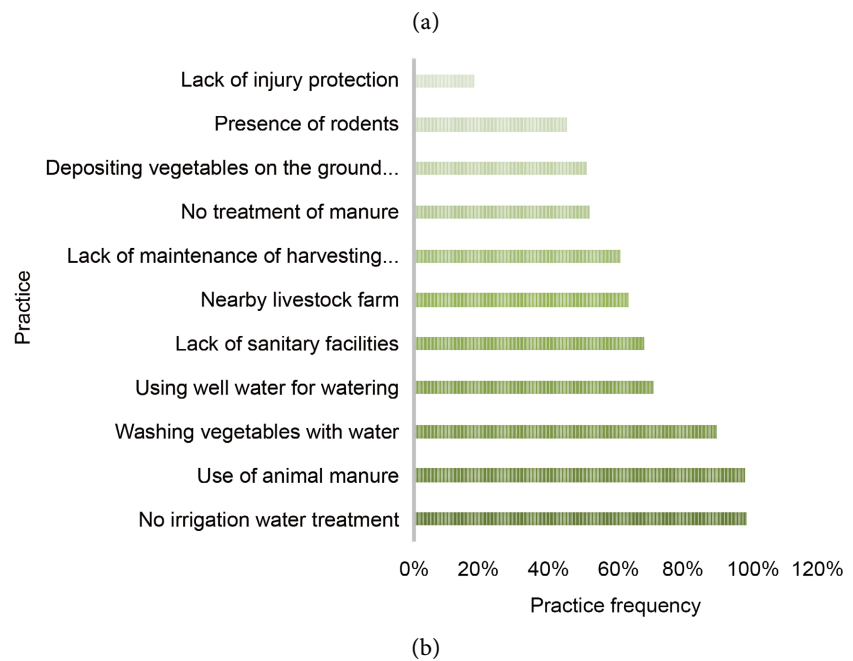
### 3.4. Biocontamination Risk Practices for Fruits and Vegetables in the Field

The majority of fields visited (68.3%) had no sanitary facilities, and 18% had no means of protection. Our survey revealed that almost all producers (98.3%) used animal manure (chicken, pig, beef, and sheep) to fertilize the soil. In addition, 63.7% and 45.3% of respondents reported the presence of livestock and rodents in and around the fruit and vegetable plant growing sites, respectively.

Producers (98.7%) mainly used water from various sources (wells, dams, canals or wastewater from abattoirs, domestic or industrial) for crop irrigation without prior treatment.

Harvested products are placed directly on the ground by 51.3% of producers, and 89.9% of producers wash them with irrigation water. During our survey, we observed that most crops are put into bags or baskets and transported to markets by motorcycle or tricycle (**Figure 5(a)**) and **Figure 5(b)**).





**Figure 5.** (a) Harvesting and transporting fruits and vegetables; (b): Biocontamination risk practices for fruits and vegetables in the field.

### 3.5. Prevalence of Adenoviruses in Fruits, Vegetables, Wastewater and Manure

A total of eight (17) samples (5.94%) (CI95, 3.2% - 8.7%) tested positive for adenoviruses (Table 3). However, 7.14% (3/42) of the tomatoes, 6.7% (2/30) of the lettuce, 20% (6/30) of the strawberries, and 7.5% (6/80) of the irrigation water tested positive for adenoviruses. No adenovirus was detected in the carrot or manure samples.

**Table 3.** Prevalence of adenovirus in fruits, vegetables, manure and irrigation water.

Product	Prevalence number and (percentage) in sampling sites				
	Boulmiougou	Silmandé	Tanghin	Loumbila	Total
Carrots	0/0 (0%)	0/0 (0%)	0/30 (0%)	0/0 (0%)	0/30 (0%)
Strawberries	6/30 (20%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	6/30(20%)

**Continued**

Tomatoes	0/11 (0%)	0/0 (0%)	0/0 (0%)	3/31 (9.7%)	3/42 (7.14%)
Lettuces	2/20 (10%)	0/10 (0%)	0/10 (0%)	0/0 (0%)	2/30 (6.7%)
Manure	0/17 (0%)	0/18 (0%)	0/24 (0%)	0/15 (0%)	0/74 (0%)
Water	4/25 (16%)	0/15 (0%)	0/15 (0%)	2/25 (8%)	6/80 (7.50%)

**4. Discussion**

Market garden production in Burkina Faso is characterized by small-scale organization of water sources on small plots with polycultures of plant species. This seasonal polyculture system is irrigated with various water sources. The plants were fertilized with animal manure without any sanitation processes. Market gardens are open-air plots characterized by the presence of weeds, urban waste and animals. These characteristics have also been observed by several authors around major urban centers, such as Bobo-Dioulasso, Ouagadougou and Benin [32]-[34]. All the market garden sites were located close to engineered water sources, such as dams. Market gardening is a dry-season activity in Burkina Faso, and water is essential to its success. However, 71% of the farmers dug shallow wells in their fields and used this untreated water for irrigation. These observations have also been made in Côte d'Ivoire, where the majority of market gardeners use well water for irrigation [35]. For most farmers, access to water from dams means extra work. They have to dig ditches to bring water from the dams to the fields. At any moment, the water level in the dam drops considerably, making it difficult for the water to reach the field [36].

The sociodemographic data in this study are consistent with those reported in Burkina [33] [37], Mali [38], Nigeria [36] and Benin [39]. This could be explained by physical constraints, namely, the drudgery of irrigation practices dominated by manual labor [40]. Indeed, market gardening, as described above, is less mechanized and requires a great deal of time and physical effort for irrigation and related activities [35]. Cultural considerations also give men more rights to the land than women. The market gardeners were mostly young (15 - 30 years old) and had a low level of education (69.7%). These rates are in agreement with those observed by Ouédraogo *et al.* [40] who reported that the median age of market gardeners is 40 and that 60% of them had no level of education. However, Hougbenou [39] showed that in Benin, 76.09% of market gardeners were educated, and 59% were over 40 years old. The high proportion of young people involved in agriculture could also be explained by rural exodus and, above all, unemployment. Indeed, young people move to cities in search of better living conditions and find themselves involved in these activities [38]. In addition, the low level of education among market gardeners could be because most young graduates only seek work in the formal sector [33].

The majority of the market garden sites (68.3%) had no sanitary facilities, were close to livestock areas and housed free-ranging domestic animals. These practices can lead to contamination of fresh produce, as the presence of pathogenic micro-

organisms in animal excrement has been reported [30] [41]. Animals such as sheep, goats, pigs, cattle, chickens and rodents excrete pathogenic agents such as *Salmonella* spp., *E. coli* and enteric viruses in their feces [42] [43]. The lack of sanitary facilities leads to open defecation around sites, which can contaminate crops through runoff [44]. Wastewater, a veritable vector for many pathogens [45], is a major source of crop biocontamination if it is used for irrigation without adequate treatment. The survey also revealed that 98.3% of the market gardeners used animal manure and that 52% of them did not treat it before use. Nabie [33] reported that 91.4% of growers used manure as fertilizer, and according to CIRAD [46], the composting phase of animal dejecta is rarely accepted by market gardeners. The use of non-composted manure as fertilizer is a risk factor for contamination of fresh produce [47] [48].

The survey results show that 77.3% of growers were unaware of the risks associated with using untreated wastewater for irrigation. The same observation was made by Wognin *et al.* [49], who reported a rate of 91.69%. These risks of biocontamination of fresh produce could be justified, on the one hand, by their low level of education and, on the other hand, by their ignorance of good farming practices and the impact of risky practices on crop quality and consumer health.

The detection rate (8.33%) observed in fresh produce is lower than that reported in Egypt (55.5%) [50]. The presence of adenovirus in fresh produce could be due, on the one hand, to the risk practices of market gardeners and, on the other hand, to the level of hygiene in the environment. According to the WHO, poor hygiene among growers, the use of soiled harvesting tools and human and animal feces are all factors in the contamination of fresh produce in the field [44]. Indeed, contamination by the main etiological agents of gastroenteritis is attributable to the environment [51]. Poor environmental hygiene can also lead to contamination of these waters by various microorganisms [52]. The detection rate of the adenovirus genome in water samples (7.5%) is in agreement with that found by Opere *et al.* [53] (5.09%) in the water of a lake in Kenya. This presence was justified by the proximity of latrines and wastewater treatment areas to the lake. However, this rate was very low compared to those reported by Ibrahim [54] for hospital wastewater in Tunisia (64%) and by Amdiouni *et al.* [55] for (untreated) wastewater in Morocco, North Africa (66%). This difference could be explained, on the one hand, by the origin or provenance of the wastewater analyzed and, on the other hand, by variations in rainfall between samplings [56] [57]. Indeed, studies in Egypt have shown higher HAdV detection rates in raw wastewater from treatment plants than in river water or treated wastewater [58]-[60]. Other studies have shown that high viral prevalence in wastewater is linked to reduced water flow during the dry season, resulting in less dilution of viral particles and, consequently, increased viral contamination when fecal matter is discharged into the river [61].

Evidence of zoonotic transmission of AdV has been documented in a meta-analysis reporting cases where AdV crossed host species barriers between hu-

mans and non-human primates, bats, felines, pigs, canids, sheep and goats [62]. However, in this study, adenovirus was not detected in any of the manure samples. This could be explained by the sensitivity of the detection method used or by the fact that virus survival is affected by composting or solar radiation [63] [64]. The survey showed that 23% of the market gardeners composted their manure, and 25% exposed it to sunlight. Another limitation of this study is the lack of clinical data. Future studies involving sequence analysis are needed to compare strains detected in clinical and environmental samples.

## 5. Conclusion

This study showed that fresh raw produce could be contaminated with adenovirus, causing a health risk to consumers. Untreated irrigation water can be one of the sources of this contamination. Animal access to market garden sites and the absence of good hygiene practices are also factors favoring biocontamination of these products during production. However, it is essential to assess the viral load and characterize these adenoviruses to implement a better sanitary strategy for fruit and vegetable production and reduce the risk associated with their consumption.

## Authors' Contributions

KAT, PR and NB: Conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, formal analysis and investigation. KAT and MS: Drafted the manuscript. KAT and NB: Data curation, investigation, writing and editing. KAT: Resources, validation, writing and editing. KAT, MS, JBO and BLO: Review and editing. All authors contributed to the article and approved the submitted version.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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## Appendix

### Survey sheet on agricultural practices of vegetable producers

LaBESTA

Ladies and Gentlemen, please answer this questionnaire. We reassure you that the processing of responses will be confidential ...

1. Sociodemographic characteristics	
<p><b>1. Vegetable production areas</b></p> <p><input type="radio"/> 1. Paspanga   <input type="radio"/> 2. Ouaga 2000   <input type="radio"/> 3. Boulmiougou <input type="radio"/> 4. Koubri   <input type="radio"/> 5. Loumbila</p>	<p><b>6. Is this your only source of income?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No</p>
<p><b>2. How is the farm size?</b></p> <p><input type="radio"/> 1. large (&gt; 0.14ha)   <input type="radio"/> 2. average (&gt; 0.03ha) <input type="radio"/> 3. small (&lt; 0.01ha)</p>	<p><b>7. Nationality</b></p> <p><input type="radio"/> 1. Burkinabè   <input type="radio"/> 2. No Burkinabè</p>
<p><b>3. Types of vegetables grown</b></p> <p><input type="radio"/> 1. lettuce   <input type="radio"/> 2. Cucumber   <input type="radio"/> 3. African eggplant <input type="radio"/> 4. Tomatoes   <input type="radio"/> 5. Strawberry   <input type="radio"/> 6. others</p>	<p><b>8. Religion</b></p> <p><input type="radio"/> 1. Muslim   <input type="radio"/> 2. Christian   <input type="radio"/> 3. Animist   <input type="radio"/> 4. Other</p>
<p><b>4. Sex</b></p> <p><input type="radio"/> 1. Male   <input type="radio"/> 2. Female</p>	<p><b>9. Duration of the activity</b> <input type="text"/></p>
<p><b>5. Age</b></p> <p><input type="radio"/> 1. 1-less than 30 years old   <input type="radio"/> 2. 30-45 years <input type="radio"/> 3. Over 45 years</p>	<p><b>10. Study level</b></p> <p><input type="radio"/> 1. Illiterate   <input type="radio"/> 2. Primary   <input type="radio"/> 3. Secondary <input type="radio"/> 4. Superior</p>
2. Identification of agricultural practices	
<p><b>11. Are there sanitary facilities (WC) in your field?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No   <input type="radio"/> 3. If not, where do you go?</p>	<p><b>19. Are you treating this water?</b></p> <p><input type="radio"/> 1. No   <input type="radio"/> 2. Yes <input type="radio"/> 3. If yes, what products do you use?.....</p>
<p><b>12. Are cuts, lesions and wounds protected by a glove or bandage when working in the field?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No</p>	<p><b>20. Do you use manure as fertilizer?</b></p> <p><input type="radio"/> 1. No <input type="radio"/> 2. Yes <input type="radio"/> 3. If yes, what type of manure do you use? Poultry droppings, Beef excrement, Pork excrement, Household waste, Others .....</p>
<p><b>13. Do you have lot of waste in the field?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No</p>	<p><b>21. Are you treating this manure?</b></p> <p><input type="radio"/> 1. No <input type="radio"/> 2. Yes <input type="radio"/> 3. If yes, by what methods? Compostin, Environmental exposure, Other .....</p>
<p><b>14. Do you have pets in your field?</b></p> <p><input type="radio"/> 1. No <input type="radio"/> 2. Yes <input type="radio"/> 3. If yes, which ones ? Poultry , Cat , Dog , Sheep , Beef , Pork , Others, .....</p>	<p><b>22. Do you apply manure to crops?</b></p> <p><input type="radio"/> 1. No <input type="radio"/> 2. Yes <input type="radio"/> 3. If yes, How do you spread manure in your field? .....</p>
<p><b>15. Do wild animals have access to your field?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No</p>	<p><b>23. Is the harvest equipment cleaned and dried?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No</p>
<p><b>16. Are there animal farms near your field?</b></p> <p><input type="radio"/> 1. No <input type="radio"/> 2. Yes <input type="radio"/> 3. if yes, Poultry, Beef, Pork, sheep, Others .....</p>	<p><b>24. Do you put the vegetables on the ground during the harvest?</b></p> <p><input type="radio"/> 1. Yes   <input type="radio"/> 2. No</p>
<p><b>17. What waters do you use to irrigate your crops</b></p> <p><input type="radio"/> 1. Well water   <input type="radio"/> 2. Dam water <input type="radio"/> 3. Canal water   <input type="radio"/> 4. Temporary pond <input type="radio"/> 5. Others .....</p>	<p><b>25. How is this production treated after harvest?</b></p> <p><input type="radio"/> 1. ....</p>
<p><b>18. How is irrigation done?</b></p> <p><input type="radio"/> 1. Pumping Irrigation   <input type="radio"/> 2. Sprinkler irrigation</p>	

**26. After harvest, how do you package the crops (or plant product)?**

- 1. ....

**27. Is the harvest product sold ?**

- 1. No
- 2. Yes
- 3. If yes, in which market?,....., distance?,.....

**28. After harvest, how do you package the vegetables ?**

- 1. Plastic bags     2. Bowls     3. Bag
- 4. Others .....

**29. How do you transport the vegetables to the places of sale (markets)?**

- 1. common vehicles     2. Carts     3. Motorcycles
- 4. Others .....