

# Influence of Seasons and Meteorological Parameters on the Circulation of Enteroviruses in Untreated Wastewater: A Nine-Year Retrospective Study in the Niger's Polio Program (2016-2024)

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## Abstract

Enteroviruses are responsible for a wide spectrum of infectious diseases worldwide. Understanding the meteorological factors influencing the environmental circulation of enteroviruses could help to improve the prevention strategy against diseases caused by these viral infectious pathogens. This retrospective study investigates the influence of seasonal and meteorological variables on the detection of enteroviruses in untreated wastewater across 11 collection sites in Niger from 2016 to 2024. Using data from the Poliovirus Surveillance System and national meteorological records, descriptive, bivariate, and multivariate statistical analyses were performed with R for Windows software. A total of 1989 laboratory test results were included in and then subjected to analysis for this study. According to a bivariate analysis, a significant association was found between the detection of enteroviruses in wastewater and season or rainfall or relative humidity (p-values < 0.05). According to multivari-

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ate logistic regression results, relative humidity was the primary meteorological factor that contributed to the peak of enteroviruses detection in untreated wastewater with Odd Ratio = 1.02, 95% Confidence Interval 95% 1.01 - 1.03, p-value < 0.0001. Temperature, rainfall and windspeed were not associated to positivity rate of enterovirus detection in wastewater (p-value > 0.05). These findings could help public health authorities to implement better prevention strategies by targeting the optimal intervention period.

## Keywords

Wastewater, Enterovirus, Seasons, Meteorological Parameters, Niger

## 1. Introduction

The genus Enterovirus belongs to the family Picornaviridae and consists of non-enveloped, positive-sense single-stranded RNA viruses. They belong to enteric viruses group and are classified into 15 species, Enterovirus A to L and Rhinovirus A to C. Within the genus Enterovirus (EV), seven species are pathogenic to humans, Enteroviruses (EVs) A to D and Rhinovirus (RV) A to C [1]. Evs are responsible for wide spectrum of infectious diseases. The most well-known EV is the poliovirus (PV), which belongs to EV-C specie. This virus is the causal agent of poliomyelitis or polio, whose main clinical manifestation is acute flaccid paralysis, particularly in unvaccinated individuals. The other EVs represent the group of non-polio enteroviruses (NPEV), among which several are emerging and becoming major public health concerns and increasingly recognized as pathogens causing morbidity in children [2] [3]. The transmission of diseases caused by EVs occurs mainly through the fecal-oral route by ingesting contaminated water or food. Wastewaters represent one of the main sources of contamination when discharged into the environment without proper treatment. According to the United Nations Environment Programme (UNEP) estimates, less than 5% of wastewaters in many developing countries is treated before reaching the environment [4]. Inadequate wastewater management exposes populations to significant public health risks, particularly in Africa, where sanitation and socio-economic conditions are poorer. Indeed, useful information about the circulation of EVs in the community can be obtained through untreated wastewater. This information includes the early detection of circulating infectious pathogens for better epidemic control, monitoring trends to observe the progression of these infectious diseases within populations, and evaluating public health measures to assess the effectiveness of interventions.

The African region started Environmental Surveillance (ES) of the poliovirus through untreated wastewater in 2011 in Kano State, Nigeria [5]. It was extended to other African countries including Niger, which started the ES in November 2015. ES program is coordinated by the Global Polio Laboratory Network (GPLN). Molecular analysis protocols used by GPLN can detect both PV and NPEV. In

addition to the inadequate wastewater management system in the majority of African countries, certain climatic factors could have an effect on the incidence of infectious diseases associated to Evs [6]. The climate of sub-Saharan Africa is highly variable and depends on factors such as temperature, rainfall and geography etc. In Niger, the impacts that meteorological parameters could have on dynamic circulation of enteroviruses in untreated wastewaters remain unknown. The current study aims to determine the influence of seasons and meteorological parameters on the circulation of enteroviruses (PV and NPEV) in untreated wastewater collected within the Poliovirus Surveillance System in Niger.

## 2. Materials and Methods

### 2.1. Study Design and Framework

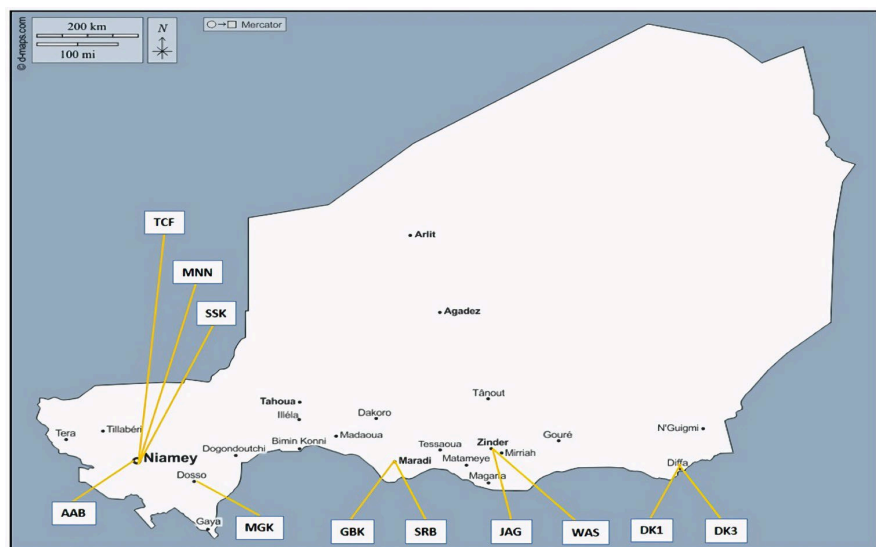
Niger is a landlocked country in sub-Saharan Africa. The country's area is 1,267,000 km<sup>2</sup>. According to data from the National Institute of Statistics, the population was estimated to be 19,865,068 people in 2016 ([https://stat-niger.org/wp-content/uploads/2020/06/TBS\\_2016.pdf](https://stat-niger.org/wp-content/uploads/2020/06/TBS_2016.pdf)) and 25,369,414 people in 2023 ([https://stat-niger.org/wp-content/uploads/tbs/tbs\\_2024.pdf](https://stat-niger.org/wp-content/uploads/tbs/tbs_2024.pdf)). The country is located in West Africa, between the parallels of 11° 37' and 23° 33' north latitude, and the meridians of 16° east and 0° 10' west longitude. Niger has a Sahelo-Saharan climate, which is characterised by high temperatures and low annual rainfall. The climate varies from desert in the north of the country, to semi-arid tropical landscape in the centre, and humid tropical climate in the far south. This retrospective study covered the period from 2016 to 2024 within the framework of environmental surveillance of poliomyelitis in Niger. Environmental surveillance of poliomyelitis was implemented in November 2015, initially with 4 operational sites in Niamey, the country's capital and was extended in 2016 with the addition of 4 new sites in Maradi and Diffa. Zinder region was included in the ES system with the opening of 2 sites in 2019 year, then Dosso region with the opening of one site in 2023 year. This makes a total of 11 open sites distributed across 5 regions of the country (**Figure 1**).

In practice one liter (L) of untreated wastewater was collected by grab sampling method from each site twice a month. For each collection, the samples were transported to the CERMES Environmental surveillance Laboratory at 4°C for laboratory analysis. Approximately 500 mL of collected samples were processed using the PEG-6000/Dextran concentration method, in accordance with the Global Polio Eradication Initiative protocol [7]. The concentrates were then sent to the World Health Organisation (WHO) Polio Regional Reference Laboratory of Ghana, where tests were carried out to viral isolation, detection and identification of PV and NPEV. Laboratory results were sent to the country every week by the Polio Regional Reference Laboratory.

### 2.2. Laboratory Data Collection

Laboratory results were stored in the databases of the Nigerien Ministry of Public

Health and WHO. To create the database for this study, these two repositories were used to ensure the similarity of the data. Were included in the study database, all wastewater samples correctly processed and identified for which the collection date and laboratory results were available. All duplicates, inconsistent laboratory results between the two repositories, and samples whose processing procedures and identifiers did not comply with WHO standards were excluded from the study database.



**Figure 1.** Localization of the 11 wastewater collection sites in Niger. The collection sites were Musée National du Niger (MNN), Stade Seyni Kountché (SSK), Ancienne ambassade de Belgique (AAB), Teminus Corniche Fleuve (TCF), Mangué Koira (MGK), Gao Barki (GBK), Soura Bildi (SRB), Jaguindi (JAG), Washington (WAS), Diffa Koura 1 (DK1) and Diffa Koura 3 (DK3).

### 2.3. Meteorological Data Collection

The meteorological data were obtained from the National Meteorology Directorate. Daily data were obtained from meteorological stations covering the locations of wastewater collection sites throughout the study period. These data refer to the maximum and minimum temperature and relative humidity in degrees Celsius ( $^{\circ}\text{C}$ ) and percentage (%) respectively, on the day of wastewater collection; Wind speed in meter per second (m/s) and rainfall in millimeter (mm) on the day of wastewater collection. Wastewater collections were carried out early in the morning at specific times, and it could rain or be windy after sample collection on the same day. This would have no impact on the detection of enteroviruses in untreated wastewater. In order to take this bias into account, average rainfall and average wind speed were calculated based on data from the day of wastewater collection (J0) and the day before (J-1). In Niger, the specific months associated with the rainy season are June, July, August, and September. The months of October to February are associated with the dry and cold season. The months of March to May are associated with the dry and hot season. The seasons were defined accord-

ing to the associations with the months mentioned above.

## 2.4. Statistical Analysis

The data concerning measured variables were collected over 9 years (2016-2024). Data were aggregated to generate a single data file. The coding of variables and descriptive analysis were carried out using this table. Descriptive, bivariate and multivariate logistic regression analyses were performed using R for Windows version 4.4.3 software, while the graphs were generated using Microsoft Excel 2021. The trend test for enterovirus positivity rate was determined using Epi Info™ 7 (chi-squared for trend). A p-value  $\leq 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Descriptive Analysis

A total of 1989 wastewater samples collected between 2016 and 2024 as part of the Polio surveillance program were included in this study. The descriptive analysis allowed to calculate absolute and relative frequencies of the measured variables. The median, mean, minimum, and maximum were also calculated for each meteorological parameter. Results were summarized on **Table 1**.

**Table 1.** Descriptive statistics results.

Characteristics	n (%)	Mean	Median	Minimum	Maximum
<b>Year (N = 1989)</b>		NA	NA	NA	NA
2016	152 (7.6%)				
2017	207 (10%)				
2018	211 (11%)				
2019	239 (12%)				
2020	160 (8.0%)				
2021	200 (10%)				
2022	255 (13%)				
2023	273 (14%)				
2024	292 (15%)				
<b>Region (N = 1989)</b>		NA	NA	NA	NA
Niamey	857 (43%)				
Maradi	409 (21%)				
Diffa	419 (21%)				
Zinder	264 (13%)				
Dosso	40 (2.0%)				

## Continued

Seasons (N = 1989)		NA	NA	NA	NA
Rainy	676 (34%)				
Dry and hot	485 (24%)				
Dry and Cold	828 (42%)				
<b>Average temperature</b>	1989 (100%)	29.4	29.7	18.6	38.8
<b>Average humidity</b>	1989 (100%)	42.8	37.0	5.5	92.5
<b>Average wind</b>	1989 (100%)	3.2	3.2	0.3	8.2
<b>Average rainfall</b>	1989 (100%)	1.3	0.0	0.0	36.9

N = Number of samples tested; n = Percentage of samples tested; NA = Not applicable.

### 3.2. Bivariate Analysis

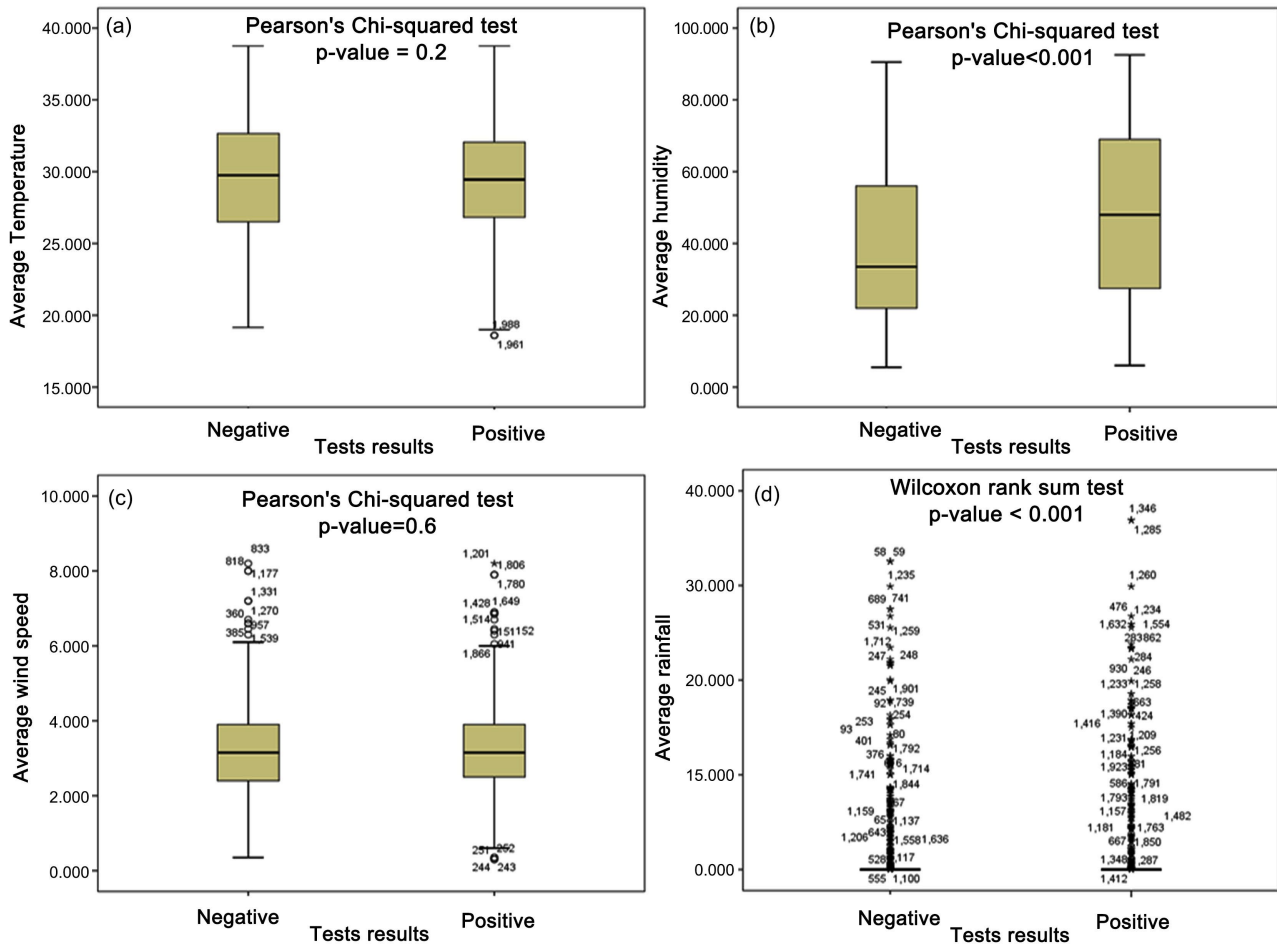
Thus, 1198 wastewater samples tested negative for the presence of enteroviruses, while the number of samples that tested positive was 791. A bivariate analysis (Pearson's Chi-squared test) was conducted and a significant association was found between the detection of enteroviruses in wastewater and the region or year covered by this study (p-values < 0.001). The findings also demonstrated a significant association between the detection of enteroviruses in wastewater and season or average humidity with p-values < 0.001 for each variable. This was not the same results for average temperature and wind speed variables with p-values of 0.2 and 0.6 respectively. The data on rainfall (daily and the day before) were paired, and the differences between the pairs followed an abnormal distribution. Consequently, Wilcoxon rank sum test (significant if p-value < 0.05) was performed for the numerical variable ("Rainfall average") to compare the medians between enterovirus negative and positive results. Results showed that there was a significant difference between these median values (p-value < 0.001). Results were summarized on **Table 2**.

To better illustrate the relationships between each measured variables (Average relative humidity, rainfall, temperature and wind speed) and the detection of enteroviruses in wastewaters, graphs were generated (**Figure 2**).

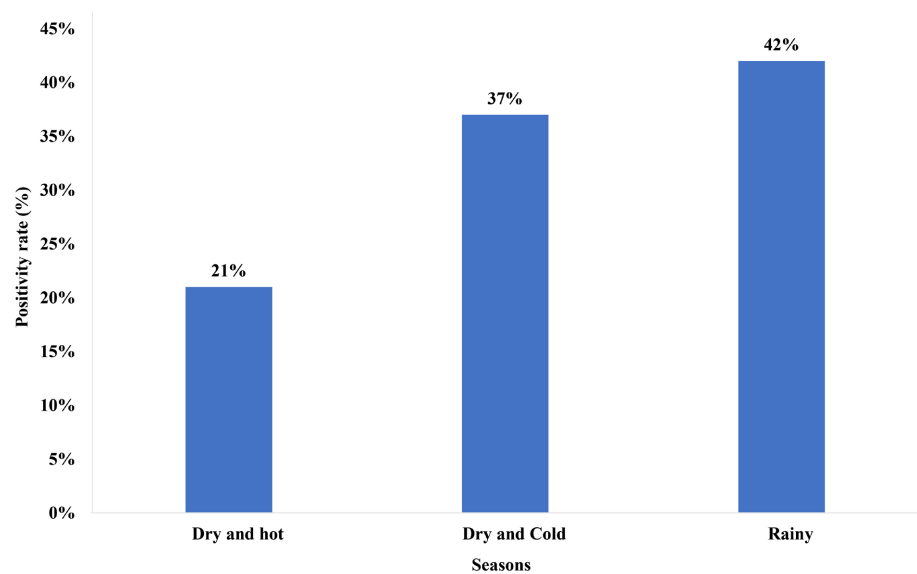
The box plot of positive samples was higher for average humidity, suggesting a clear tendency toward higher values compared to negative cases. The median temperatures and wind speed for enterovirus-negative and enterovirus-positive results seemed similar, suggesting that these two parameters did not differ significantly between the two groups.

Also, a histogram was generated to illustrate the positivity rate according to the season. High positivity rate was observed during rainy season (**Figure 3**).

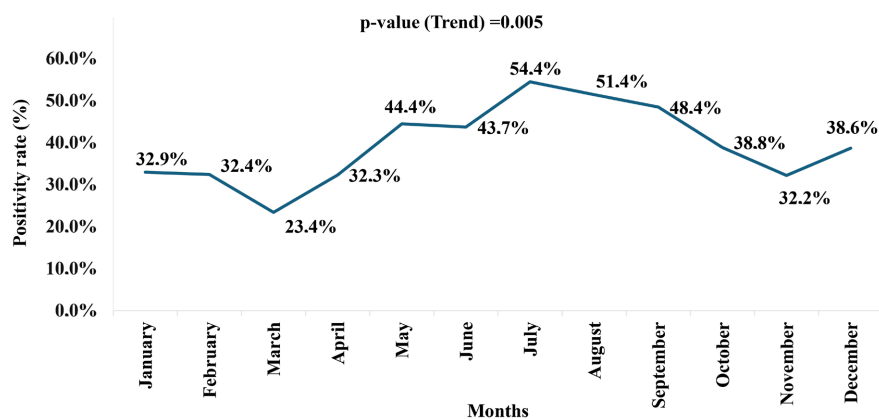
Then, a trend test was carried out to determine the rate of enterovirus positivity over months during the study's period. A significant trend test (p-value = 0.005) was obtained after statistical analysis and results showed that the peaks of positivity rate were observed between July and August (**Figure 4**).



**Figure 2.** Relationships between measured variables and the detection of enteroviruses in untreated wastewaters. Box plots comparing the (a) average temperature; (b) average humidity; (c) average windspeed for negative and positive results; (d) dot plots comparing the average rainfall for negative and positive results.



**Figure 3.** Enterovirus positivity rate according to the season.



**Figure 4.** Evolution of positivity rate over months.

**Table 2.** Bivariate analysis results.

Variables	N	Enteroviruses		p-value*
		Negative	Positive	
		N = 1198	N = 791	
		N (%) / Median (IQR)	N (%) / Median (IQR)	
<b>Years</b>	1989			<0.001
2016		98 (8.2%)	54 (6.8%)	
2017		115 (9.6%)	92 (12%)	
2018		160 (13%)	51 (6.4%)	
2019		162 (14%)	77 (9.7%)	
2020		102 (8.5%)	58 (7.3%)	
2021		152 (13%)	48 (6.1%)	
2022		146 (12%)	109 (14%)	
2023		157 (13%)	116 (15%)	
2024		106 (8.8%)	186 (24%)	
<b>Regions</b>	1989			<0.001
Niamey		553 (46%)	304 (38%)	
Maradi		165 (14%)	244 (31%)	
Diffa		293 (24%)	126 (16%)	
Zinder		164 (14%)	100 (13%)	
Dosso		23 (1.9%)	17 (2.1%)	
<b>Seasons</b>	1989			<0.001
Rainy		340 (28%)	336 (42%)	
Dry and hot		321 (27%)	164 (21%)	
Dry and Cold		537 (45%)	291 (37%)	
<b>Average temperature</b>	1989	29.8 (19.6)	29.5 (20.2)	0.2
<b>Average humidity</b>	1989	33.5 (85.0)	48 (86.5)	<0.001
<b>Average wind</b>	1989	3.2 (7.9)	3.2 (7.9)	0.6
<b>Average rainfall</b>	1989	0.0 (32.6)	0.0 (36.9)	<0.001

\*p-value: Pearson's Chi-squared test; Wilcoxon rank sum test for Average rainfall, N = Number of samples tested; n = Percentage of samples tested; IQR = Interquartile range.

### 3.3. Multivariate Logistic Regression Analysis

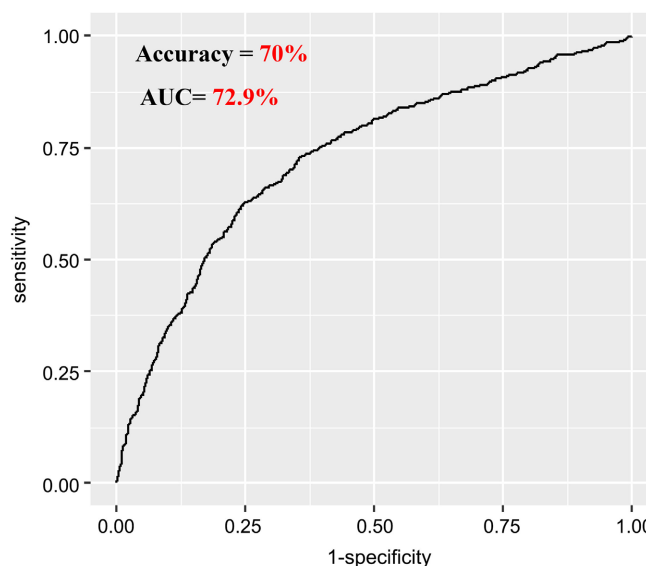
A multivariate logistic regression analysis was then conducted to assess the independent influence meteorological parameters on enterovirus detection in untreated wastewater. The Odds Ratio (OR), confidence interval (CI) and p-value were determined. For categorical variables, Year 2016, rainy season and Niamey region were fixed as reference. Multivariate analysis showed that, Average Humidity (OR = 1.02, [1.01; 1.03],  $p < 0.0001$ ), Maradi region (OR = 2.91, [2.23; 3.81],  $p < 0.0001$ ) and years 2024 (OR = 4.17, [2.69; 6.53],  $p < 0.0001$ ), 2023 (OR = 1.61, [1.04; 2.50],  $p = 0.034$ ), 2022 (OR = 1.62, [1.04; 2.52],  $p = 0.034$ ) and 2017 (OR = 1.71, [1.08; 2.70],  $p = 0.022$ ), were associated with higher rates of Enterovirus detection in wastewater. The years 2018 (OR = 0.61, [0.38; 0.98],  $p = 0.043$ ), 2021 (OR = 0.6, [0.37; 0.97],  $p = 0.04$ ), Diffa region (OR = 0.73, [0.55; 0.95],  $p = 0.022$ ) were associated with lower rates of Enterovirus detection in wastewater. The other variables were not associated with the rate of Enterovirus detection of enterovirus in untreated wastewater ( $p > 0.05$ ). Results were summarized in **Table 3**.

**Table 3.** Multivariate logistic regression results.

Variables	OR*	95% CI**	p-value
<b>Years</b>			
2016	—	—	
2017	1.71	1.08, 2.70	<b>0.022</b>
2018	0.61	0.38, 0.98	<b>0.043</b>
2019	0.95	0.60, 1.50	0.8
2020	1.28	0.78, 2.09	0.3
2021	0.6	0.37, 0.97	<b>0.04</b>
2022	1.62	1.04, 2.52	<b>0.034</b>
2023	1.61	1.04, 2.50	<b>0.034</b>
2024	4.17	2.69, 6.53	<b>&lt;0.001</b>
<b>Regions</b>			
Niamey	—	—	
Maradi	2.91	2.23, 3.81	<b>&lt;0.001</b>
Diffa	0.73	0.55, 0.95	<b>0.022</b>
Zinder	1	0.73, 1.36	>0.9
Dosso	0.54	0.27, 1.07	0.081
<b>Seasons</b>			
Rainy	—	—	—
Dry and hot	0.72	0.50, 1.04	0.077
Dry and Cold	0.83	0.60, 1.17	0.3
<b>Average temperature</b>	1.01	0.98, 1.04	0.5
<b>Average humidity</b>	1.02	1.01, 1.03	<b>&lt;0.0001</b>
<b>Average wind</b>	1.02	0.93, 1.12	0.7
<b>Average rainfall</b>	1	0.97, 1.02	>0.9

\*OR = Odds Ratio; \*\*CI = Confidence Interval.

The performance indicators of the model used were Accuracy = 70% and the Area Under the Curve (AUC) = 72.9% (**Figure 5**).



**Figure 5.** Performance of the multivariable logistic regression mode.

#### 4. Discussion

Niamey is the capital of Niger and the largest city, producing the largest amount of discharged wastewater. This justifies the four collection sites included in this study. The collection of samples at the four sites in the city of Niamey was carried out on different days. This would reduce the bias related to the generalizability of meteorological correlations. Niamey region has the largest number of analysed and positive samples in this study, followed by Maradi, Diffa, Zinder, and Dosso (**Figure S1**). The Niamey region also has the highest population density followed by Maradi region. This could explain the significant association found between the detection of enteroviruses in wastewater and the region. Moreover, several factors could contribute to the fluctuations in the positivity rate across different years (**Figure S2**). Among these factors were the low number of samples tested at the beginning of the ES implementation in the country, the decrease of ES activities during the COVID-19 pandemic, the surge in environmental cases of type 2 vaccine-derived poliovirus during the years 2022 and 2024. These factors could explain the association found between the year and the detection of EVs in untreated wastewaters.

A predictive study revealed that in certain localities, climate change could increase enterovirus outbreak peaks by up to 40% [8]. Consequently the prevalence of enteroviruses in surface waters, including wastewater could depends on the specifics of climate according to different regions of the world. In temperate climate areas, there is seasonality in high detection rates of enteroviruses through wastewater, with a peak observed in summer [9]. As illustrated, some studies in Italy showed that there was a high percentage of positivity for detecting enterovi-

ruses in wastewater during the spring and summer [10] [11]. In a study conducted in India, the annual peaks of EV cases in wastewater and clinical samples occurred at the end of summer or early fall [12]. According to a study conducted in United States, the seasonality of positivity for enterovirus detection in wastewater varied depending on enterovirus species (EV-A to D) [13]. Other studies had shown that there was a significant association between the water contamination by EV and heavy rainfall [14] [15], which could lead to severe illnesses and deaths. But in Africa, some studies that have addressed this topic have revealed that there was a significant association between rainfall and the detection of enteroviruses in surface water [15] [16]. In Niger, most of ES wastewater drainage sites were open systems, and there was poor implementation of hygiene and sanitation infrastructure in the areas surrounding these sites. This implied that enterovirus contamination would particularly be higher in wastewater during the rainy season because of environmental or climatic factors such as runoff or flooding. Runoff water from these sites was usually untreated and released into the environment, posing a risk of contaminating irrigation and drinking water. The contamination could also be exacerbated by open defecation, which is widely practiced in sub-Saharan Africa, with negative consequences for the health and well-being of populations [17]. Furthermore, the period of high humidity occurs during the country's rainy season. Based on the results obtained in this study, this context could explain the finding of a significant association between the detection of enteroviruses in untreated wastewater and the rainy season, rainfall or humidity according to bivariate analyses.

Indeed, temperature could be one of the main factors of adaptation, leading to increased thermotolerance of some adapted enteroviruses at 30°C [18]. According to a study conducted in Nigeria, univariate analysis showed that the detection of enteroviruses in wastewater was associated to high temperatures ( $\geq 27^{\circ}\text{C}$  vs.  $< 22^{\circ}\text{C}$ ) [19]. In a study conducted in Ivory Coast, enteroviruses were detected in lagoon water at temperatures ranging from 26°C to 30°C [20]. A study conducted in the United States of America (USA) showed that temperature and dew point were strongly correlated to the seasonal detection of some enteroviruses in wastewater [21]. This study conducted in USA revealed that warm and humid climates lead to earlier outbreaks and that virus survival would be prolonged in densely populated urban areas. Niger is a country with a consistently warm climate throughout the year, compared to countries in temperate regions. Consequently, humidity, which varies greatly throughout the seasons, would be the main weather factor influencing the detection of enteroviruses in wastewater. Multivariate logistic regression results, obtained in our study, seemed to support that.

Also current research in Africa on wastewater surveillance of enteroviruses does not show a direct role of wind speed in viral detection. Results obtained in our study would support this trend. Wind may indirectly influence detection only through its effect on surface water mixing or aerosolization resulting in the contamination of nearby people [22], but this is not a primary factor in wastewater-based epidemiology of enteroviruses.

## 5. Conclusion

Monitoring enteroviruses through untreated wastewater revealed a seasonal peak in environmental circulation in Niger, along with some associated climatic parameters. Relative humidity is the main climatic factor that would influence the detection of enteroviruses in wastewater in Niger. This finding provides a better understanding of the dynamics of enterovirus circulation within communities. The study showed that environmental surveillance of EV could be a useful tool to help public health authorities develop better prevention strategies. These strategies include vaccination and raising awareness among populations about maintaining good practices for water, sanitation, and hygiene (WASH), while targeting the most effective time for intervention.

## Ethical Approval

Environmental surveillance of poliovirus was implemented in November 2015 with the authorization of the Ministry of Public Health. Approval from the ethics committee is not required.

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## Authors Contributions

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- **Supervision of environmental surveillance activities:** Rakia Boubacar, Goubékoye Boubacar, Mohamed Bargaz, Bintou Kiari, Hamidou Harouna, Daouda Chaïbou Gallo, Idrissa Moussa Barazé and Issifi Kollo Abdoukader.
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- **Critical analysis of the manuscript:** Ousmane Diouf, Haoua Seini Sabo, Adamou Lagaré.

## Conflicts of Interest

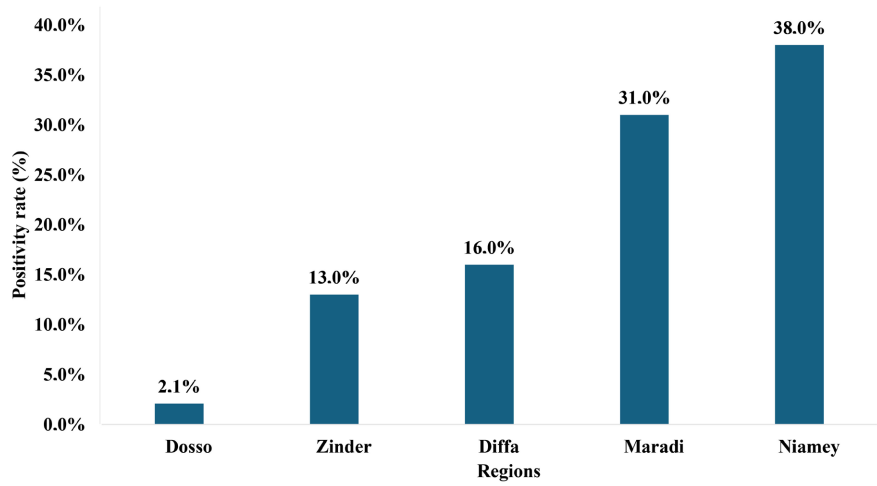
The authors declare no competing interests.

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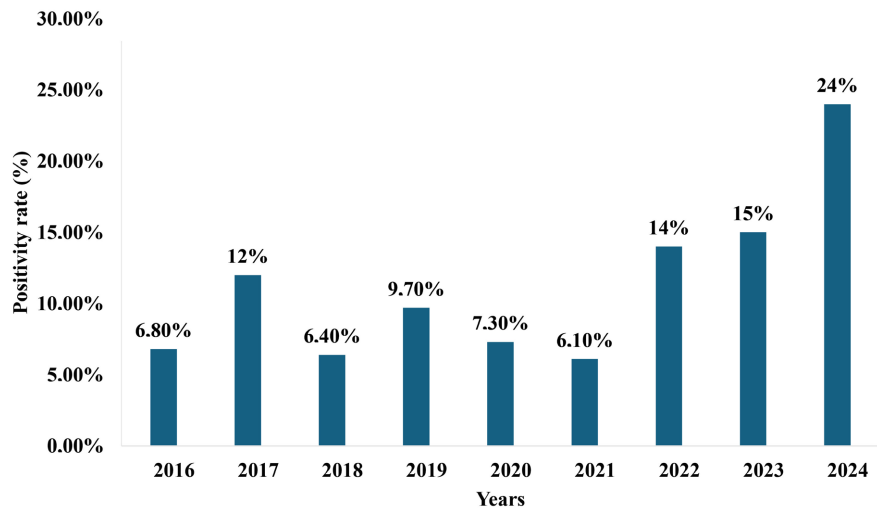
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### Supplemental Files



**Figure S1.** Positivity rate according to the regions.



**Figure S2.** Positivity rate over years.