Analysis of the impact of African dust storms on the presence of enteric viruses in the atmosphere in Tenerife, Spain

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Abstract

Airborne viruses and their relation to dust storms, as a possible route for dispersion, has not been widely investigated. There are, however, studies that have described airborne dispersal of pathogenic viruses and their potential impact on public and agronomical health. Atmospheric samples were collected in an urban area of Tenerife during 2009, 2010, 2012 and 2013; and screened for the presence of enteric viruses using PCR and sequencing. The potential relationship of viral data with African dust storms and other climatic variables (seasonality, origin of the air mass and PM levels) was analyzed. Enteroviruses and Rotaviruses were detected in 15.4% (20/130) and 36.9% (48/130) of the samples, respectively. No significant statistical relationships were observed with African dust storms or the origin of the air masses, although higher percentages of positives were obtained for dust days. Enterovirus detection was significantly linked to warmer seasons and PM2.5 levels showed an inverse correlation to Rotavirus presence. This is the first multi-year report to describe the presence of Enterovirus and Rotavirus genetic sequences in air samples collected in an outdoor urban environment. The data illustrates the need for source region sampling to determine links and the influence of weather, climatic and regional wind patterns on long-range atmospheric dispersion of viruses in future research efforts.

Keywords: Enteric viruses, Canary Islands, African dust storms, Airborne dispersion, Particulate matter

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INTRODUCTION

Dust storms are a climatic phenomenon, originating in arid and desert regions of the planet and are the main source of atmospheric dust on Earth (Middleton and Goudie, 2001). On a global scale, the primary source region is the Sahara – Sahel, which is believed to transmit more than 50% of all aerosolized dust to Earth's atmosphere at an estimated range of 400 to 2,200 Tg · year^{-1} (Huneeus *et al*., 2011). Atmospheric dust carries diverse materials, including minerals, anthropogenic aerosols, and microorganisms that may be aerosolized from soils and congregated from various ecosystems during dust cloud movement. Bacterial concentrations in soil samples have been estimated in ≈ 10^6 microorganisms per gram (Kellogg and Griffin, 2006). Fungal concentrations may be at a similar magnitude, and virus concentrations are typically one to two orders of magnitude lower than bacterial concentrations (Gonzalez-Martin et al., 2013; Griffin, 2007). Most microorganisms are probably only transported relatively short distances due to their attachment to large soil particulate matter (PM) and many others may lose their viability during transport due to physical sources of stress (ultraviolet exposure, dehydration, etc.) (Griffin *et al*., 2011). However, some are able to resist adverse conditions experienced during transport and reach new niches many kilometers away (Hara *et al*., 2015). Knowledge about Saharan air mass transportation routes and the possible dispersion of microorganisms has driven studies about the potential relationship between dust storm events and the spread of pathogens (Gonzalez-Martin *et al*., 2014).

Among microorganisms, viruses may be more easily aerosolized due to their smaller size and potential attachment to finer sized particulate matter, but they are also very sensitive to adverse conditions during transport. For example, UV radiation has been identified as an effective way to inactive viral particles (Tseng and Li, 2005), but it

does not eliminate the possibility to detect their genomes, as previously demonstrated by Lee and colleagues. They tested an aerosolized vaccine with inactivated Influenza virus and the viral RNA was easily identified by regular molecular biology methods (Lee *et al.*, 2011) . So, there is, however, airborne dispersal of viruses. There are a large number of viruses that have been detected in atmospheric samples, such as the Newcastle virus (Hietala *et al*., 2005) that infects poultry; or the porcine viruses PRRSV (Porcine Reproductive and Respiratory Syndrome Virus) and SIV (Porcine Influenza Virus) (Hermann *et al*., 2006; Weesendorp *et al*., 2008). These viruses have been studied to determine their dissemination routes inside of farms to limit substantial economic losses. In the eighties, transmission and dispersion of FMDV (Foot – and – Mouth Disease Virus) throughout Europe became a huge problem, and Gloster demonstrated that under certain circumstances, long – range (~60 km) atmospheric transmission of the virus was possible and it was recently demonstrated using statistical methodology (Gloster *et al*., 2005; Sanson *et al*., 2011). Research in Asia have indicated a similar trend of airborne dispersion of FMDV in Korea and Japan and the potential relationship with dust storms emanating from Asian deserts (Joo et al., 2002; Ozawa et al., 2001; Sakamoto and Yoshida, 2002). Studies that have shown that some viruses are still viable after extended long-range transport have been recently reviewed (Gonzalez-Martin *et al*., 2014).

Regarding human health, pathogenic viruses like Enteroviruses or Adenoviruses, whose mode of transmission is the fecal – oral route, have been isolated from aerosols sampled within hospital facilities (Tseng, 2010). Although to-date there is no confirmation of their capacity to be transported long distances, there are studies on Enterovirus outbreaks that point to the atmosphere as route of transmission (Ho *et al*., 1999; Lin *et al*., 2002). Other viruses responsible for epidemics have been investigated

to identify its routes of dispersion. The Severe Acute Respiratory Syndrome (SARS) was found in air samples taken inside a clinic where infected individuals were hospitalized (Booth *et al*., 2005). Influenza viruses have been identified in aerosolized particles in a hospital emergency department, whose size was within the "breathable" fraction (Blachere *et al*., 2009) and other hospital environments (Tseng, 2010). Respiratory Syncytial Virus (RSV) has been isolated from air filters in a children's daycare facility (Prussin *et al*., 2016). Moreover, recent studies have addressed possible long – range transmission of these types of virus. Chen detected Ambient Influenza and Avian Influenza viruses in air samples collected from farms and a year later they confirmed their presence in Asian dust storms impacting Taiwan (Chen *et al*., 2009, 2010).

The Canary Islands are a Spanish archipelago formed by seven main islands, located between 100 – 500 km off the West Coast of North Africa and they are frequently affected by African dust storms (Dorta et al., 2002). To date, studies conducted on the microbiological aspects associated with dust storms in the Canary Islands are practically nonexistent, although there are some reports about the potential consequences on human health such as possible correlations with elevated rates of allergies and asthma (Garcia-Carrasco *et al*., 2001; Sanchez-Lerma *et al*., 2009). In addition, previous studies have focused on the idea of the airborne route as an alternative dispersion of the human enteric viruses (Dennehy, 2000; Tseng, 2010; Thornley *et al*., 2011; Wan *et al*., 2012). These data initiated our interest in the research presented here whose focus was the presence ofenteric viruses in the atmosphere over Tenerife during several years of study and their potential sources. The two main objectives were: to detect and identify viral genomes of enteric viruses (Adenovirus, Enterovirus, Rotavirus and Norovirus) present in the air during different atmospheric

conditions (with and without the influence of African dust); and to analyze possible correlation between the results observed and the climatic variables (frequency of dust storms, origin of air masses, PM levels).

METHODS

Air sampling

Air samples were collected on the roof of the University Institute of Tropical Diseases and Public Health of the Canary Islands (≈550 masl.; 28º28'43.71''N; 16º19'17.27''O). This facility is in an urban area within the center of San Cristobal of La Laguna city, located close to Tenerife North Airport. Two different air samplers were used, a Mattson – Garvin sampler (Barramundi Corporation, Homosassa, FL, USA) and an Omni 3000 (Innovaprep, Drexel, MO, USA). The switch to the Omni 3000 was due to a malfunction of the Mattson-Garvin unit. The Mattson – Garvin sampler used a 140 mm Petri dish containing 45 mL of glycine buffer, pH 6.5. Sampling was performed continuously for two hours at flow rate of 28,2 L min^{-1} resulting in the screening of 3,396 L of air. Afterwards, the remaining volume of glycine buffer, ranging from 30 – 40 mL, was transferred aseptically to a 50 mL sterile tube. The Omni 3000 (Innovaprep, Drexel, MO, USA) collects air samples at a flow rate of 300 L min^{-1}, using cartridges containing 10 mL of distilled water. Sampling was performed for 20 min, which resulted in the screening of 6,000 L of air. All samples were kept under refrigeration until processed and the time between sampling and processing never exceeded 72 hr.

Samples concentration

Concentration of air samples was performed through centrifugation at 5000 X g for 15 min in a Heraeus® Multifuge® 1 L – R (Thermo – Scientific, Waltham, MA, USA) using Macrosep® Advance Centrifugal 30 K tubes obtaining a final volume of ≈

300 μL (PALL, Port Washington, NY, USA). Samples collected with the Mattson - Garvin were split into two sub – samples (≈ 20 – 25 mL each) for the centrifugation step and the recovered volume was later unified (≈ 500 μL).

Nucleic acid extraction

The QIAamp® Ultrasens® Virus kit (Qiagen, Valencia, CA, USA) was used as it allows the joint extraction of RNA and DNA. The protocol was performed following the manufacturer instructions. Quantification of nucleic acids was conducted using a NanoDrop ND – 1000 (Thermo – Scientific) spectrophotometer, and ranged from 8 – 228 ng μL^{-1} for DNA and from 6 – 130 ng μL^{-1} for RNA.

PCR and sequencing

Samples were screened for Human Adenoviruses, Enteroviruses, Rotaviruses and Noroviruses. The Adenovirus primer target was the hypervariable region 1 – 6 (HVR_{1-6}), based on Lu and Erdman (2006). Enterovirus detection was based on the amplification of the untranslated region UTR – 5' (Casas et al., 1997). Rotavirus identification was performed using a VP6 gene target (Gray and Iturriza-Gómara, 2011). Prior to amplification, the rotavirus sample aliquot was mixed with DMSO to a final concentration of 7%, heated at 97ºC for 5 min and chilled afterwards in an ice-ethanol bath to achieve the complete denaturalization of double stranded RNA (Gouvea *et al.*, 1990; Iturriza-Gómara *et al.*, 1999). Detection of Noroviruses was performed by targeting the RNA polymerase gene, following Moe and Wang publications (Moe et al., 1994; Wang et al., 1994). Primer sequences are detailed on Table S1 of Supplementary Material. QIAquick® PCR Purification kit (Qiagen, Valencia, CA, USA) was used to purified amplicons of expected band sizes and were later sequenced in a Genetic Analyzer Applied 3500 (Life Technologies, Carlsbad, CA, USA) using a Big Dye Terminator V 3.1 sequencing (Life Techonologies, Carlsbad, CA) kit. Sequences were

analyzed with MEGA software and screened within the GenBank database using BLAST software from NCBI.

Climatic variables

African dust intrusions were monitored over four different years (2009, 2010, 2012 and 2013) using the forecasts provided by AEMET (Spanish Meteorological Agency) based on climatic models: NAAPS, BSC– DREAM8b V2.0, Skiron, and ECMWF (CALIMA, 2013). Origin of all air masses sampled was verified using the same forecast models as well as the retro-trajectories provided by the HYSPLIT model. Air masses trajectories were classified into three categories, Africa, Marine and European. Africa corresponded to those aerosols that originated and whose trajectories crossed part of the North African continent. Marine air masses had a predominant trajectory over the Atlantic Ocean; and European's were considered those European and Marine aerosols, whose trajectories crossed the European continent and the Atlantic Ocean. PM values were obtained from the Network for the Control and Surveillance of Air Quality in the Canary Islands (Canary Islands Government, 2013).

Statistics

Comparison between viral results and climatic variables was performed using IBM-SPSS Statistics (version 20.0) software using a level of significance of $p < 0.05$. Values of quantitative variables did not have a normal distribution; therefore, they were expressed as median and rank (minimum and maximum values). Values of qualitative variables were expressed as frequency and percentage, and comparisons were made using Pearson's Chi-squared test. Fisher's exact test was used in those cases were the expected frequency was lower than five.

RESULTS AND DISCUSSION

196
197 The study of long-range atmospheric transmission of microorganisms is an
198 emerging field of research. Recent interest regarding their capacity to survive long-
199 range dispersion, the possibility of airborne metabolic activity, their role in atmospheric
200 nucleation and the significant role that dust storms serve as a vector has fostered a
201 number of scientific endeavors (Amato *et al*., 2005; Bowers *et al*., 2011; Gonzalez-
202 Martin *et al*., 2014; Griffin, 2007; Joly *et al*., 2013; Schuerger *et al*., 2013). However,
203 airborne viral composition in open – field studies is still a lightly explored field. Here,
204 we investigated the presence of enteric viruses in the outdoor atmospheric environment
205 and the possible influence of African dust storms in the Canary Islands. Sixty – five
206 samples were collected with Mattson – Garvin sampler during 2009 – 2010 and another
207 sixty – five were collected using the Omni 3000 during 2012 and 2013, generating a
208 total of one hundred and thirty air samples. Detailed information about positive samples
209 is shown in Table 1.

210 ***Viral results***

211 Two out of the four enteric viral groups analyzed were detected in the air
212 samples collected, Enteroviruses and Rotaviruses. Regarding Enteroviruses, 15.4%
213 (20/130) of the samples were classified within the *Picornavirales* order. Eleven positive
214 samples were collected with the Mattson – Garvin sampler (55%, 11/20) and the other
215 nine with the Omni 3000 sampler (45%, 9/20). Nineteen out of the twenty positive
216 samples were identified within the *Enterovirus* genus, and among them, ten samples
217 corresponded to Enterovirus A, five to Enterovirus B and two to Enterovirus C.
218 Although one sample could not be classified as Enterovirus due to their low identity
219 value to the GenBank results, in all cases, the closest matches were among the
220 Enterovirus genus.

Previous research has studied aerial dispersion of Enteroviruses, but mostly in indoor environments (Couch et al., 1970; Dick et al., 1987; Jennings et al., 1988; Meschievitz et al., 1984; Myatt et al., 2003). Several studies were conducted in the 1970's in which air samples from wastewater-irrigated fields were screened using culture-based and fluorescent antibody methods and different Enteroviruses species were identified, to include Echovirus 7 and Coxsackievirus type B (Teltsch et al., 1980; Teltsch and Katzenelson, 1978). In 2010, Tseng detected Enteroviruses in a pediatric area of a hospital in Taiwan at similar rates as observed in this study, 15% (n=33) (Tseng, 2010).

Forty-eight samples out of the 130 (36.9%) were positive for Rotaviruses and twenty-nine of them (29/48) were identified as Rotavirus A. Thirty three (50.8%, 33/48) of the positives were samples collected with the Mattson – Garvin sampler and fifteen (23.1%, 15/48) with the Omni 3000. Seven samples (7/130) were simultaneously positive for both targets (Enterovirus and Rotavirus), five of them collected with the Mattson – Garvin sampler and two with the Omni 3000 sampler. Sequences over 200 bp were submitted to GenBank (Ac. No. KU821663 – KU821677).

Regarding Rotaviruses, reports are scarcer in relation to their airborne dispersal. A recent study in which air samples were collected in hospital rooms with Rotavirus infected children, 75% of samples (n=61) were positive for the pathogen's genome. This data indicates that the airborne route could be an important way of dispersion and would explain the explosive outbreaks that this virus is known to cause (Dennehy, 2000; Dennehy et al., 1998). Nevertheless, until this date, no study has been published regarding the presence of this viral group in outdoor atmospheric environments. However, despite identifying this group of viral genomes in the air samples described in this report, the issue of their viability, and therefore their potential pathogenicity,

246 remains unsolved and will be addressed in future efforts. If viability were demonstrated
247 for the viral groups detected in this study, then co – presence would indicate an increase
248 in health risk if the viruses where proven to be pathogenic. Considering this issue, Ijaz
249 and colleagues performed several indoor atmosphere studies focused on the influence of
250 climatic conditions on Rotavirus survival (Ijaz et al., 1994, 1985). They found an 80%
251 survival of airborne Rotaviruses when temperatures were ~20ºC and relative humidity
252 (HR) was 50%. Mean values of temperature and HR in Tenerife are 21.2ºC and 63%,
253 respectively, (AEMET, 2014) which is suitable for Rotavirus atmospheric survival.
254 Furthermore, a prediction model has been recently developed to assess hospitalizations
255 due to Rotavirus gastroenteritis (Hervás et al., 2014). In that study, association of
256 different climatic variables with Rotaviruses was evaluated and a positive correlation to
257 atmospheric pressure was noted (higher pressures were related to higher viral
258 prevalence). The authors theorized that elevated atmospheric pressure decreases the
259 mobility of airborne particles, favoring their settlement. Additionally, since high
260 pressure decreases evaporation, resulting humidity levels would favor survival of
261 viruses (Hervás et al., 2014). Accordingly, the frequent influence of the Azores High
262 over the Canary Islands archipelago (Dorta Antequera, 1996) could enhance
263 atmospheric survival for Rotaviruses.

264 Positive samples for Enteroviruses and Rotaviruses were detected from both
265 samplers used, but differences were noticed. Statistical analyses showed a positive
266 correlation between the Mattson – Garvin sampler and Rotavirus detection, which may
267 be due to the glycine buffer used. Glycine buffer could act as a better preservative for
268 Rotaviruses (or genomes) than distilled water utilized by the Omni 3000.

269 Regarding Adenoviruses and Noroviruses, both have been detected in previous indoor
270 studies and the airborne route has been considered in multiple gastrointestinal outbreaks

(Aziz, 2010; Kuo et al., 2009; Marks et al., 2003, 2000; Moon et al., 2013; Nenonen et al., 2014; Said et al., 2008; Tseng, 2010; Wan et al., 2012; Xu et al., 2013). Only one publication has reported the detection of Norovirus genomes in an outdoor environment (Brooks et al., 2005). The study analyzed the influence and risks from aerosols generated during the application of biosolids and three out of 350 air samples collected were positive for Noroviruses; however, no positive sample was detected further than 5 m from the application sites.

Climatic variables and viral data comparison

While differences in the frequency of viral detection between variables were noted, statistical analysis indicated that the observation were heterogeneous in nature. Considering weather conditions, 46.2% (60/130) of the samples were collected on days under the influence of a dust storm, while 53.8% (70/130) were collected on days under different climatic conditions. No significant differences were noted for dust vs non-dust days regarding the viral results observed, although, 55% (11/20) of Enterovirus and 54.2% (26/48) of Rotavirus were detected during dust days. Previous publications have reported higher concentrations of microorganisms during dust storms days, using culture-based and real-time PCR methods (Griffin *et al.*, 2001, 2003, 2006; Chen *et al.,* 2010) while others, besides perceiving the same increase, found similar composition patterns for different climatic conditions (Smith et al., 2013). However, simultaneous detection of Enteroviruses and Rotaviruses in the same sample was more frequent during dust days (6/7).

Regarding the origin of the air masses, 46.2% (60/130) of the samples were considered to be from Africa (equal percentage of dust days samples), 45.4% (59/130) were catalogued as Marine and 8.4% (11/130) as European. Similar percentages of positives were observed for both, Enteroviruses and Rotaviruses, in relation to origin.

Thus, 55 (11/20) and 54.2% (26/48) of the positives were obtained from African air masses, 40 (8/20) and 42% (20/48) from marine air masses and 5 (1/20) and 4% (2/48) from European air masses. No significant differences were noted that could infer a relationship between the origin of the air mass and the viral data. Considering the specific origin of African air masses and results obtained, with Morocco being the most frequent source of African dust, these data correlate with other previously published analyses, where it was considered the primary source of African dust in the Canary Islands, based on fingerprinting of deposited dust and analyses of back – trajectory data (Bergametti et al., 1989; Coude-Gaussen et al., 1987; Grousset et al., 1992; Kandler et al., 2007; Muhs, 2012).

Warmer seasons produced a higher relative percentage of positive samples (63.4%, 26/41), than cooler ones (39.2%, 35/89). This trend is opposite to observations obtained in Korea, using metagenomics, by Whon and colleagues, who found fluctuations in the airborne viral concentrations, with a peak during autumn – winter (Whon et al., 2012). Two seasonal groups were analyzed (autumn/winter vs spring/summer) to perform statistical analysis and significant differences were obtained for Enteroviruses, with a higher probability of being detected during spring/summer season, using Pearson's chi – squared test ($p = 0.003$; RR 0.239; IC95% 0.089 – 0.642). These results seem to correlate with prior analyses of seasonality, where Enterovirus outbreaks have been linked to summer and autumn (Stalkup and Chilukuri, 2002) and Rotaviruses outbreaks (in temperate climates) linked to autumn and winter, with a peak at the end of the winter (in tropical environments they can occur throughout the year) (Blacklow, 2013). Although the airborne transmission route is not currently considered as substantial, the data obtained in the present work seems to coincide with those seasonal trends.

Mean PM10 value for dust days was four times higher than for non-dust days (44.2 and 11.6 μg m^{-3}, respectively), and the limit recommended by the European Directive (European Commission, 2008), 50 μg m^{-3}, was exceeded 12.3% (16/130) of the sampled days. In the case of PM2.5, the mean value for the samples collected during dust days, 18.4 μg m^{-3}, was more than twice the value obtained for non-dust days, 7 μg m^{-3}. Regarding the limit recommended by the World Health Organization (WHO, 2006) 25 μg m^{-3}, it was surpassed 10% (13/130) of the days. When viral results were contrasted with the PM10 levels, no statistical difference was observed, while when analyzed regarding PM2.5 values, an inverse correlation was detected for Rotaviruses ($p = 0.047$; RR 2.186; IC95% 1.005-4.578). Levels of particulate matter and their influence on morbidity and mortality have been previously reviewed (de Longueville et al., 2013) and conflicting results have been noted. While some authors have found a clear correlation between parameters (Cadelis et al., 2014; Chan and Ng, 2011; López-Villarrubia et al., 2010), others have reported negative correlation (Kashima et al., 2012). Considering the methodology used to detect airborne microorganisms, there is the potential for high concentrations of particulate matter acting as an inhibitor for PCR-based methods. In this case, experiments to detect viral, bacterial and fungal genomes from air samples proved to be successful with concentrations of particulate matter up to 50 μg per sample (Fabian et al., 2010; Hospodsky et al., 2010). In the present work, a significant correlation was noted between PM2.5 and Rotavirus results, showing that lower PM levels increased the probability of detection. This trend would agree with other studies that have observed that high concentrations of particulate matter may inhibit molecular biology tools utilized to detect airborne microorganisms (McDevitt et al., 2007). In our sample set, no positive sample was obtained when the highest PM levels were recorded.

CONCLUSIONS

Although viability issues are yet to be examined, results presented in this work report the presence of Enterovirus and Rotavirus genomic sequences in air samples in an outdoor urban environment. Hypothetical influence of African dust storms over the airborne viral composition was not confirmed. Increasing the sampling period and including collection from source regions could provide information about whether microorganisms detected originated from Africa and or other locations. Detection of Enteroviruses was influenced seasonally, but this apparent trend should be examined in future efforts in consideration of outbreaks occurring in potential source regions. Earlier described associations between desert dust storms and an increase of particulate matter levels (PM) in the Canary Islands was confirmed and trends were noted with viral presence, although not statistically significant. In addition, results warrant investigations into modifications of existing nucleic extraction methodologies to increase extraction efficiencies and to limit PCR inhibitor carryover.

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DISCLAIMER

The use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Table 1. List of positive samples and associated climatic variables.

SAMPLE CODE[a]	YEAR	SEASON[b]	DUST[c]	ORIGIN[d]	PM 10 (µg m^{-3})[c]	PM 2.5 (µg m^{-3})[c]	ENTEROVIRUSES[f]	ROTAVIRUSES[f]
A1	2009	WIN	YES	AFR	27,8	14,5	**Enterovirus B** (KU821663)	**Rotavirus A**
A2	2009	WIN	YES	AFR	52,2	27,9	**Enterovirus A** (KU821664)	**Rotavirus A**
A3	2009	WIN	YES	AFR	23,6	17,6	NEG	**Rotavirus A**
A4	2009	WIN	YES	AFR	64,1	31,9	**Picornaviridae** (KU821665)	NEG
A5	2009	WIN	YES	AFR	59,3	28,4	NEG	**Rotavirus A**
A6	2009	SPR	NO	MAR	12,3	9,4	NEG	**Rotavirus A**
A7	2009	SPR	NO	EUR	14,8	10	NEG	**Rotavirus A**
A9	2009	SPR	NO	MAR	11,3	3,5	**Enterovirus B** (KU821666)	**Rotavirus**
A10	2009	SPR	NO	EUR	24,1	12,5	NEG	**Rotavirus A**
A12	2009	SPR	YES	AFR	32,9	16,8	NEG	**Rotavirus**
A14	2009	SPR	YES	AFR	46,8	23,3	**Enterovirus A**	**Rotavirus A**
A15	2009	SPR	YES	AFR	46,8	23,3	NEG	**Rotavirus A**
A16	2009	SPR	YES	AFR	31	25,3	**Enterovirus A** (KU821667)	NEG
A17	2009	SPR	YES	AFR	23,1	7,8	**Enterovirus** (KU821668)	**Rotavirus A**
A19	2009	SPR	NO	MAR	19,4	N	**Enterovirus** (KU821669)	NEG
A20	2009	SPR	NO	MAR	N	3,5	NEG	**Rotavirus A**
A23	2009	SPR	NO	MAR	N	7,4	NEG	**Rotavirus A**
A24	2009	SPR	NO	MAR	N	9,1	NEG	**Rotavirus A**
A25	2009	SPR	NO	MAR	N	11,1	NEG	**Rotavirus A**
A26	2009	SPR	NO	MAR	N	12,8	**Enterovirus B**	NEG
A27	2009	SPR	NO	MAR	N	9,5	**Enterovirus B** (KU821670)	NEG
A28	2009	AUT	YES	AFR	N	18,3	NEG	**Rotavirus A**
A29	2009	AUT	YES	AFR	N	22,6	NEG	**Rotavirus A**
A30	2009	AUT	NO	MAR	2	4,6	NEG	**Rotavirus A**
A31	2009	AUT	NO	MAR	7,2	1,2	NEG	**Rotavirus A**
A32	2009	AUT	YES	AFR	15,3	2,5	NEG	**Rotavirus A**
A34	2009	AUT	NO	MAR	9	2,3	NEG	**Rotavirus A**
A35	2009	AUT	NO	MAR	9	2	**Enterovirus B** (KU821671)	NEG
A36	2009	AUT	NO	MAR	8,2	2,5	NEG	**Rotavirus A**
A40	2010	WIN	NO	MAR	N	N	NEG	**Rotavirus A**
A43	2010	WIN	YES	AFR	N	N	NEG	**Rotavirus A**
A44	2010	WIN	YES	AFR	N	N	NEG	**Rotavirus A**
A46	2010	WIN	NO	MAR	25,5	15,3	NEG	**Rotavirus A**
A51	2010	WIN	NO	MAR	28,3	20,8	NEG	**Rotavirus A**

A54	2010	WIN	YES	AFR	147,5	47,2	NEG	**Rotavirus A**
A55	2010	SPR	NO	MAR	13,6	7,2	NEG	**Rotavirus A**
A59	2010	SPR	NO	MAR	16,4	10,3	NEG	**Rotavirus A**
A61	2010	SPR	YES	AFR	77,3	17	NEG	**Rotavirus A**
A63	2010	SPR	YES	AFR	N	N	NEG	**Rotavirus A**
B1	2012	WIN	YES	AFR	125,5	38,8	NEG	**Rotavirus A**
B10	2012	SPR	YES	AFR	33,5	11	**Enterovirus C**	**Rotavirus**
B12	2012	SPR	YES	AFR	62,2	22,3	NEG	**Rotavirus A**
B13	2012	SPR	YES	AFR	67,2	23,8	**Enterovirus C** (KU821672)	NEG
B14	2012	SPR	YES	AFR	26	10	**Enterovirus A** (KU821673)	NEG
B15	2012	SUM	YES	AFR	N	N	**Enterovirus A** (KU821674)	**Rotavirus A**
B16	2012	SUM	YES	AFR	N	N	**Enterovirus A**	NEG
B28	2013	WIN	YES	AFR	17,5	10,6	NEG	**Rotavirus A**
B29	2013	WIN	YES	AFR	14,3	9,5	NEG	**Rotavirus A**
B30	2013	WIN	YES	AFR	7,2	4,8	NEG	**Rotavirus A**
B31	2013	WIN	NO	MAR	10,2	6,7	NEG	**Rotavirus A**
B33	2013	WIN	YES	AFR	28,5	16,1	NEG	**Rotavirus A**
B34	2013	WIN	YES	AFR	15,8	8,3	NEG	**Rotavirus A**
B38	2013	WIN	NO	MAR	10,5	6	NEG	**Rotavirus A**
B40	2013	WIN	NO	MAR	6	3,5	NEG	**Rotavirus A**
B43	2013	WIN	YES	AFR	31	15,2	NEG	**Rotavirus A**
B51	2013	WIN	NO	MAR	3	1,5	NEG	**Rotavirus A**
B54	2013	WIN	NO	MAR	16,8	9	NEG	**Rotavirus A**
B60	2013	WIN	NO	EUR	6,8	5	**Enterovirus A** (KU821675)	NEG
B61	2013	WIN	NO	MAR	7,6	5,3	**Enterovirus A** (KU821676)	NEG
B63	2013	WIN	NO	MAR	4,5	3,1	**Enterovirus A**	NEG
B64	2013	WIN	NO	MAR	7,6	6	**Enterovirus A** (KU821677)	NEG

[a] Sample code: *A* = Mattson – Garvin samples and *B* = Omni3000. [b] Seasons: *SPR* = spring, *SUM* = summer, *AUT* = autumn, *WIN* = winter. [c] DUST: *YES* = dust day, *NO* = no dust day. [d] Origin: *AFR* = African, *MAR* = Marine, *EUR* = European. [e] PM levels: *N* = null value. [f] Viruses: *NEG* = Negative result. Positive results are indicated with the species identification. Sequences over 200 bp were submitted to GenBank and accession numbers are attached to the virus ID. The remaining sequences that could not be deposited are available by contacting the author. For additional information see Table S2 in the Supplementary Material.

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649 **List of tables and figures**

650

651 Table 1: List of positive samples and associated climatic variables.

652

653 Figure 1: Examples of air mass trajectories (HYSPLIT). On the left, an air mass
654 collected on March 30th 2009 originated over the North Atlantic Ocean. On the right, an
655 air mass collected on June 26th 2012, originated over North Africa.

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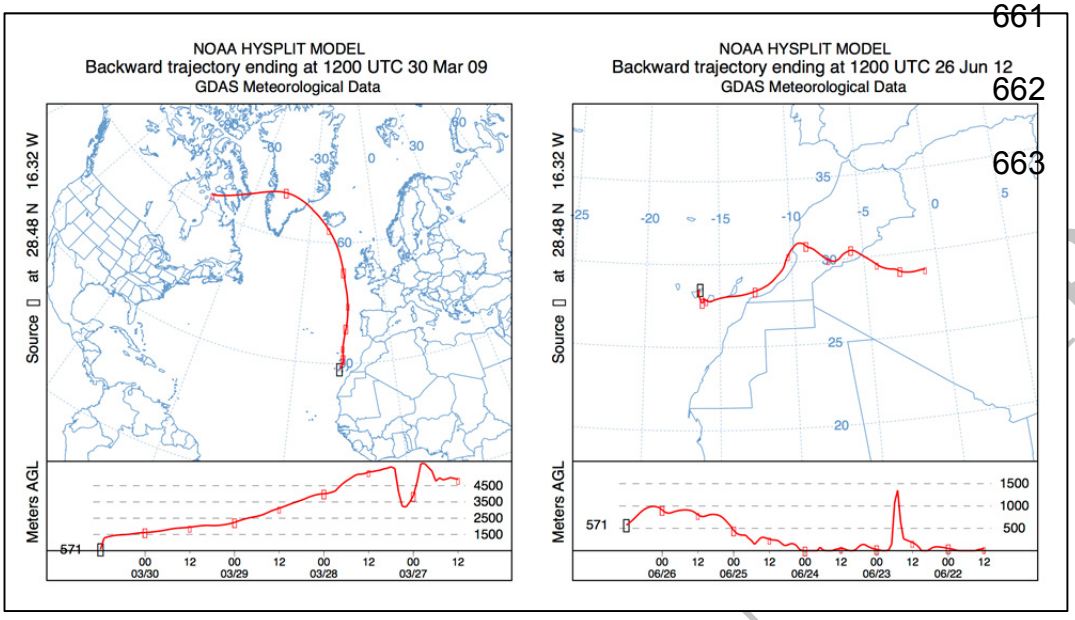
660 Figure 1



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