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Assessing environmental exposure to viruses in wastewater treatment plant and swine farm scenarios with next-generation sequencing and occupational risk approaches

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ABSTRACT

Occupational exposure to pathogens can pose health risks. This study investigates the viral exposure of workers in a wastewater treatment plant (WWTP) and a swine farm by analyzing aerosol and surfaces samples. Viral contamination was evaluated using quantitative polymerase chain reaction (qPCR) assays, and target enrichment sequencing (TES) was performed to identify the vertebrate viruses to which workers might be exposed. Additionally, Quantitative Microbial Risk Assessment (QMRA) was conducted to estimate the occupational risk associated with viral exposure for WWTP workers, choosing Human Adenovirus (HAdV) as the reference pathogen. In the swine farm, QMRA was performed as an extrapolation, considering a hypothetical zoonotic virus with characteristics similar to Porcine Adenovirus (PAdV). The modelled exposure routes included aerosol inhalation and oral ingestion through contaminated surfaces and hand-to-mouth contact.

HAdV and PAdV were widespread viruses in the WWTP and the swine farm, respectively, by qPCR assays. TES identified human and other vertebrate viruses WWTP samples, including viruses from families such as *Adenoviridae*, *Circoviridae*, *Orthoherpesviridae*, *Papillomaviridae,* and *Parvoviridae*. In the swine farm, most of the identified vertebrate viruses were porcine viruses belonging to *Adenoviridae*, *Astroviridae*, *Circoviridae*, *Herpesviridae*, *Papillomaviridae*, *Parvoviridae*, *Picornaviridae,* and *Retroviridae*.

QMRA analysis revealed noteworthy risks of viral infections for WWTP workers if safety measures are not taken. The probability of illness due to HAdV inhalation was higher in summer compared to winter, while the greatest risk from oral ingestion was observed in workspaces during winter. Swine farm QMRA simulation suggested a potential occupational risk in the case of exposure to a hypothetical zoonotic virus.

This study provides valuable insights into WWTP and swine farm worker's occupational exposure to human and other vertebrate viruses. QMRA and NGS analyses conducted in this study will assist managers in making evidence-based decisions, facilitating the implementation of protection measures, and risk mitigation practices for workers.

1. Introduction

People can be exposed to various risk factors in workplaces, with biological factors being of particular importance. Exposure to bioaerosols in the occupational environment has garnered attention due to its association with various health effects, including infectious diseases, acute toxic effects, allergies, and cancer (Douwes et al., 2003). Bioaerosols are composed of small particles containing microorganisms

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such as viruses, bacteria, and fungi, as well as organic compounds derived from microorganisms (Mandal and Brandl, 2011).

Numerous processes and activities can lead to the generation of bioaerosols in occupational settings, including manufacturing, agriculture, waste and wastewater treatment, farming, and laboratory environments (Mirskaya and Agranovski, 2018). In a Wastewater Treatment Plant (WWTP), most of the adverse health conditions reported among workers have been attributed to bioaerosols (Amoah et al., 2022; Carducci et al., 2016; Corrao et al., 2012). These aerosols can be generated by several wastewater treatment processes, and pathogenic microorganisms from wastewater can be easily released into the atmosphere in aerosols (Kataki et al., 2022).

Wastewater contains a diverse range of viruses, with geographical and seasonal differences observed among specific viral groups (Nieuwenhuijse et al., 2020). The abundance and diversity of pathogenic viruses in wastewater have been shown to reflect the pattern of infection in the human population, and some of the principal human pathogenic waterborne viruses include Human Adenovirus (HAdV), Rotavirus (RoV), Hepatitis A Virus (HAV), and other enteric viruses, such as Norovirus (NoV), Coxsackievirus (CV), and Astrovirus (AstV) (Corpuz et al., 2020).

Aerosols of different sizes produced in WWTPs and all airborne biological agents can subsequently settle on surfaces (Han et al., 2013). Therefore, WWTP workers may be exposed to viruses through either the inhalation of bioaerosols generated during technological processes in the WWTP or oral ingestion after direct contact with contaminated surfaces, clothes, or tools. In fact, WWTP workers have been found to be at a higher risk of developing a wide range of work-related symptoms compared to the general population. These symptoms may include respiratory and gastrointestinal effects, such as diarrhea (Masclaux et al., 2014). The level of risk faced by WWTP workers also depends on factors such as the infectivity of the pathogen, its concentration, the duration of exposure, and the immunity (Carducci et al., 2016, 2018).

With the rapid expansion of large-scale and intensive swine production, the emission of aerosols from swine farms has also become a growing concern (Liu et al., 2023). This concern has intensified especially in the context of the One Health era, which emphasizes the interdependence of human, animal, plant, and environment health (One Health Commission, 2018). Aerosols produced in swine farms mainly originate from sources such as manure, feed, swine hair and skin, secondary production, and waste treatment (Liu et al., 2023). Airborne pathogenic viruses, including Influenza A Virus (IAV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Epidemic Diarrhea Virus (PEDV), Classical Swine Fever Virus (CSFV) and African Swine Fever Virus (ASFV), have been detected in the air of swine farms (Alonso et al., 2015; Corzo et al., 2013; Dee et al., 2009; Neira et al., 2016; O'Brien and Nonnenmann, 2016; Olesen et al., 2017; Weesendorp et al., 2009). Additionally, long-distance airborne transport of pathogens such as IAV and PRRSV has been demonstrated (Corzo et al., 2013; Dee et al., 2009) and IAV has also been detected on surfaces in swine production facilities during outbreaks (Neira et al., 2016). These findings imply potential risks, which align with data suggesting that swine workers and their non-swine-exposed families are at an increased risk of zoonotic influenza virus infections (Gray et al., 2007), leading to calls for the inclusion of these workers in pandemic surveillance and in antiviral and immunization strategies (Myers et al., 2006).

Next-generation sequencing (NGS) is a valuable and highly effective tool for detecting viral pathogens within a sample by simultaneously analyzing the sequences present. The metagenomic information obtained from these samples is essential for investigating potential biological exposures. To date, only a few studies have employed NGS techniques to characterize viral communities in aerosols and surfaces in the context of occupational exposure, such as aerosol samples from an animal slaughterhouse (Hall et al., 2013) and WWTP (Han et al., 2019), as well as surface and mobile phone swab samples from healthcare workers in the Emergency unit of a tertiary care facility (Boucherabine et al., 2022). These studies utilized NGS to characterize the profile of viruses present in aerosol and surface samples, providing a deeper understanding of the viruses to which workers are exposed.

Quantitative Microbial Risk Assessment (QMRA) approaches can be used to evaluate the risk of infection within those occupational settings. The use of QMRA involves assessing the risk of harmful microorganisms through hazard identification, exposure assessment, dose-response modeling, and risk characterization (Haas et al., 1999). QMRA is commonly estimated through Monte Carlo simulations to quantitatively assess the range and probability of health risks (Chen et al., 2021; Lim et al., 2015; Shi et al., 2018; Yan et al., 2021). For risk characterization, the two primary health risk benchmarks widely used in describing the magnitude of QMRA outcomes are the acceptable annual infection risk level proposed by the U.S. Environmental Protection Agency (U.S. EPA, 2005) ($\leq$10E−4 pppy, per-person-per-year) and the acceptable disability-adjusted life years (DALYs) proposed by the World Health Organization (WHO, 2008) ($\leq$10E−6 DALYs pppy) (Chen et al., 2021; Yan et al., 2021). Additionally, the importance of all input variables can be identified through sensitivity analysis, which tests the relative impacts of stochastic input parameters on health risks (Chen et al., 2021). QMRA has been applied to assess the risk of viral infections associated with drinking water and food (Deere and Ryan, 2022; Petterson, 2016; Schijven et al., 2019), reclaimed water (Schoen et al., 2018), as well as in various working environments, such as WWTPs (Carducci et al., 2018; Dada and Gyawali, 2021; Medema et al., 2004; Zaneti et al., 2021), agricultural areas utilizing reclaimed wastewater for irrigation (Antwi-Agyei et al., 2016) or fertilizer applications (Brooks et al., 2005, 2012; Tanner et al., 2008), and scenarios dealing with leachate from municipal solid waste (MSW) (Lanzarini et al., 2022).

This study aims to provide valuable insights into the occupational exposure of workers to viruses in two different settings, WWTPs and swine farms. The first objective is to evaluate viral contamination through quantitative polymerase chain reaction (qPCR) assays and investigate the presence of vertebrate pathogenic viruses in bioaerosols and contaminated surfaces from these workplaces by employing NGS. To our knowledge, this is the first study to characterize the profiles of viruses infecting vertebrates present in aerosol and surface samples using a target enrichment sequencing (TES) approach. Additionally, the second objective of this study is to estimate the risk of exposure to viral pathogens with QMRA analysis in a WWTP, with HAdV as a model virus transmitted through mixed oral-fecal and respiratory routes. In the swine farm scenario, the risk is intended to be estimated through a QMRA simulation, considering the inhalation or oral ingestion of a hypothetical virus with assumed zoonotic potential and characteristics similar to Porcine Adenovirus (PAdV).

2. Material and methods

2.1. Sampling sites and samples

Two workplace scenarios located in Catalonia were chosen for this study: a WWTP and a swine farm. The selected WWTP is a conventional plant that treats wastewater from 183,517 inhabitants with a flow capacity of 57,000 m^3/day. On the other hand, the selected swine farm is a breeding and gestation barn with an occupancy of 2,000 piglets, where newborn pigs spend the first weeks of life before being moved to a fattening farm. The sampling campaigns included both winter and a summer seasons, with three sampling events for each workplace scenario and season. Table 1 and Table 2 provide a summary of the exposure scenarios, samples analyzed, seasons, and the conducted analysis in the WWTP and the swine farm, respectively.

For aerosol sampling, a Coriolis μ air sampler (Bertin Technologies, France), designed to capture particles ranging from 0.5 to 20 μm, was used at a flow rate of 300 L/min during 60 min in specific areas where a higher exposure to aerosols by workers was identified. Aerosol samples were collected in sterile collection cones, pre-filled with 15 ml of a saline

Table 1
WWTP exposure scenarios, specifying the type of samples analyzed, seasons and the type of analysis conducted.

		Samples analyzed	Season	Analysis
Workspaces	Centrifuge zone (indoor)	Aerosol	Winter	qPCR, NGS, QMRA
			Summer	qPCR, NGS, QMRA
		Surface	Winter	qPCR, NGS, QMRA
			Summer	qPCR, NGS, QMRA
	Reactor zone (outdoor)	Aerosol	Summer	qPCR, NGS
		Surface	Winter	qPCR, NGS, QMRA
			Summer	qPCR, NGS, QMRA
	Other	Surface	Winter	qPCR, NGS, QMRA
			Summer	qPCR, NGS, QMRA
Break room		Surface	Winter	qPCR, NGS, QMRA
			Summer	qPCR, NGS, QMRA

Table 2
Swine farm exposure scenario, specifying the type of samples analyzed, seasons and the type of analysis conducted.

Samples analyzed	Season	Analysis
Aerosol	Winter	qPCR, NGS, QMRA
	Summer	qPCR, NGS, QMRA
Surface	Winter	qPCR, NGS, QMRA
	Summer	qPCR, NGS, QMRA

phosphate buffer solution (PBS). At the WWTP, aerosol samples were collected in the centrifuge zone (indoor) during winter (3 samples) and summer (4 samples). Additionally, in summer, 3 long-monitoring aerosol samples lasting 6 h were collected in the reactor zone (outdoor). At the swine farm, 3 aerosol samples were collected from the breeding area for each season. In all aerosol samplings, the manufacturer's accessory to maintain a constant liquid volume and compensate evaporation was used.

For surface sampling, samples were collected following the strategy described by Sommer and coworkers as a long-term sampling approach to study viruses on surfaces (Sommer et al., 2021), technique previously outlined by Bobal and Witte for the detection and monitoring of bacterial pathogens (Bobal et al., 2019). Commercially paper-based stickers (Markierungspunkte ø 8 mm, permanent, ref. PSA08J 301; Avery of CCL Industries, Inc., Toronto, Canada), sterilized with UV-C radiation for a minimum of 15 min, were applied to frequently touched surfaces by workers, such as switches, doorknobs, and working tools, using sterile tweezers.

After 7 days, the stickers were removed and transferred to 2 ml Eppendorf tubes. At the WWTP, two scenarios were differentiated: the workspaces and the break room, which serves as the kitchen where workers take a break to eat. During winter, 12 surfaces samples from the workspaces (5 from the filtering zone, 2 from the centrifuge zone, 4 from the main building and 1 from the reactor zone) and 6 surface samples from the break room were collected on each sampling event of the season's campaign. In summer, a selection of surface samples that showed higher contamination during winter was collected, consisting of 5 workspaces surface samples (2 from the filtering zone, 1 from the centrifuge zone, 1 from the main building and 1 from the reactor zone) and 2 break room samples. For the swine farm, each sampling event involved collecting 6 surface samples distributed on switches and doorknobs around the entire farm. Stickers were also collected from surfaces on upper walls, serving as a negative control for sampling and subsequent sample processing.

Both aerosol and surfaces samples were transported at 4 °C to the laboratory and processed on the same day of collection.

2.2. Sample processing and viral quantification

The concentration of viral particles from aerosol samples was performed by ultrafiltering the volume from the collection cones using the automatic Concentration Pipette (CP-Select™) with 150 KDa tips (InnovaPrep, Drexel, MO, USA) to obtain a final volume of 300 μL, following the procedure previously described for viral concentration from wastewater (Forés et al., 2021). The viral nucleic acids from the concentrated aerosol samples were then extracted using the QIAamp® Viral RNA Mini Kit from QIAGEN (QIAGEN, Germantown, MD, USA). The extracted nucleic acids were eluted in 70 μl and stored at −80 °C for further analysis in qPCR and NGS assays.

For the surface samples, the viral nucleic acids were extracted using the RNeasy® PowerMicrobiome® Kit (QIAGEN, Germantown, MD, USA) with a final volume of 50 μl, following the manufacturer's instructions. An additional pre-step of bead-beating for 30 s at 4 m s^{-1} using FastPrep-24™ (MP Bio, USA) was included, as previously described (Mejías-Molina et al., 2023). The extracted nucleic acids were stored at −80 °C for further analysis.

For evaluating the viral contamination in the WWTP and swine farm samples, specific qPCR assays were performed for Human Adenovirus (HAdV) and Porcine Adenovirus (PAdV), respectively. TaqMan® Environmental Master Mix 2.0 (Applied Biosystems, Waltham, MA, USA) was used, along with specific primers and probes previously described (Bofill-Mas et al., 2006; Hernroth et al., 2002; Hundesa et al., 2009). Additionally, all samples, from both the WWTP and the swine farm, were analyzed using the SARS-CoV-2 N1 assay (CDC Division of Viral Diseases, 2023) with the RNA UltraSense™ One-Step qRT-PCR System (Applied Biosystems, Waltham, MA, USA). With the exception of the synthetic SARS-CoV-2 control (control 51 from Twist Biosciences), the qPCR standards were prepared using synthetic gBlocks® Gene fragments (IDT, Coralville, IA, USA), which were quantified with a Qubit 3.0 dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) and serially diluted from 10° to 10^{7} copies per reaction. All qPCR assays were performed using the QuantStudio™ Real-Time PCR System from ThermoFisher Scientific. Both undiluted and 10-fold diluted nucleic acid extractions were analyzed, and non-template controls were included in each of the assays.

2.3. Metagenomic analysis: target enrichment sequencing (TES)

Nucleic acid extractions from aerosol and surface samples were pooled, considering the type of sample, the season of the year, and the workplace zone studied (further details can be found in the Supplementary Material, Table S1 and Table S2). The nucleic acid pools were analyzed using the NGS approach target enrichment sequencing (TES) to explore the diversity of vertebrate viral pathogens present in the aerosol and surface samples, aiming for a better characterization of the viruses to which WWTP and swine farm workers are exposed.

2.3.1. Sequence-independent, single-primer amplification (SISPA)

A total of 11 nucleic acid pools were prepared prior to library construction following a sequence-independent, single-primer amplification (SISPA) method, which has been previously described for studying both RNA and DNA viruses (Fernandez-Cassi et al., 2018, 2018b; Itarte et al., 2021; Martínez-Puchol et al., 2020; Mejías-Molina et al., 2023). Briefly,

a retrotranscription step was performed using a random nonamer primer and SuperScript IV (Invitrogen, Carlsbad, CA, USA), followed by a second-strand synthesis with Sequenase 2.0 (Applied Biosystems, Waltham, MA, USA). To obtain sufficient dsDNA for library construction, nucleic acids were amplified using AmpliTaq Gold DNA polymerase (Applied Biosystems, Waltham, MA, USA), and the resulting PCR products were purified with the Zymo DNA Clean & Concentrate kit (Zymo Research, Irvine, CA, USA). The purified PCR products were quantified using the Qubit 3.0 dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

2.3.2. Library construction

For each pool of samples, libraries were constructed using the KAPA HyperPlus Library Preparation Kit (KAPA Biosystems, Roche, Basel, Switzerland). Following the manufacturer's instructions, the library construction consisted of enzymatic fragmentation, end repair and A-tailing reaction, and adapter ligation. Then, samples were amplified using the KAPA UDI Primer Mixes (KAPA Biosystems, Roche, Basel, Switzerland). Afterwards, the resulting libraries were quantified using the Qubit 3.0 dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

2.3.3. Capture of viral sequences by VirCapSeq-VERT Capture Panel

Libraries were equimolarly pooled and captured using the VirCapSeq-VERT Capture Panel (Roche, Basel, Switzerland). This panel consists of probes designed to capture sequences from 207 viral taxa known to infect vertebrates through hybridization and a following post-capture PCR amplification, enabling the detection of viral sequences in complex sample types as described in previous studies (Briese et al., 2015; Filipa-Silva et al., 2020; Hjelmsø et al., 2019; Itarte et al., 2021; Martínez-Puchol et al., 2020, 2022; Mejías-Molina et al., 2023; Strubbia et al., 2020). Captured libraries were sequenced using an Illumina NextSeq 500 platform for a 2 × 150 cycles Mid Output run.

2.3.4. TES bioinformatic processing

Bioinformatic analysis was performed using CZ ID portal (https://czid.org/, accessed on November 30, 2023), an open-source cloud-based pipeline and service for metagenomic pathogen detection and monitoring (Kalantar et al., 2020). Briefly, this portal performs host and quality filtration steps, followed by an assembly-based alignment pipeline, which results in the assignment of reads and contigs to taxonomic categories. Only viral assignments with a nucleotide identity above 70% and >100 bp alignment length were considered.

2.4. Quantitative Microbial Risk Assessment

The QMRA was constructed, as described in the following paragraphs, to estimate the individual-level occupational risk faced by workers in WWTPs and also as an extrapolation for workers in swine farms.

2.4.1. Risk assessment framework and hazard identification

The reference viruses HAdV and PAdV were selected for WWTP and swine farm risk assessment, respectively. Both HAdV and PAdV are double-stranded DNA viruses belonging to the *Adenoviridae* family and they are commonly found in the environment due to their high resistance to environmental conditions (Hijnen et al., 2006). HAdV has been suggested as a human fecal indicator since it is persistently excreted in feces or urine by infected humans, both with and without clinical symptoms (Bofill-Mas et al., 2013), causing various conditions such as gastroenteritis, respiratory infections, eye infections, acute hemorrhagic cystitis, and meningoencephalitis (Allard and Vantarakis, 2017). On the other hand, PAdV has been used as a Microbial Source Tracking (MST) tool to identify sources of porcine fecal contamination (Rusiñol et al., 2014). Although PAdV infection in pigs is often subclinical, it can cause mild diarrhea, anorexia and dehydration (Kumthip et al., 2019).

PAdV is a virus that infects pigs and not humans but, as an assumption, it was chosen as a model of a hypothetical virus with zoonotic potential for the following reasons: (1) no potential zoonotic viruses were detected in these samples, (2) it was prevalently excreted and present on the farm, and 3) it allows the application of well-known parameters to construct a robust model (adenovirus parameters).

In the WWTP scenario, the QMRA analysis focuses on estimating the occupational risk of viral infections from pathogens present in wastewater. In contrast, the QMRA for the swine farm is an approximate analysis to assess the risk of workers getting infected with a hypothetical virus with an assumed zoonotic potential and characteristics similar to PAdV, in a One Health context. The exposure pathways considered for both scenarios include inhalation and oral ingestion, as these are known transmission routes for both HAdV and PAdV (Kumthip et al., 2019; Teunis et al., 2016).

2.4.2. Exposure assessment

The objective of the exposure assessment models was to estimate the dose of the selected virus to which WWTP and swine farm workers are exposed during their work-related tasks at different seasons of the year. The study considered two main exposure pathways: inhalation of aerosols and oral ingestion through contaminated surfaces. Additionally, for the WWTP scenario, a distinction was made between the surfaces in the workspaces and those in a break room where the workers take breaks to eat. This differentiation was made because it was considered that the workers' activities and behaviors in the break room were different compared to the workspaces.

2.4.2.1. Aerosol model. The daily HAdV or PAdV dose to which each WWTP or swine farm worker is exposed by inhalation (d_s) was estimated from Equation (1), adapted from (Carducci et al., 2018) and the input parameters detailed in Table 3.

$$d_s = C_{AdV} \times \frac{f_{conv}}{r_{eff}} \times t_{exp} \times r_{in} \times cf \quad (1)$$

In Equation (1), the concentration C_{AdV} represents the number of genomic copies (GC) of HAdV or PAdV per m^3 detected in WWTP or swine farm air samples, respectively, and is expressed in GC/m^3. These

Table 3
Input exposure parameters used in the QMRA model for the aerosol inhalation pathway.

Model inputs	Notation	Distribution/value	Unit	Source
Concentration of HAdV (WWTP) or PAdV (farm) in air	C_{AdV}	WWTP winter: Uniform (3.09, 6.34) WWTP summer: Uniform (86.13, 96.24) Farm winter: Uniform (1.53, 126.96) Farm summer: Uniform (6.15, 128.23)	GC/m^3	This study (see sections 2.1. and 2.2.)
Conversion factor	f_{conv}	1/700	$TCID_{50}$/GC	McBride et al. (2013)
Recovery efficiency	r_{eff}	WWTP: 80 Farm: 75	%	Estimated in section 2.4.3.2.
Exposure time	t_{exp}	WWTP: Uniform (60,120) Farm: Uniform (240,360)	minutes	Observed in this study (see section 2.4.3.3.)
Inhalation rate	r_{in}	Lognormal ($\mu = 1.4$, $\sigma = 0.51$)	m^3/hour	EPA (2011)
Conversion factor	cf	1/60	hour/minutes	–

data were obtained in this study as explained before in sections 2.1. and 2.2. and follow a uniform distribution (see section 2.4.3.1. for boundary estimations). Since the qPCR data does not provide quantification of infectious particles, the constant $f_{conv} = 1/700$ is the ratio between tissue culture infective dose ($TCID_{50}$) and GCs, assuming 700 GC = 1 $TCID_{50}$ (McBride et al., 2013). The constant r_{eff} represents the recovery efficiency, which is the recoverable amount of HAdV or PAdV present in the sample. This efficiency slightly varies between the WWTP and the swine farm (see section 2.4.3.2. for estimations). The random variable t_{exp} corresponds to the amount of time that the worker is exposed to aerosols and is expressed in minutes per day, following a uniform distribution (see section 2.4.3.3.). The random variable inhalation rate r_{in}, represents the inhalation rate, expressed in m^3/hour, which is the volume of air inhaled per unit of time and it follows a lognormal distribution with $\mu = 1.4$ and $\sigma = 0.51$ (EPA, 2011). The constant conversion factor *cf* is equal to 1/60, which is used to transform minutes to hours.

2.4.2.2. Surface model. For the surface risk equations, considering oral ingestion through hand-to-mouth contact, the dose was estimated using the model from Lanzarini et al. (2022), a conceptual model developed by the Institute for Occupational Medicine (Cherrie et al., 2006; HSE et al., 2007), and adapted to the specific exposure scenarios of this study using the input parameters detailed in Table 4. Oral exposure of WWTP and swine farm workers relies on the oral ingestion of HAdV or PAdV, respectively, through the contact of contaminated hands with the mouth after touching contaminated surfaces with bare hands. Therefore, the daily exposure dose for the surface risk is given by:

$$d_s = C_{AdV} \times \frac{f_{conv}}{r_{eff}} \times f_{cont|surf} \times TE_{surf|hand} \times TE_{hand|mouth} \times f_{cont|mouth} \times n_{hours} \quad (2)$$

C_{AdV} represents the concentration of HAdV or PAdV expressed in GC/cm^2, data obtained in this study (sections 2.1. and 2.2.) and in this case, follows a lognormal distribution (see section 2.4.3.1. for the parameter estimations). The constant f_{conv} is also 1/700, and the constant r_{eff} is now 5.95%, experimentally determined (data not shown). The random variable $f_{cont|surf}$ represents the frequency of hand contact with any surface, and it follows a uniform distribution (see 2.4.3.3. for the estimation). The random variable $TE_{surf|hand}$ models the percentage of viral transfer efficiency from stainless steel surfaces to hands and follows a normal distribution (Lopez et al., 2013). $TE_{hand|mouth}$ is the constant percentage of viral transfer efficiency from bare hands to mouth (Rusin et al., 2002). The number of hand-to-mouth contacts ($f_{cont|mouth}$) follows a discrete distribution (see section 2.4.3.3) and finally, n_{hours} represents the hours the worker spends in each studied workplace zone (workspaces or break room in WWTP and general workspaces on farm). The skin contact area between the hand and peri-oral compartment for each exposure event was assumed to be 1 cm^2.

2.4.3. Fitting parameters in aerosol and surface exposure models

2.4.3.1. Fitting the virus concentration parameters in aerosol and surfaces. As explained in the preceding subsection, both aerosol and surface concentrations in equations (1) and (2) are treated as random variables following a suitable probabilistic distribution. In the case of aerosol concentrations, where the number of samples was small, a uniform distribution was used (see Carducci et al., 2018) for both seasons and both scenarios. The parameters of these situations were trivially computed as the minimum and maximum values registered in each case.

On the other hand, the number of surface samples was larger, enabling the adjustment of a lognormal distribution that fits the observed data. It is important to note that all the samples, including those recorded below the limit of detection, were used for these estimations. The limit of detection was determined based on the minimum value reported for each virus assay and each type of sample. Such samples were treated as censored data (see Canales et al. 2018), allowing the fitting of the lognormal distribution using the "fitdistrplus" package in R (R Core Team, 2023). The "fitdistr" method of this package fits a univariate distribution for censored data using maximum likelihood estimation.

2.4.3.2. Fitting the recovery efficiency in aerosol exposure model. A relationship between the diameter of the particles suspended in the air and the collection efficiency was established from the information provided in a white paper supplied by the air sampler's manufacturer, Bertin Technologies (https://www.bertin-technologies.com/, accessed on November 30, 2023), about the particle collection efficiency according to size and flow rate. On the logarithmic scale, this relation appears to be approximately linear. Assuming that the diameter of the particles follows a lognormal distribution, the two parameters of the density can be obtained from Gholipour et al. (2021); Agranovski et al. (2004), which

Table 4
Input exposure parameters used in the QMRA model for the oral ingestion pathway from contaminated surfaces.

Model inputs	Notation	Distribution/value	Unit	Source
Concentration of HAdV (WWTP) or PAdV (farm) in surfaces	C_{AdV}	WWTP winter workspaces: Lognormal ($\mu = 1.42$, $\sigma = 3.16$) WWTP summer workspaces: Lognormal ($\mu = 0.21$, $\sigma = 3.61$) WWTP winter break room: Lognormal ($\mu = 0.89$, $\sigma = 3.13$) WWTP summer break room: Lognormal ($\mu = 0.51$, $\sigma = 2.31$) Farm winter: Lognormal ($\mu = 3.81$, $\sigma = 1.81$) Farm summer: Lognormal ($\mu = 9.17$, $\sigma = 2.57$)	GC/cm^2	This study (see sections 2.1. and 2.2.)
Conversion factor	f_{conv}	1/700	$TCID_{50}$/GC	McBride et al. (2013)
Recovery efficiency	r_{eff}	5.95	%	This study (data not down)
Frequency of hand-to-surface contacts	$f_{cont\|surf}$	WWTP workspaces: Uniform (6, 11) WWTP break room: Uniform (4.5, 10) Farm: Uniform (8, 12)	events/hour	Observed in this study (see section 2.4.3.3.)
Viral transfer efficiency from surface to bare hands	$TE_{surf\|hand}$	Normal (37, 16)	%	Lopez et al. (2013)
Viral transfer efficiency from hands to mouth	$TE_{hand\|mouth}$	34	%	Rusin et al. (2002)
Frequency of hand-to-mouth contacts	$f_{cont\|mouth}$	WWTP workspaces: $\mu \simeq 2.8$, $\sigma \simeq 2.7$ WWTP break room: $\mu \simeq 7.6$, $\sigma \simeq 3.6$ Farm: $\mu \simeq 2.8$, $\sigma \simeq 2.7$	events/hour	(see section 2.4.3.3.)
Number of hours spent at workplace	n_{hours}	WWTP workspaces: Uniform (2, 5) WWTP break room: Uniform (0.1, 1) Farm: Uniform (4, 6)	hour	Observed in this study (see 2.4.3.3.)

characterize the size particle range generated in WWTPs and swine farms, respectively. By applying the linear transformation, a mean recovery efficiency was obtained for the WWTP and the farm scenarios.

2.4.3.3. Fitting the exposure times and frequency of contacts in aerosol and surface exposure models. In the aerosol model, the parameters of the uniform distribution for t_{exp} were chosen based on the observed behavior of the workers. Similarly, in the surface model, the parameters for n_{hours} and $f_{cont|surf}$ were obtained in an analogous manner. Since these three variables were modelled as uniform distributions, with t_{exp} and n_{hours} being continuous, and $f_{cont|surf}$ being discrete, their boundaries were estimated as the minimum and maximum values in the observed data.

As for the frequency $f_{cont|mouth}$, the number of hand-to-mouth contacts was modelled as a discrete variable. The jump points and cumulative distribution were fitted to the quantiles in the adult's rows in the eating and non-eating macro-activity of Table 1 from Wilson et al. (2021).

2.4.4. Dose-response model

Dose-response models describe the relationship between exposure and the probability of infection and illness. The dose-response model developed by Teunis et al. (2016) was adopted in this study for HAdV and PAdV risk models, and it was used before in other QMRA studies (Carducci et al., 2018; Lanzarini et al., 2022). Briefly, this response model describes the distributions of infectivity and pathogenicity in adenovirus studies, incorporating differences in inoculation route (aerosol inhalation, oral ingestion and intranasal or intraocular droplet inoculation) as shift in average infectivity and pathogenicity. The daily dose variable allowed to compute the probability of infection (P_{inf}):

$$P_{inf}(d_s|\alpha,\beta) = 1 - {}_1F_1(\alpha, \alpha+\beta, -d_s) \quad (3)$$

where ${}_1F_1$ is the Kummer confluent hypergeometric function, and α and β are infectivity (infection dose-response) parameters of the distribution that refer to the inhalation route or the oral ingestion route. The infectivity parameters for the adenovirus dose-response were established as $\alpha = 5.24$ and $\beta = 2.95$ for the inhalation route (aerosol model), whereas $\alpha = 5.11$ and $\beta = 2.80$ were the parameters for the oral ingestion route (surface model) (Teunis et al., 2016). The daily probability of illness conditioned to be infected, denoted by $P_{ill|inf}$, was:

$$P_{ill|inf}(d_s) = 1 - \left(1 + \frac{d_s}{\eta}\right)^{-r} \quad (4)$$

where the pathogenicity parameters for the adenovirus dose-response used were $r = 3.04$ and $\eta = 3.36$ for the inhalation route (aerosol model), whereas $r = 0.41$ and $\eta = 6.53$ (Teunis et al., 2016) were the parameters for the oral ingestion route (surface model). Finally, the daily probability of illness (P_{ill}) was given by the following relation:

$$P_{ill}(d_s) = P_{ill|inf}(d_s) \times P_{inf}(d_s) \quad (5)$$

For any of the situations, the seasonal probability of illness was computed as:

$$P_{ill\ season} = 1 - \prod_{1}^{70} (1 - Random(P_{ill})) \quad (6)$$

where Random(P_{ill}) is a random sample from the distribution of P_{ill} (Karavarsamis and Hamilton, 2010). Both the winter and summer season were assumed to comprise 70 working days.

2.4.5. Risk characterization

Hazard identification, exposure assessment and dose-response assessment were combined to determine the probability of infection with HAdV in WWTPs and to provide an approximation for the probability of infection with a hypothetical virus with zoonotic potential and characteristics similar to PAdV in swine farms. A Monte Carlo analysis was conducted for 10,000 simulations for each of the 10 different scenarios considered, obtaining their daily probability of illness and a suitable set of quantiles.

Following Lanzarini et al., 2022, a sensitivity analysis was conducted to assess the relative importance of the stochastic variables on the results of both models: the concentration C_{AdV} in both exposure models, the exposure time t_{exp} and the inhalation rate r_{in} in the aerosol model, whereas the transfer efficiency $TE_{surf|hand}$, the frequencies of contacts ($f_{cont|surf}$ hands-surface, and $f_{cont|mouth}$ hands-mouth) and the exposure time n_{hours}, in the surface model. To determine the effect of any variable -for instance, the variable v_i-on the final risk estimate, all the other input parameters in the model equation were fixed to their average values, while v_i was treated as a random variable in the Monte Carlo runs. This computation was conducted over all the random variables v_i in the equation, fixing in each case all the parameters except the one of interest. Sensitivity analysis was also computed based on the median values, as some C_{AdV} distributions exhibit long-left-tailed asymmetric shapes.

All the simulations were conducted using R 4.3.0 (R Core Team, 2023).

3. Results

3.1. Viral quantification in aerosol and surface samples

Aerosol and surface samples from the WWTP and the swine farm were analyzed using specific qPCR assays for the detection and quantification of HAdV and PAdV, respectively. Additionally, all samples were analyzed for the presence of SARS-CoV-2. The qPCR assay results are summarized in Table 5 and Table 6, with further details available in the Supplementary Material (Table S1 and Table S2).

HAdV was detected in 60% of WWTP aerosol samples. In the winter season, all indoor samples from the centrifuge zone tested positive, with a mean concentration of 4.87 E+00 GC/m^3. In the summer season, 2 out of 4 samples were positive with a mean value of 9.12 E+01 GC/m^3, resulting in higher HAdV concentrations in summer centrifuge aerosol samples. For outdoor samples in the reactor zone during the summer, 1 out of 3 samples tested positive, with a concentration value of 9.04E-01 GC/m^3. Moreover, HAdV was also detected in 65% of workspaces surface samples and in 50% of the surface samples in the break room. Among these surface samples, those collected in the workspaces during winter had the highest HAdV concentrations, with a mean value of 1.29 E+02 GC/cm^2.

PAdV was detected in 83% of the swine farm aerosol samples and 86% of the surface samples. Notably, all surface samples from summer tested positive and exhibited higher concentrations (mean value of 5.73 E+04 GC/cm^2) compared to those reported in winter (mean value of 1.89 E+02 GC/cm^2).

On the other hand, SARS-CoV-2 was not detected in any of the

Table 5
Quantification of HAdV in WWTP, respectively, during winter and summer seasons using qPCR, including mean concentrations (standard deviations, positive samples vs tested samples). HAdV concentrations in aerosol samples are expressed as GC/m^3, while surface concentrations values are in GC/cm^2. Additional details are available in the Supplementary Material (Table S1).

	Winter	Summer
Aerosol (GC/m^3)	Centrifuge zone (indoor): 4.87 E+00 (1.64 E+00, 3/3)	Centrifuge zone (indoor): 9.12 E+01 (7.15 E+00, 2/4) Reactor zone (outdoor): 9.04E-01 (0, 1/3)
Surface (GC/cm^2)	Workspaces: 1.29 E+02 (3.29 E+02, 23/32) Break room: 1.77 E+01 (3.04 E+01, 8/18)	Workspaces: 2.94 E+01 (2.38 E+01, 8/16) Break room: 1.27 E+01 (1.35 E+01, 4/6)

Table 6
Quantification of PAdV in swine farm samples, respectively, during winter and summer seasons using qPCR, including mean concentrations (standard deviations, positive samples vs tested samples). PAdV concentrations in aerosol samples are expressed as GC/m^3, while surface concentrations values are in GC/cm^2. Additional details are available in the Supplementary Material (Table S2).

	Winter	Summer
Aerosol (GC/m^3)	4.98 E+01 (6.75 E+01, 3/3)	6.72 E+01 (8.63 E+01, 2/3)
Surface (GC/cm^2)	1.89 E+02 (1.96 E+02, 14/19)	5.73 E+04 (7.42 E+04, 18/18)

aerosol and surface samples from the WWTP. In the case of the swine farm, two surface samples tested positive for the presence of SARS-CoV-2 RNA, which was linked to an outbreak among the workers (further discussed in the discussion section). The viral concentrations quantified in these samples were 1.35 E+01 and 2.32 E+02 GC/cm^2.

3.2. NGS results

Aerosol and surface samples from the WWTP and the swine farm were analyzed using the TES approach to explore the presence of vertebrate viruses within these environments. For this purpose, nucleic acids extractions were pooled, considering the type of sample, season of the year, and workplace zone studied. The TES approach involved capturing sequences from vertebrate viruses during library preparation by employing the VirCapSeq-VERT Capture Panel (Roche, Basel, Switzerland).

The viral assignments and reads sequenced in WWTP samples are presented in Fig. 1, and additional details can be found in the Supplementary Material (Tables S3–S9). TES enabled the detection of human and other vertebrate-infecting viruses in aerosol and surface samples from the WWTP. The sequencing reads obtained from the WWTP were assigned to viruses from *Adenoviridae*, *Circoviridae*, *Orthoherpesviridae*, *Papillomaviridae,* and *Parvoviridae* families. Additionally, several families, including *Anelloviridae*, *Astroviridae* (Human astrovirus), *Caliciviridae* (Norwalk virus), *Coronaviridae* (Avian coronavirus) and *Picornaviridae*, were only sequenced in aerosol samples, while *Herpesviridae* (Porcine lymphotropic herpesvirus 3), *Polyomaviridae* (Merkel cell polyomavirus, MCPyV) and *Retroviridae* were exclusively found in surface samples. The highest viral diversity, considering the number of different viral assignments, was observed at the indoor samples (centrifuge zone) collected in the summer months, followed by workspaces surfaces in winter.

The *Parvoviridae* family accounted for the highest number of reads at the indoor aerosol samples in both seasons, as well as at the surfaces of workspaces in winter with Adeno-associated virus accounting for the majority of reads. Indoor aerosols from summer presented a high viral diversity within this family including a variety of known hosts susceptible to these viruses, such as humans, birds, dogs, pigs, and rodents.

The highest diversity of *Circoviridae* was also found in summer indoor aerosols, including human circovirus and cyclovirus, and pigeon circovirus.

A notable viral diversity of *Papillomaviridae, Orthoherpesviridae* and *Retroviridae* members was detected in surface samples, in both workplaces and the break room. Most of the *Papillomaviridae* reads were assigned to alpha, beta, gamma and mu Human Papillomavirus (HPV) genera, and also to Canine Papillomavirus. Epstein-Barr virus (EBV), belonging to *Orthoherpesviridae* family, was the most ubiquitous viral assignment within this viral family, as it was also detected in most of the surface samples. *Retroviridae*-assigned reads belonged to avian, sheep and goat viruses. MCPyV reads were detected only in workspace surfaces in winter.

HAdV, used as a reference virus in the QMRA analysis, was detected in all aerosol and surface samples, except for the break room samples, and the main serotype identified in all samples was HAdV-41. Other members of *Adenoviridae* family were detected, such as Fowl adenovirus in summer aerosol and workspaces surface samples and Turkey adenovirus only in the break room surfaces in winter.

Fig. 2 presents the viral assignments and reads obtained from swine farm samples, and additional information is available in the Supplementary Material (Tables S10–S13).

TES also enabled the detection of vertebrate viruses in aerosol and surface samples from the swine farm. Most of the sequenced reads in aerosol and surface samples were assigned to porcine viruses, belonging to families like *Adenoviridae* (PAdV), *Astroviridae*, *Circoviridae*, *Herpesviridae*, *Papillomaviridae*, *Parvoviridae*, *Picornaviridae* and *Retroviridae* (Porcine type-C oncovirus). Additional families, including *Anelloviridae* (Torque teno sus virus), *Genomoviridae* (Porcine feces-associated gemycircularvirus) and *Tobaniviridae* (Bovine torovirus and Porcine torovirus), were exclusively detected in aerosol samples, while *Polyomaviridae* and *Sedoreoviridae* members (Porcine polyomavirus and Porcine Rotavirus A, respectively) were only found in surface samples. The greatest viral diversity was observed in winter aerosol samples, followed by summer surface samples.

Porcine bocavirus (PBoV) was the viral assignment that accounted for the highest number of reads in aerosol samples, whereas in surface samples, it was Porcine type-C oncovirus. Aerosol samples from winter and summer showed a high similarity in viral assignments between them. The aerosol sample collected in winter only showed few additional viral assignments compared to the summer sample, including PAdV, Porcine circovirus, Porcine feces-associated gemycircularvirus, Adeno-associated virus, Porcine parvovirus, and Porcine enterovirus. Similarly, high similarity was observed between surface samples from winter and summer, with the summer sample having a few more viral assignments, including Porcine astrovirus, Porcine stool-associated circular virus, Gamapapillomavirus, Porcine parvovirus, the bat-infecting virus Rhinolophus sinicus bocaparvovirus 2, Porcine enterovirus and Porcine Rotavirus A. In contrast, Porcine circovirus was the only viral assignment detected in winter samples.

The reference virus PAdV, used in the swine farm QMRA simulation as a model virus, was detected in all surface samples and in the winter aerosol sample.

3.3. QMRA

3.3.1. QMRA analysis in the WWTP

Fig. 3 presents the distributions of the daily illness probability (P_{ill}) for a WWTP worker, considering the inhalation or oral ingestion of HAdV. The scenario involving inhalation of HAdV through aerosols in the reactor zone (outdoor) was not included in the QMRA analysis because only one sample tested positive for HAdV, which did not provide sufficient data for the model.

When inhaling aerosols in the centrifuge zone (indoor), the highest probability of illness occurs during the summer season, with a mean value of 5.4%. In contrast, the highest probability of illness resulting from oral ingestion due to contaminated surfaces and hand-to-mouth contact was observed in the workspaces during the winter, with mean and median values of 13% and 0.14%, respectively. Surfaces in the workspaces exhibited a higher probability of illness compared to those in the break room.

The mean seasonal probability of illness for a WWTP worker exceeded 90% for both HAdV transmission routes in both seasons, expect for oral ingestion from contaminated surfaces in the break room and inhalation during winter (88.48% and 1.47%, respectively).

3.3.2. QMRA simulation in the swine farm

The distributions of daily illness probability (P_{ill}) for a swine farm worker, considering the inhalation or oral ingestion of a hypothetical virus with an assumed zoonotic potential and with characteristics similar to PAdV, are depicted in Fig. 4. The graph suggests that, assuming the presence of a potential zoonotic virus with similar characteristics to PAdV, the highest probability of illness would occur

Fig. 1. Viral assignments and reads sequenced in aerosol and surface samples from the WWTP using target enrichment sequencing. Centrifuge zone aerosols (indoor) were analyzed in both winter and summer, while reactor zone aerosols (outdoor) were only analyzed in summer. Regarding surface samples, which were also analyzed in both winter and summer, two scenarios were distinguished: the workspaces and the break room.

through oral ingestion from contaminated surfaces, particularly during the summer season, with mean and median risk values of 63.6% and 87.5%, respectively. The probability of illness due to the hypothetic virus inhalation would be similar between winter and summer, with mean values of 20.8% and 21.8%, respectively, both of which are lower than the values associated with the oral ingestion route.

As an approximation, the mean seasonal probability of illness for a swine worker, in the hypothetical scenario of the presence of a virus with zoonotic potential and characteristics similar to PAdV, would be 100% for both transmission routes in both seasons.

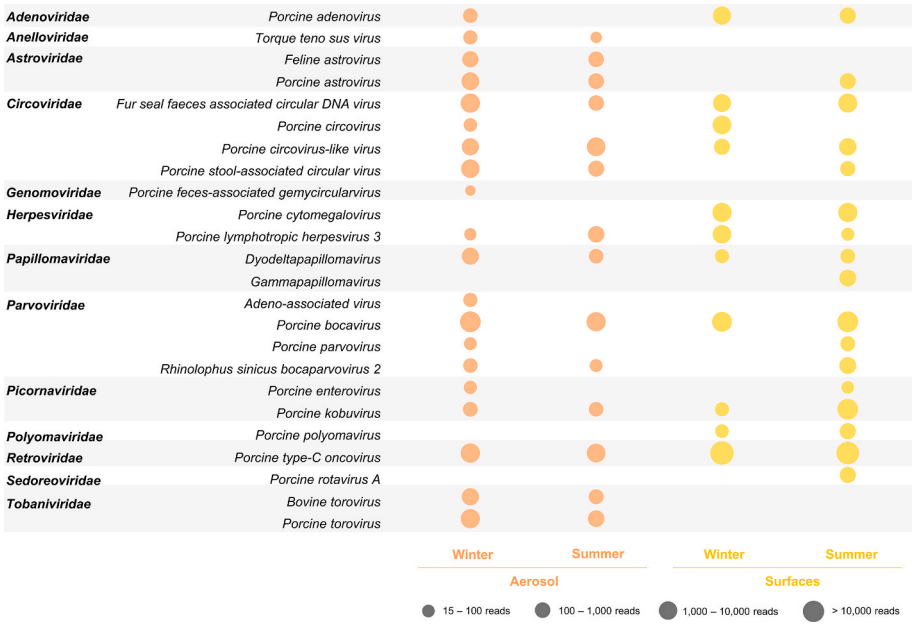

Fig. 2. Viral assignments and reads sequenced in aerosol and surface samples from the swine farm using target enrichment sequencing. Both aerosol and farm samples were analyzed in winter and summer.

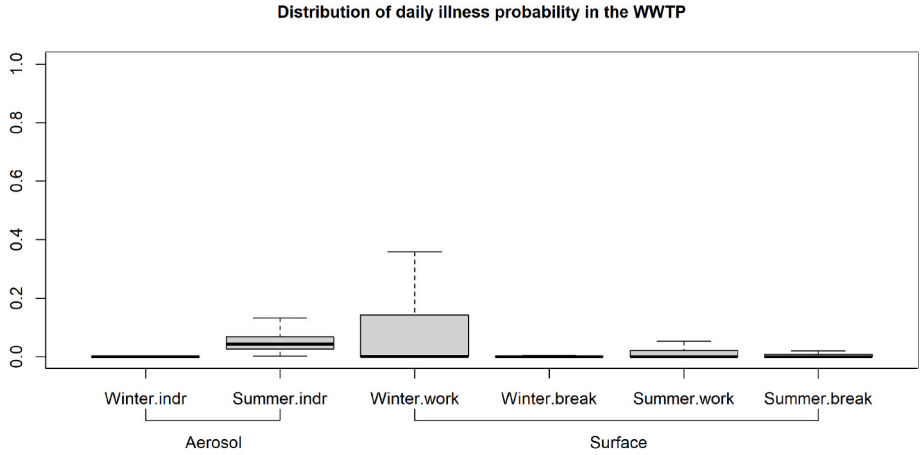

Fig. 3. Boxplots showing the distribution of daily illness probability in the WWTP, considering inhalation of HAdV from indoor aerosols in winter (Winter.indr) and summer season (Summer.indr), as well as oral ingestion of HAdV from contaminated surfaces in the workspaces and the break room in winter (Winter.work, Winter.break) and summer seasons (Summer.work, Summer.break).

3.3.3. Sensitivity analysis

Table 7 provides a summary of sensitivity analysis results, in terms of variance proportion of P_{ill} for the aerosol and surface models across the different scenarios and seasons. In the aerosol model, the analysis revealed that the parameter with the greatest influence on the model output's P_{ill} variations was the inhalation rate (r_{in}) in WWTP scenarios. On the other hand, in swine farm, P_{ill} was most sensitive to variations in the concentration of PAdV (C_{PAdV}) parameter.

In the surface model, the parameter with the highest influence was the number of hand-to-mouth contacts ($f_{cont|mouth}$) in nearly all scenarios for both WWTP and the swine farm, except for the break room in the WWTP during both seasons, where the concentration of HAdV (C_{HAdV}) parameter held the greatest influence.

4. Discussion

Using suitable sampling and monitoring tools is essential for virus detection and understanding their transmission routes. The Coriolis μ air sampler enabled the detection of viruses in both indoor and outdoor environments at the WWTP, as well as in the swine farm. Other studies have also employed the Coriolis μ sampler to detect viruses in WWTP (Brisebois et al., 2018; Stobnicka-Kupiec et al., 2022) and in swine, cattle and poultry farms (Prost et al., 2019; Salem et al., 2019; Scoizec et al., 2018).

Surface samples were collected following the long-term strategy described by Sommer and colleagues (Sommer et al., 2021). In the present study, this paper-based sticker approach demonstrated to be a successful method for detecting viruses on surfaces in the WWTP, as well as in the swine farm, using qPCR and NGS assays. A few prior studies have examined the presence of viruses on WWTP and swine farm surfaces, with most utilizing swabs for sampling (Anderson et al., 2021; López-Lorenzo et al., 2022; Prost et al., 2019; Stobnicka-Kupiec et al., 2022) while Neira and colleagues employed a sterile gauze dipped in their sampling procedures (Neira et al., 2016). To the best of our knowledge, this is the first study to utilize paper-based sticker strategy for surface sampling and subsequently perform NGS and QMRA analysis.

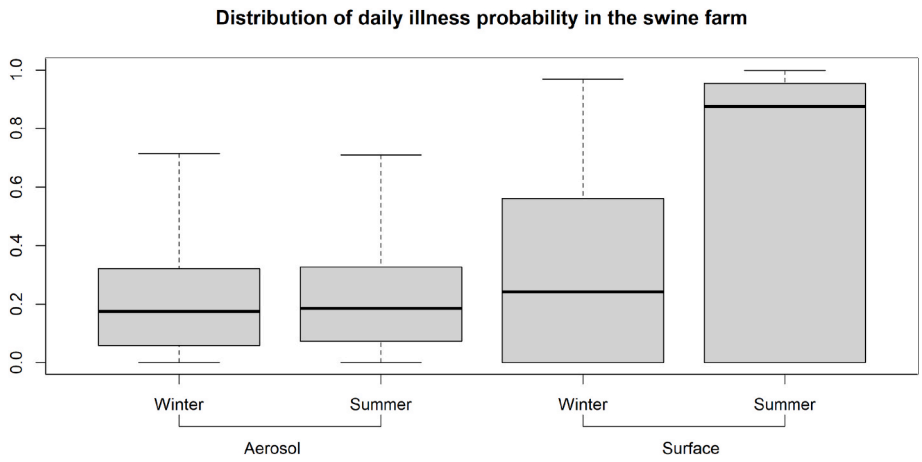

Fig. 4. Boxplots showing the distribution of daily illness probability in the swine farm, considering inhalation or oral ingestion from contaminated surfaces of a hypothetical virus with an assumed zoonotic potential and characteristics similar to PAdV.

Table 7
Sensitivity analysis in terms of variance proportion for each input parameter in the aerosol and surface models that affects the change of the probability of illness. Aerosol model (a) parameters: C_{AdV} (concentration of HAdV, WWTP, or PAdV, farm, in air), t_{exp} (exposure time) and r_{in} (inhalation rate); scenarios: WWTP.wtr.indr (WWTP winter indoor), WWTP.smr.indr (WWTP summer indoor), Farm.wtr (farm winter) and Farm.smr (farm summer). Surface model (b) parameters: C_{AdV} (concentration of HAdV, WWTP, or PAdV, farm, in surface), $TE_{surf|hand}$ (viral transfer efficiency from surface to bare hands), $f_{cont|surf}$ (frequency of hand-to-surface contacts), $f_{cont|mouth}$ (frequency of hand-to-mouth contacts) and n_{hours} (number of hours spent at workplace); scenarios: WWTP.wtr.wrk (WWTP winter work spaces), WWTP.wtr.break (WWTP winter break room), WWTP.smr.wrk (WWTP summer work spaces), WWTP.smr.break (WWTP summer break room), Farm.wtr (Farm winter) and Farm.smr (Farm summer).

a) Aerosol

	WWTP.wtr.indr	WWTP.smr.indr	Farm.wtr	Farm.smr
C_{AdV}	10.34	0.48	70.90	70.77
t_{exp}	9.62	17.20	3.58	4.05
r_{in}	49.67	73.46	32.08	34.32

b) Surface

	WWTP.wtr.wrk	WWTP.wtr.break	WWTP.smr.wrk	WWTP.smr.break	Farm.wtr	Farm.smr
C_{AdV}	116.46	105.9	123.32	105.05	70.76	10.26
$TE_{surf\|hand}$	18.81	47.1	29.38	27.82	12.87	1.28
$f_{cont\|surf}$	0.62	11.8	0.99	8.02	0.23	0.0005
$f_{cont\|mouth}$	204.90	58.9	296.56	36.30	115.42	112.47
n_{hours}	1.43	60.3	2.30	35.65	0.23	0.0004

All in all, both aerosol and surface sampling strategies allowed the detection and quantification of viral contamination using qPCR assays in samples from the WWTP and the swine farm, and the detection of sequences from vertebrate-infecting viruses through NGS analysis using TES. The viral contamination of aerosol and surface samples from the WWTP and the swine farm was evaluated by analyzing the prevalence of HAdV and PAdV, respectively. HAdV was detected in both aerosol and surface samples from the WWTP. This human virus is known to be shed in high concentrations and exhibits stability under various environmental conditions and disinfection treatments (Allard and Vantarakis, 2017). HAdV was detected in 60% of the WWTP aerosol samples. Samples collected from the centrifuge zone (indoor) exhibited higher HAdV concentration during summer (mean value of 9.12 E+01 GC/m^3) compared to winter, which is consistent with previous findings, (Masclaux et al., 2014) and it has also been described for other microbial aerosols in WWTP (Grisoli et al., 2009; Oppliger et al., 2005). This could be related to the expected high evaporation of the wastewater and the possible increased emission of bioaerosols. HAdV was also detected in aerosol samples from the reactor zone (outdoor) at lower concentrations, as expected, due to its location in an open-air area. Other studies have detected higher concentration of HAdV in indoor WWTP air samples and observed slower concentrations outdoors (Carducci et al., 2016; Masclaux et al., 2014).

As for surfaces, HAdV was detected in 65% of the WWTP workspaces samples and in 50% of the surface samples in the break room. In contrast to aerosol samples, the surface samples collected from the WWTP workspaces in summer exhibited lower concentrations of HAdV compared to winter. One possible explanation for this is that since most of the workspace surface samples were located outdoors and exposed to direct sunlight for an extended period, factors such as intensive solar radiation and high temperatures could have reduced the viral load on the paper-based stickers.

A recent investigation has explored the use of qPCR in combination with propidium monoazide (PMA) dye pretreatment, also known as viability-PCR (v-qPCR), as a method to distinguish potentially infectious and non-infectious viral particles at WWTP workplaces (Stobnicka-Kupiec et al., 2022). The study reported a mean concentration of potentially infectious AdV of 4.41 E+03 GC/m^3 in air samples, as well as 2.22 E+01 GC/cm^2 on steel and 6.68 E+00 GC/cm^2 on plastic swab surfaces samples. These findings suggest that HAdV detected in WWTP aerosol and surface samples in this study could be potentially infectious, although infectivity potential was not assessed.

In the swine farm, PAdV was detected in both aerosol and surface samples. PAdV is commonly found within swine populations, present in feces, residual water and sludge (De Motes et al., 2004; Hundesa et al., 2006; Rusiñol et al., 2014). PAdV is primarily transmitted through inhalation and the fecal-oral route, causing asymptomatic or mild illness in pigs. Typical symptoms include watery diarrhea, dehydration,

depression, and vomiting. In some cases, PAdV infection can lead to glomerulonephritis, chronic pneumonitis, and even death (Benfield and Richard, 2012; Kumthip et al., 2019; Nietfeld and Leslie-Steen, 1993). PAdV was detected in 83% of the swine farm aerosol samples, with mean concentrations values of 4.98 E+01 GC/m^3 in winter and 6.72 E+01 GC/m^3 in summer. PAdV detection in aerosol samples from a pig handling facility has been described before using conventional PCR (Poh et al., 2017) but to our knowledge, no data on PAdV concentration in swine farm aerosols have been reported to date. Regarding surfaces, PAdV was detected in 86% of the surface samples. All surface samples from summer tested positive and exhibited higher concentrations of PAdV (mean value of 5.73 E+04 GC/cm^2) compared to those reported in winter (mean value of 1.89 E+02 GC/cm^2). The presence of PAdV genetic material on swine farm surfaces may result from the deposition of airborne PAdV, as well as contact from contaminated hands. Upon observing the behavior of workers, it was noted that workers tended to wear gloves during winter but not during summer. This could provide a possible explanation for the higher PAdV concentrations detected on surfaces in the summer season. To date, studies analyzing the presence of viruses on surfaces in swine farms have primarily focused on Influenza A virus, porcine circovirus type 2 (PCV2), and porcine circovirus type 3 (PCV3) (Anderson et al., 2021; López-Lorenzo et al., 2022; Neira et al., 2016; Prost et al., 2019). Like aerosols, the concentration of PAdV on swine farm surfaces has not been previously described.

SARS-CoV-2 RNA was detected in two surface samples collected from the swine farm. Importantly, both samples were obtained on a day when there was an ongoing outbreak of the virus among the workers. While it appears that the risk of SARS-CoV-2 transmission through contaminated surfaces is low, the role of fomite transmission has been widely discussed, and the plausibility of a SARS-CoV-2 transmission chain from fomites has been suggested (Onakpoya et al., 2022). Furthermore, the susceptibility of pigs to SARS-CoV-2 is a subject of controversy (Frazzini et al., 2022). In this study, NGS data were obtained using a targeted assay (TES) with the VirCapSeq-VERT Capture Panel (Roche, Basel, Switzerland) to characterize the presence of vertebrate viral pathogens. This panel comprises approximately 2 million biotinylated oligonucleotide probes designed to bind coding sequences of all known viral taxa that infect vertebrates. While TES has been successfully employed in previous studies (Briese et al., 2015; Filipa-Silva et al., 2020; Hjelmsø et al., 2019; Itarte et al., 2021; Martínez-Puchol et al., 2020, 2022; Mejías-Molina et al., 2023; Strubbia et al., 2020), this is the first study to utilize this approach for the analysis of aerosol and surface samples.

The TES approach allowed the detection of vertebrate viruses in aerosol and surface samples from both the WWTP and the swine farm. In the case of the WWTP, the reference pathogen HAdV was detected in all aerosol and surface samples from the WWTP, except for the break room samples. The primary serotype detected was HAdV-41. Interestingly, HAdV-41 was also the predominant serotype identified through HAdV nested PCR and subsequent Sanger analysis of wastewater samples collected during the same period as the aerosol and surface sampling events in the WWTP (data not shown). HAdV-41 is etiologically associated with gastroenteritis and its high prevalence in environmental samples has been identified previously (Bofill-Mas et al., 2013). In addition to HAdV, other human and vertebrate viruses belonging to different viral families were detected in aerosol and surface samples from the WWTP. In the study conducted by Han et al. (2019), the metagenomic analysis of viral population in submicron aerosols emitted during wastewater treatment resulted in the detection of viruses primarily having bacteria or archaea as natural hosts. In this study, the TES approach facilitated the detection of vertebrate-infecting viruses, including HAdV, Human astrovirus, Norwalk virus, Human circovirus, Human cyclovirus, beta-HPV, Adeno-associated virus, and Human bocavirus. These viruses have been described as excreted and waterborne viruses, reinforcing that the source of these viruses would be the aerosolization from the wastewater. Some of these viruses cause asymptomatic infections and also outbreaks or sporadic cases with a wide range of symptoms, from mild to severe gastroenteritis to meningitis, respiratory disease, conjunctivitis, myocarditis, paralysis, or hepatitis (Rusiñol and Girones, 2017). HPVs were initially believed to be mainly epitheliotropic, but some studies have reported their presence in raw sewage and river waters, including high- and low-risk oncogenic types (Iaconelli et al., 2015; Itarte et al., 2021; La Rosa et al., 2013; Martínez-Puchol et al., 2020; Rusiñol et al., 2020). Additionally, evidence of their excretion in the feces of patients with diarrhea has been documented (Di Bonito et al., 2015; Iaconelli et al., 2015; La Rosa et al., 2013), suggesting a potential transmission route through fecal shedding. A diverse array of avian viruses was detected in both aerosol and surface samples, aligning with prior studies that found avian viruses in wastewater samples from the same WWTP and confirming the impact of poultry industry effluents (Carratalà et al., 2012).

If the viruses detected in aerosol samples are suspected to originate from wastewater aerosolization, the profile of viruses found in surface samples provided insights suggesting that the presence of most of these viruses may be more likely caused by the contaminated hands of WWTP workers. A variety of different genera of HPVs were detected in all surface samples from the WWTP. HPVs infect the skin and mucosa epithelia, with effects ranging from benign lesions, such as common warts, to malignant carcinomas (Egawa et al., 2015). In most of the surface samples, EBV was also detected. This virus, mainly transmitted through saliva, is highly prevalent in the human population and can cause latent infection, being associated with many diseases, from mild asymptomatic infection to tumorigenesis (Huang et al., 2023). Finally, another human virus detected in workspaces surfaces in winter was MCPyV. This virus is the first polyomavirus to be associated with human cancer (Liu et al., 2018). It is highly prevalent in the general population, and nearly all healthy adults asymptomatically shed MCPyV from their skin. However, this infection can lead to a lethal form of skin cancer in elderly and immunosuppressed individuals (Liu et al., 2016). Further investigation should be conducted to explore the seasonality of these viruses. The SISPA protocol, performed before library preparation to overcome the limitation of low quantities of viral genomes, may introduce bias by amplifying sequences in a random manner, as previously reported (Duhaime et al., 2012; Itarte et al., 2021; Karlsson et al., 2013). Therefore, additional research is required to elucidate whether the variations in virus detection between seasons are attributable to SISPA bias or are due to the seasonal behavior of the viruses.

Regarding the swine farm, the TES approach also enabled the detection of PAdV, in all surface samples and in the winter aerosol sample. PAdV infections are common in swine, and this virus appears to persist in swine tissues for extended periods (Paul et al., 2003). Additionally, other viruses were detected in both aerosol and surface samples from the swine farm, with most of them being described as common viruses infecting swine and belonging to different viral families. Among the detected viruses, PBoV was the viral assignment with the highest number of reads in aerosol samples. PBoV has been reported worldwide, primarily in weaning piglets, and exhibits a wide tissue tropism (Aryal and Liu, 2021). Porcine type-C oncovirus species, which accounted for the highest number of reads in surface samples, consists of Porcine Endogenous Retroviruses (PERVs) and constitute an integral part of the porcine genome (Lopata et al., 2018). Other swine viruses of veterinary interest found in both aerosol and surface samples include porcine circoviruses, parvoviruses and bocaviruses, all of which are associated with porcine respiratory disease complex (PRDC) (Qin et al., 2018). Porcine astroviruses and porcine kobuviruses have been associated with neonatal piglet diarrhea (Qiu et al., 2022). Porcine lymphotropic herpesviruses (PLHVs) are widespread in pigs and, although no association between PLHVs and any pig diseases has been described Denner (2021); Halecker et al. (2022) suggested that they could be involved in the pathogenesis of erythema multiforme diagnosed in sows. *Sus scrofa* papillomavirus 1 (SsPV1), a member of Dyodeltapapillomavirus 1 species, has previously been described in domestic pigs and has been attributed to papillomatosis tumor (Li et al., 2023; Link et al., 2017).

Additional families, including *Anelloviridae*, *Genomoviridae* and *Tobaniviridae*, were exclusively detected in aerosol samples. Among these viral families, Torque teno sus virus (TTSuV) has also been associated with PRDC (Qin et al., 2018). Porcine torovirus is another potential enteric swine pathogen, and its interspecies recombinant nature with Bovine torovirus has been revealed (Ito et al., 2016). Both toroviruses were detected in aerosol samples. Members of *Polyomaviridae* and *Sedoreoviridae* families, Porcine polyomavirus (PPyV) and Porcine rotavirus A (RVA) respectively, were found only in surface samples. A previous study described PPyV in nasal swabs of pigs with respiratory disease (Hause et al., 2018), while RVA has primarily been associated with diarrhea but also has been identified in pulmonary infections in pigs with respiratory diseases (Nelsen et al., 2022).

All these NGS results provided essential information about the vertebrate viruses to which WWTP and swine farm workers may potentially be exposed. TES can be employed as a tool for WWTPs and farms to screen for the most significant pathogenic viruses present in aerosols and/or surfaces for QMRA. Additionally, it helps identify the most abundant genotypes and assess the emergence of zoonotic viruses on farms or the evolution of animal viruses, potentially increasing the zoonotic risk.

The QMRA analyses conducted in this study aimed to estimate the occupational risk of WWTP workers' exposure to aerosols and contaminated surfaces during their work-related tasks in different seasons of the year. These analyses revealed a noteworthy risk of illness for WWTP workers if safety measures are not taken, due to the inhalation and oral ingestion of HAdV through exposure to workplace bioaerosols and contaminated surfaces. In contrast, the QMRA analysis in the swine farm is intended to be a simulation, as a hypothetical scenario that considers the presence of a virus with zoonotic potential and characteristics similar to PAdV. This extrapolation suggested a possible risk to swine farm workers if all these factors occur and if safety measures are not implemented. These findings align with previous epidemiological studies that have reported work-related symptoms and health effects among WWTP (Al-Batanony and El-Shafie, 2011; Douwes et al., 2001; Thorn and Kerekes, 2001) and swine farms workers (Andersen et al., 2004; Donham et al., 1995; Samadi et al., 2013). These studies further support the association between occupational hazards in these workplaces and adverse health outcomes.

In the WWTP setting, the daily probability of illness due to HAdV inhalation was found to be higher during summer compared to winter, while the higher risk resulting from HAdV oral ingestion was observed in the workspaces during winter. This difference in risk can be attributed to the variations in the concentrations of HAdV in the WWTP aerosols and surfaces explained before. The mean seasonal probability of illness for a WWTP worker exceeded 90% for both transmission routes in both seasons, except for oral ingestion from contaminated surfaces in the break room and inhalation during winter. All these seasonal probabilities would be exceeding the U.S. EPA benchmark ($\leq$10E−4 pppy). Inhalation of aerosols produced in a WWTP has been suggested as the primary exposure pathway for WWTP workers (Hsiao et al., 2020) and several studies have conducted QMRA to determine the risk of illness caused by inhalation exposure to HAdV (Carducci et al., 2016, 2018), and SARS-CoV-2 (Dada and Gyawali, 2021; Gholipour et al., 2021). The QMRA analyses by Carducci and colleagues also indicated a high-risk of illness for wastewater workers due to exposure to bioaerosols, reporting a higher average risk in sewage influent and biological oxidation tanks (15.64% and 12.73% for an exposure of 3 min) (Carducci et al., 2018). On the other hand, the study conducted by Amoah and coworkers reported that hand-to-mouth ingestion was the major route of exposure during untreated wastewater exposition at the head of the works (Amoah et al., 2022).

In the swine farm, a hypothetical virus with zoonotic potential and similar characteristics to PAdV was chosen for the model. It is important to note that PAdV is a virus that infects pigs and not humans, and the QMRA analysis conducted in the swine farm scenario approximates the risk of workers getting infected with a hypothetical zoonotic virus. The highest probability of illness occurred through this hypothetical virus oral ingestion from contaminated surfaces, especially during the summer season. The mean seasonal probability of illness for a swine farm worker was 100% for both transmission routes in both seasons, exceeding the U.S. EPA benchmark of $\leq$10E−4 pppy. A study assessed the occupational risk of zoonotic influenza infection in swine workers, and it was found that spending 25 min working in a barn during an influenza outbreak in a swine herd could be sufficient to cause zoonotic infection in a worker through airborne transmission (Paccha et al., 2016).

The importance of the input variables in QMRA analysis was identified through a sensitivity analysis, which tests the relative impact of stochastic input variables on the result of the models (Federigi et al., 2019). In the aerosol model, the analysis revealed that the parameter with the greatest influence on the model's output, P_{ill} variations, was the inhalation rate (r_{in}) in WWTP scenarios and the concentration of PAdV (C_{PAdV}) in swine farm. HAdV concentration was the predominant factor in the sensitivity analysis in the estimated risk described by Carducci et al. (2018). In the surface model, the parameter with the highest influence was the number of hand-to-mouth contacts ($f_{cont|mouth}$) in nearly all scenarios for both WWTP and the swine farm, except for the break room in the WWTP during both seasons, where the concentration of HAdV (C_{HAdV}) parameter had the greatest influence. These results are consistent with the sensitivity analysis performed by Lanzarini et al. (2022), which reported that the most impacting parameters in the hand-to-mouth model were the frequency of hand-to-mouth contact and the concentration of HAdV.

While QMRA is a valuable tool for estimating occupational risk, it is essential to acknowledge the limitations of the analysis. Firstly, although this study is focused on HAdV and PAdV, the QMRA analysis could potentially be applied to other biological agents that pose an occupational risk for WWTP and swine farm workers. However, selecting adenoviruses as an index pathogen due to their prevalence and environmental persistence allows for a conservative estimation of the levels of other pathogens in the environment (Carducci et al., 2018). It is important to note that PAdV is a virus that infects pigs and not humans. It was chosen as a model to approximate the risk of workers getting infected with a hypothetical virus assumed to have zoonotic potential and infectivity equivalent to adenovirus types. Nevertheless, the probability of spillover for this virus was not incorporated into the models. Additionally, the concentration of the viral pathogens was determined using qPCR, but the infectivity potential was not verified. Therefore, the assumption of a fixed ratio of 700 GC = 1 $TCID_{50}$ across all scenarios in this study might not accurately reflect the true infectivity potential. Furthermore, the dose-response models used from Teunis et al. (2016) were applied indiscriminately to HAdV and PAdV, even though the study focused on AdV-4, AdV-7, and AdV-16 adenovirus types. Important factors such as secondary transmission of infection, immunity, and variations among workers based on gender, age or health conditions were not considered in the analysis. The deposition efficiency of aerosols in the respiratory tract was also not contemplated in the model for the inhalation route. These limitations highlight the need for further investigation in future studies to enhance the accuracy of QMRA.

The QMRA and NGS analysis performed in this study will facilitate evidence-based decision-making by managers and provide new insights into protection measures and good practices for workers. Assessing occupational risk is essential, and the measures to control it must be defined, as established by the European Union (EU) Directive 2000/54/EC on the protection of workers in the case of activities involving exposure to biological agents (European Commission, 2000). Nevertheless, it is important to note that the directive does not specify exposure limits. The application of QMRA can provide valuable insights for the decision-making process in occupational risk management. By understanding the risks associated with microbial hazards in the workplace, decision-makers can effectively identify and prioritize these risks.

This enables the development of appropriate management strategies and the implementation of safety measures to protect workers.

From a risk management perspective, controlling key factors such as pathogen concentration and exposure time can help mitigate the risks associated with exposure to biological agents. Additionally, increasing the frequency of professional trainings on workplace hazards, implementing protective measures for workers like masks and gloves, and enforcing mandatory vaccinations among WWTP workers can reduce potential risk effects (Jaremków and Agata Kawalec, 2018). The use of personal protective equipment (PPE), including respirators and eye protection, is recommended for swine workers when working with sick animal to reduce risk of cross-species transmission of IAV as well as exposure to other contaminants on swine facilities (Paccha et al., 2016).

These measures collectively contribute to a safer working environment and better protection for workers. The aerosol and surface sampling methods used in the present study could be employed in swine farm as a noninvasive and efficient means to detect and characterize circulating virus in these facilities, as previously suggested for conducting surveillance of novel influenza viruses and other animal viruses through bioaerosol sampling (Anderson et al., 2016). Other pathogenic swine viruses of veterinary interest that could be monitored are PRRSV, PEDV, CSF and ASFV.

Detecting specific viruses in both swine farm aerosol and surface samples, specially through NGS approaches, could serve as an early warning method for the detection of emerging zoonotic pathogens.

5. Conclusions

WWTP and swine farm workers are exposed to viruses during their workday and could face occupational risks associated with exposure to viral pathogens if measures are not implemented. Viral contamination of aerosol and surface samples from these workplaces was identified by qPCR assays, and vertebrate pathogenic viruses were detected through TES.

The application of QMRA models to evaluate the risks associated with aerosol and surface exposures provides valuable estimations of the occupational risk in these workplaces. The daily illness probability due to HAdV inhalation in WWTP was higher in summer, whereas the greatest risk for HAdV oral ingestion from contaminated surfaces was observed in the workspaces during winter. In the swine farm QMRA simulation, considering a hypothetic virus with zoonotic potential and characteristic similar to PAdV, the highest probability of illness occurred through oral ingestion from contaminated surfaces, especially during the summer season.

Overall, this study highlights the significance of evaluating and managing viral pathogen exposure risks in occupational settings to protect the health and well-being of WWTP and swine farm workers. To minimize the risk of virus transmission, it is advisable to implement effective cleaning procedures capable of degrading viral particles on frequently touched surfaces and objects. Additionally, the use of personal protective equipment, especially in areas where bioaerosol particles are aerosolized and can pose a risk, should be made compulsory. Aerosol and surface sampling of WWTP and swine facilities could be implemented as monitoring tools for conducting surveillance to detect emerging zoonotic pathogens.

Data availability statement

The raw sequencing data generated during the current study are available in Zenodo under the DOI number 10.5281/zenodo.8424685.

Patents

The use of paper-based stickers used in this study as a sampling method for long-term monitoring of surfaces is disclosed on Patent Application Publication No. WO 2020/182,924 A1, titled 'A Novel Sampling Method for Long-Term Monitoring of Microbes' (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020182924). The inventors of this patent application are Martin Bobal, Anna Witte, Patrick Mester, and Peter Rossmanith, and the current applicant is Merck Patent GmbH (Frankfurter Strasse 250, 64,293 Darmstadt).

CRediT authorship contribution statement

Marta Itarte: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Miquel Calvo:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Formal analysis, Data curation. **Lola Martínez-Frago:** Methodology, Investigation. **Cristina Mejías-Molina:** Writing – review & editing, Visualization, Investigation. **Sandra Martínez-Puchol:** Writing – review & editing, Visualization, Methodology, Investigation. **Rosina Girones:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Gertjan Medema:** Writing – review & editing, Validation, Supervision, Formal analysis. **Sílvia Bofill-Mas:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Marta Rusiñol:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2024.114360.

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