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The virucidal effect of 405 nm visible light on selected viruses

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Abstract

Visible light with a wavelength of 405 nm has emerged as a promising alternative to ultraviolet radiation for environmental disinfection due to its antimicrobial efficacy and safety for higher organisms. This study investigated the virucidal potential of 405 nm light on two structurally distinct model viruses: feline calicivirus (FCV), an RNA virus, and feline parvovirus (FPV), a non-enveloped DNA virus. Viral suspensions were exposed to 405 nm light for 8 h using a commercial germicidal LED device. Samples were prepared both in a dried state and resuspended in an organically rich culture medium (DMEM with 2% FCS) to assess the influence of environmental conditions and media composition. The results demonstrated that 405 nm irradiation significantly reduced FCV infectivity, particularly in resuspended samples (up to $10^{2.3}$ $TCID_{50}$/ml). In contrast, FPV showed no significant reduction in infectivity or genome quantity under the same conditions, regardless of sample state. These findings indicate that the virucidal efficacy of 405 nm light is strongly influenced by viral structure, character and size of the genome, and the surrounding medium.

Blue light, viral inactivation, antiviral phototherapy

Visible light with a wavelength of 405 nm has garnered increasing scientific attention as a potential alternative to traditional ultraviolet (UV) disinfection methods. Unlike UV-C radiation, which inactivates microorganisms through direct DNA damage but poses safety risks to higher organisms, 405 nm light operates via a different mechanism: the photoexcitation of endogenous or exogenous porphyrins and flavins. This excitation leads to the generation of reactive oxygen species (ROS), which subsequently cause oxidative damage to cellular structures, resulting in microbial inactivation (Plavskii et al. 2018).

Numerous studies have confirmed the broad-spectrum antimicrobial efficacy of 405 nm light against bacteria, bacterial spores, and fungi (Zhang et al. 2016; Bumah et al. 2017; Tomb et al. 2017). Its germicidal action, although generally less potent than UV-C light, offers a key advantage in its safety profile, making it suitable for continuous use in occupied environments (Sliney 2013; Maclean et al. 2014). However, its virucidal potential remains less well characterized, especially due to the structural differences between viruses and other microorganisms. Viruses typically lack endogenous porphyrins, which may limit their sensitivity to this form of photoinactivation.

Recent research suggests that the virucidal activity of 405 nm light can be enhanced in the presence of photosensitizers or complex organic media, which facilitate the formation of ROS (Wu et al. 2015; Tomb et al. 2017; Ho et al. 2020). This raises the possibility of using such media to increase viral susceptibility, especially for enveloped viruses whose lipid membranes may be more prone to oxidative damage. However, the precise mechanisms remain under investigation, and the effectiveness of 405 nm light on non-enveloped, environmentally resistant viruses is largely unexplored.

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The present study aimed to evaluate the virucidal efficacy of 405 nm visible light on two model viruses of veterinary importance: feline calicivirus (FCV) and feline parvovirus (FPV). These viruses were chosen for their differing structural characteristics and clinical relevance in multi-animal settings. FCV, an RNA virus, is known for causing respiratory and oral disease in cats and tends to be moderately stable in the environment (Radford et al. 2007). In contrast, FPV is a small, non-enveloped DNA virus that is highly resilient and can persist on surfaces for extended periods (Gordon and Angrick 1986). Given the challenges of environmental decontamination in shelters, clinics, and breeding facilities, this study investigates whether 405 nm light, in combination with medium composition, could offer a viable disinfection strategy for these pathogens.

Materials and Methods

Viruses

Field strains of FPV and FCV isolated in the Czech Republic were propagated and titrated in CRFK (Crandell-Rees feline kidney) cells using standard virological protocol. Cultivation flasks were inoculated with the virus and when 80 to 100% of the monolayer showed a viral cytopathic effect, cells were frozen, thawed clarified by centrifugation at 300 × *g* for 5 min, titrated, aliquoted and stored at −80 °C until further use. The FCV titre was 10^6 $TCID_{50}$/ml, the FPV titre was 10^4 $TCID_{50}$/ml.

Cells

Crandell-Rees feline kidney cells, were obtained from the American Tissue Culture Collection and were cultivated using standard protocols in growth Dulbecco’s Modified Eagle’s medium (DMEM) (Biosera, Cholet, France), enriched with 10% antibiotic solution (Antibiotic-Antimycotic, Biosera) and 5% foetal calf serum (FCS). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. After the viral infection, the concentration of FCS was reduced to 2%.

Light source and irradiation setup

The visible light disinfection product used in this study was a commercially available Germi line 405 lamp. The viruses were exposed to radiation for 8 h, which is the time recommended by the manufacturer of the equipment used. The maximum emitted power of the lamp is in the region of 405 nm, the maximum power is 22 W. The SPECTRA 1 spectrometer (KVANT, s.r.o., Bratislava, Slovakia) was used to verify the wavelength of the emitted radiation.

Viruses were suspended in DMEM medium supplemented with 2% FCS. Suspensions of both viruses were pipetted in an amount of 150 µl onto Petri dishes and allowed to dry for 4 h to achieve a dry state of the sample before starting irradiation. A total of 10 replicates of dried samples of each of the two viruses were applied to the surface of the Petri dish. Before starting the irradiation, 10 replicate samples of each of the two viruses, resuspended in 150 µl of culture medium, were further applied to the surface of the Petri dish (resuspended samples). The exposure of pathogens to the application of radiation was carried out in an experimental room with an average temperature of 24 °C and a humidity of 40%. The virus samples were placed in such a way that the distance between the samples and the light-generating device was 1 m so that no light radiation fell on them and at the same time the same environmental conditions (temperature, humidity) were maintained. Control non-irradiated samples (dried virus and virus resuspended in culture medium) were placed under the same conditions. After irradiation, the samples were transferred to 1,000 µl DMEM with 2% FCS and filtered through a 0.25 µm bacteriological filter (Sarstedt AG & Co. KG, Nümbrecht, Germany).

Virus quantification

Quantitation of viruses before and following irradiation was performed by virus titration and a quantitative PCR/RT-PCR tests. While quantification of the virus by qPCR/qRT-PCR assay allowed the detection of viral particles containing the genome, titration of the virus by cell culture then enabled the determination of infectious virus particles.

Virus titration

Serial 10-fold dilutions of the virus samples (50 µl) were used to infect CRFK cells seeded in 96-well tissue culture plates in triplicate. Cell culture control (cells maintained in cultivation medium without the addition of the virus) and virus control (FPV and FCV of a known titre) were included with each titration. The plates were assessed for the presence of viral CPE after four days of incubation at 37 °C in a humidified atmosphere with 5% CO_2. The titres were calculated using the method of Reed-Muench (Reed and Muench 1938) and presented as tissue culture infective dose 50% ($TCID_{50}$).

qPCR test for feline parvovirus

Primers and probe for qPCR test were designed according to Calatayud et al. (2019) and synthesized (Generi Biotech, Hradec Králové, Czech Republic) (Table 1). The qPCR was carried out in 20 µl reaction mixtures consisting of 10 µl of 2 × concentrated Luna Universal Probe qPCR Mastermix (BioLabs, Long Island City, NY, USA), 1 µl of each primer (40 µM final concentrations) and 0.5 µl of probe, 2 µl of DNA template, and dH_2O. The thermocycling profile for FPV included initial denaturation 95 °C for 3 min, then followed by 45 cycles of 95 °C for 5 s, 60 °C for 30 s.

Table 1. Primers and probe for feline parvovirus (FPV) amplification.

Name of primer	Sequence	Direction
FPV for	5’-TGGAACTAGTGGCACACCAA-3’	forward
FPV rev	5’-AAATGGTGGTAAGCCCAATG-3	reverse
FPV probe	5’-CAGGTGATGAATTTGCTACAGG-3’	

RT-qPCR test for feline calicivirus

The RT-qPCR method with SYBR Green intercalation dye was chosen to compare the survival ability of calicivirus. Degenerated primers for RT-qPCR test were designed according Helps et al. (2002), Wilhelm and Truyen (2006) and synthesized (Generi Biotech) (Table 2). The RT-qPCR was carried out in 20 µl reaction mixtures consisting of 10 µl Xceed qPCR SG 1-step 2 × Mix Lo-ROX (IAB, Praha, Czech Republic), 0.8 µl of each primer (40 µM final concentrations), 1 µl of RNase (IAB), 2 µl of RNA template and dH_2O. The thermocycling profile for FCV included reverse transcription 50 °C for 10 min, initial denaturation 95 °C for 3 min, then followed by 40 cycles of 95 °C for 5 s, 60 °C for 25 s, melting curve of 90 °C for 10 min.

Table 2. Primers for feline calicivirus (FCV) amplification.

Name of primer	Sequence	Direction
FCV1	5’-TAATTCGGTGTTTGSTTTGGCCTGGGCT -3’	forward
FCV2	5’-CATATGCGGCTCTGATGGCTTGAAACTG-3’	reverse
FCV3	5’-GNAAAGCWCAACAAATTGAAT -3’	forward
FCV4	5’-CHTGTACCCTYTGCTCAAG -3’	reverse

Results

The results demonstrated a substantial reduction in feline calicivirus infectivity following exposure to 405 nm light, particularly in resuspended samples (Figs 1 and 3). In contrast, feline parvovirus exhibited no significant reduction in either infectivity or genomic copy number under the same experimental conditions, irrespective of the sample state (Figs 2 and 4).

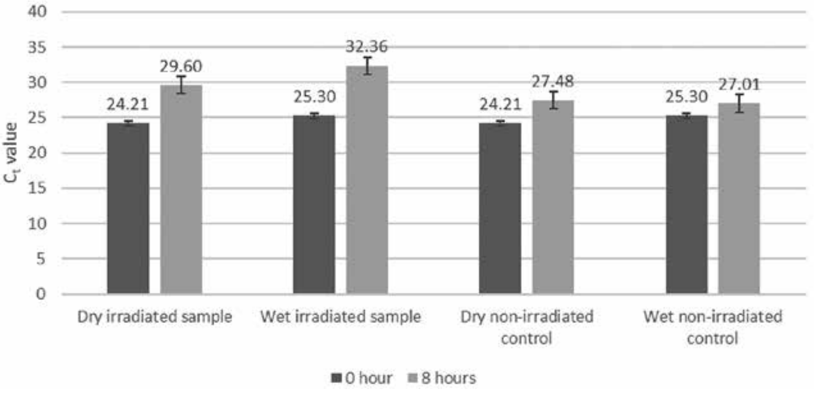

Fig. 1. Threshold cycle (C_t) values of feline calicivirus (FCV) samples before (0 hour) and after 8 hours of irradiation, and of non-irradiated controls before (0 hour) and after an 8-hour interval.

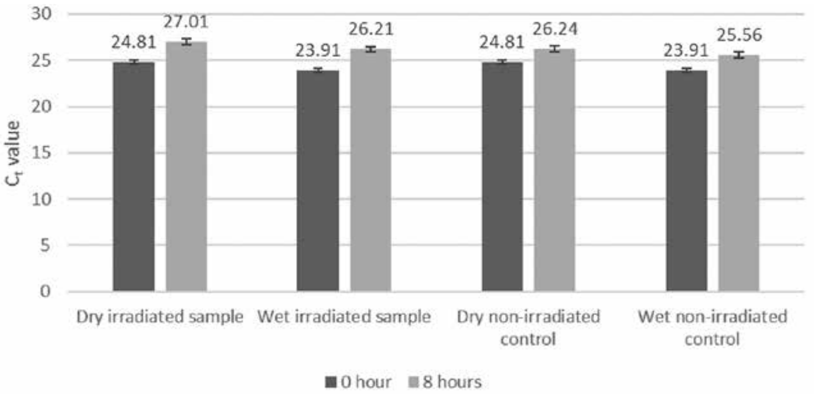

Fig. 2. Threshold cycle (C_t) values of feline parvovirus (FPV) samples before (0 hour) and after 8 hours of irradiation, and of non-irradiated controls before (0 hour) and after an 8-hour interval.

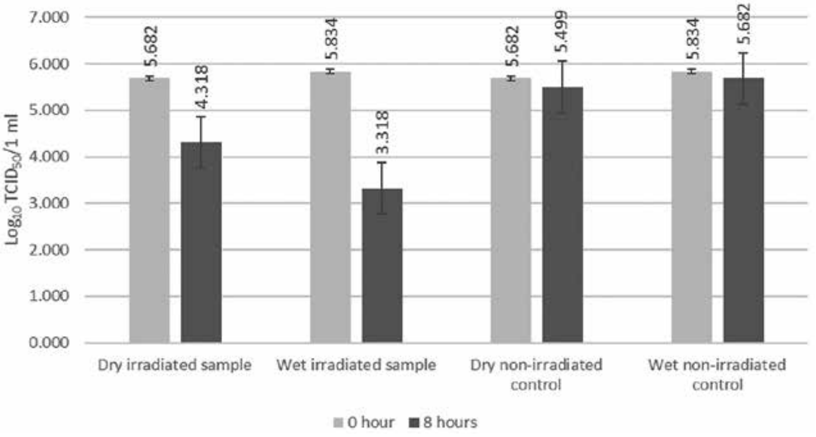

Fig. 3. 50% tissue culture infectious dose (TCID_{50}) values of feline calicivirus (FCV) samples measured before (0 hour) and after 8 hours of irradiation, and of non-irradiated controls before (0 hour) and after an 8-hour interval.

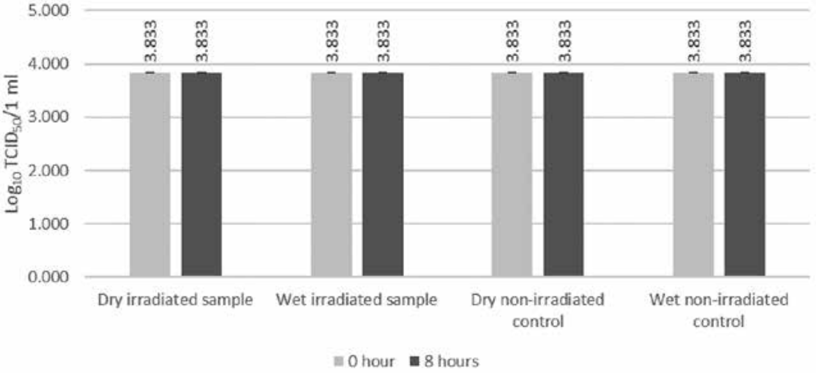

Fig. 4. 50% tissue culture infectious dose (TCID_{50}) values of feline parvovirus (FPV) samples measured before (0 hour) and after 8 hours of irradiation, and of non-irradiated controls before (0 hour) and after an 8-hour interval.

Discussion

The results of several studies show that light with a wavelength of 405 nm yields promising outcomes in environmental and air decontamination and demonstrates significant antimicrobial properties against a broad spectrum of bacterial and fungal pathogens, including spores (Zhang et al. 2016; Tomb et al. 2018). Although the germicidal efficacy of 405 nm light is generally lower than that of UV radiation, it offers the notable advantage of being safer for higher organisms, including humans and animals (Maclean et al. 2014). In our experiments, we concentrated on two viral agents with distinct structural and biological properties: FPV and FCV. These viruses differ markedly in their genome size, composition, and particle size. FPV is a small, non-enveloped DNA virus measuring approximately 25 nm in diameter. It is characterized by exceptional environmental stability and is capable of persisting for several weeks to months in various settings (Gordon and Angrick 1986). In contrast, FCV is a virus with larger RNA genome, rendering it more susceptible to degradation and disinfection strategies. Both viruses are commonly encountered in animal shelters, breeding stations, and veterinary clinics, where their resilience in the environment poses significant biosecurity risks.

We specifically evaluated the virucidal effect of 405 nm light on both dried viral samples and samples suspended in an organically rich culture medium (DMEM supplemented with 2% FCS). Organic components can play a crucial role in modulating the efficacy of photoinactivation, as they may either absorb light or act as sources of photosensitizers that enhance ROS generation.

A fundamental challenge in evaluating the virucidal efficacy of visible light is the distinction between physical presence and biological viability of viral particles. Ionizing radiation may induce genome damage or structural protein modifications, rendering the virus non-infectious while preserving the integrity of its genetic material. Therefore, we employed a dual-method approach to quantify the impact of irradiation: RT-qPCR/qPCR for genome detection and virus titration in cell cultures for assessing infectivity. This strategy enabled us to differentiate between structural persistence and functional inactivation.

Our results showed that after 8 h of exposure to 405 nm radiation, a significant reduction in FCV infectivity was observed. The RT-qPCR analysis of irradiated FCV samples showed only a minimal change in the number of detectable viral genomes, whereas titration data revealed a 100-fold reduction in viable particles. This disparity indicates that FCV particles were rendered non-infectious, despite retaining their RNA genomes, likely due to damage to viral structures or subtle changes at the genomic level.

Conversely, FPV exhibited remarkable resistance to 405 nm irradiation. Neither qPCR analysis nor virus titration indicated any significant reduction in viral load after exposure, under both dried and resuspended conditions. This robustness could stem from FPV's compact structure, DNA-based genome, and lack of a lipid envelope, all of which contribute to its intrinsic resilience.

Our findings align with previous work by Tomb et al. (2017), who demonstrated effective inactivation of FCV using 405 nm light, though their setup involved a significantly shorter distance (4 cm) between light source and sample. Ash et al. (2017) further confirmed the penetration potential of 405 nm light in aqueous media, which increases with exposure duration. In our experiments, this was evident in the enhanced reduction of FCV titre in resuspended samples compared to dried ones. Specifically, the FCV titre in the resuspended state decreased by approximately $10^{2.3}$ $TCID_{50}$/ml, while the dried sample showed a less pronounced reduction of $10^{1.2}$ $TCID_{50}$/ml. Notably, this effect was only apparent in titration data, as RT-qPCR measurements did not reflect changes in genome presence.

Despite promising results with FCV, the mechanism of action of 405 nm light against viruses remains incompletely understood. Prior studies suggest that viruses are inherently less susceptible to 405 nm photoinactivation due to the scarcity of endogenous porphyrins and flavins, which are necessary for ROS production (Rathnasinghe et al. 2021). Nonetheless, suspending viruses in organically rich media such as DMEM may overcome this limitation by providing exogenous photosensitizers. Indeed, studies by Wu et al. (2015) and Tomb et al. (2017) demonstrated that curcumin-supplemented media or complex media enabled effective inactivation of noroviruses and other small, hard-to-kill viruses.

Another possible mechanism involves indirect effects of the emission spectrum, particularly if it extends into the UVA region, as suggested by Girard et al. (2011). UVA photons may induce oxidative damage to viral surface proteins. In our FCV experiments, the more substantial reduction in the virus titre in resuspended conditions supports this hypothesis, although further controlled experiments would be necessary to confirm this pathway.

Feline parvovirus presented a more challenging target. Its exceptional environmental stability is well-documented, with the ability to remain infectious for months or even years (Gordon and Angrick 1986). Even prolonged irradiation for 8 h failed to induce significant changes in viral infectivity. However, a slight trend toward reduced infectivity in the resuspended FPV samples compared to dried ones was observed. This observation suggests that the culture medium may have marginally enhanced the ROS production, though not to a degree sufficient for effective inactivation.

These findings correspond with those of Tomb et al. (2014), who used bacteriophage φC31 as a model non-enveloped virus and found that irradiation in the presence of enriched media was necessary for any detectable effect. Similarly, Rathnasinghe et al. (2021) emphasized the need for significantly higher irradiation doses and longer exposure times when targeting non-enveloped viruses. One plausible explanation for this discrepancy is that the lipid envelope in viruses acts as a photosensitive structure that can absorb radiation and facilitate either ROS generation or direct envelope degradation.

To date, no other studies have reported the effects of 405 nm visible light on parvoviruses specifically. Our work thus contributes a novel insight into the resistance profile of FPV under such conditions. By contrast, the virucidal effect of 405 nm light on enveloped viruses has been replicated in several models, including SARS-CoV-2, influenza A, and noroviruses (Tomb et al. 2017; Hadi et al. 2020; Gardner et al. 2021; Rathnasinghe et al. 2021; Hessling et al. 2022).

Taken together, our study highlights the potential of 405 nm visible light for inactivating viruses under appropriate conditions, particularly when combined with photosensitizer-rich media. However, its effect on non-enveloped viruses remains limited, requiring optimization of irradiation protocols or adjunctive strategies to improve virucidal efficacy.

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