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Short communication

Effective inactivation of porcine epidemic diarrhea virus on contaminated surgery masks by low-concentrated sodium hypochlorite dispersion

M. Antas[1], A. Szczotka-Bochniarz[1], G. Woźniakowski[2]

[1] Department of Swine Diseases,
National Veterinary Research Institute, Partyzantow 57 Avenue, 24-100 Pulawy, Poland
[2] Department of Diagnostics and Clinical Sciences, Institute of Veterinary Medicine,
Nicolaus Copernicus University in Torun,
Faculty of Biological and Veterinary Sciences, Gargina 7, 87-100 Torun, Poland

Abstract

Coronaviruses present a considerable concern for humans and animals. The current worldwide pandemic of SARS-CoV-2 virus showed many gaps in understanding of coronaviruses spread and transmission. Because of lack of effective vaccine against SARS-CoV-2 the only preventive measures are represented by wearing protective masks and gloves thus limiting potential risk of contact with the airborne virus. Inversely, the limited time of protective function of the masks presents another drawback of their use. Therefore, the application of disinfection agent dispersed on the surface of protective masks may enhance their effectivity and safety of their application. The aim of the study was to examine the virucidal efficacy of low-concentrated sodium hypochlorite dispersed using ultrasonic humidifier on the surface of surgery masks. The study was conducted using SARS-CoV-2 surrogate virus, namely porcine epidemic diarrhea virus (PEDV) representing a model with similar biophysical properties and genomic structure to human coronaviruses. Five different concentrations of the disinfectant with different content of sodium hypochlorite were selected for the study. A final concentration of 0.228 g/L sodium hypochlorite effectively inactivated the PED virus and may support the biosafety of masks usage.

Key words: coronaviruses, SARS-CoV-2 disinfection, surgery/protective masks, dispersion, portable humidifier, porcine epidemic diarrhea, PEDV, sodium hypochlorite.

Correspondence to: M. Antas, e-mail: marta.antas@piwet.pulawy.pl

Introduction

The coronaviruses of humans and animals are the serious threat for health and life, causing not only infections of the respiratory tract but also digestive system. Some of them, such as SARS-CoV (severe acute respiratory syndrome coronavirus), were first found in 2002 in Guangdong Province, China. The estimated origin of SARS-CoV is associated with the street market where civets, mammals belonging to the family of warvery *(Viveridae*), were traded. The meat of these animals is a valued culinary specialty in the People's Republic of China (PRC). Ten years later, in 2012, a new variant of the coronavirus called MERS-CoV (middle east respiratory syndrome coronavirus) appeared (Fong 2017, Kampf et al. 2020). Both SARS and MERS have a zoonotic potential. MERS coronavirus has a genomic sequence similar to that found in bats and monocampal camels. Despite the initial 'warnings' related to the threat of meat consumption from non-farmed animals, such as civets or predators, a new variant of coronavirus (COVID-19 or SARS-CoV-2) emerged in 2019. SARS--CoV-2 probably originates from a species of placental mammals called pangolins (*Pholidota*), from which the virus was probably transmitted to humans (Fong 2017, Cui et al. 2019, Kampf et al. 2020). Since the first SARS epidemic in 2002, coronaviruses may caused many disease cases with high mortality. It is estimated that currently the only strategy that may stop the crisis with COVID-19 pandemics in the world is to develop a vaccine with introduction of effective pharmacological agents.

Coronaviruses have a one of the largest genomes among RNA viruses, which in combination with high genetic variability leads to changes in the genomic sequence. This may lead to frequent mutations, changes in pathogenicity, changes in cellular tropism, and the ability to adapt to new hosts. The main source of mutations are errors resulting from the rapid and inaccurate replication of ribonucleic acid by RNA-dependent RNA polymerase (RdRP) (Jackwood and Wit 2013, Fehr and Perlman 2015, Rasmussen et al. 2018, Cui at al. 2019).

Porcine epidemic diarrhea (PED) is highly contagious and devastating enteric disease of pigs. Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded RNA virus, belonging to *Coronaviridae* family, *Alphacoronavirus* genus (Rasmussen et al. 2018, Vlasova et al. 2020). The virus causes acute diarrhea, vomiting and dehydration with mortality reaching up to 100%. For the first time PED has been observed in 1971 in the United Kingdom and spread to other European countries. PEDV reemerged in China in 2010, resulting in significant economic losses. Then in 2013 the epidemic occurred in the United States. The losses during this epidemic were estimated as 7 million pigs (Wang et al. 2019, Vlasova et al. 2020).

Face masks play an important role in protecting against COVID-19 virus transmission. Reusable masks are good alternative to disposable masks but after taking off the mask, it should be effectively disinfected before using it again. Not all reusable masks may be washed or ironed therefore a basic humidifier and disinfectant with disinfection agent e.g sodium hypochlorite may be a good alternative to home disinfection of masks.

SARS and MERS can persist on various surfaces such as metal, glass or plastic up to 9 days. They can be effectively inactivated with 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite in 1 minute. Other biocides, such as 0.05-0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate, are not as effective (Kampf et al. 2020).

Hypochlorites are most commonly used chlorine disinfectants that may be available as liquids (e.g. sodium hypochlorite) or solids (e.g. calcium). Chlorine compounds are very effective disinfectants and are widely used for disinfecting hard surfaces as well as in food and dairy industry and for drinking water treatment (McDonnell and Russell 1999). Sodium hypochlorite has a broad spectrum of antimicrobial activity and is effective against viruses, bacteria, fungi and spores. It exhibits relatively low residual toxicity and can be produced in large quantities at low production costs. Sodium hypochlorite is one of the agents recommended by WHO as having virucidal activity against SARS and other human coronaviruses (McDonnell and Russell 1999, Juszkiewicz et al. 2019, World Health Organization 2020).

Materials and Methods

Vero-81 (ATCC No. CCL-81) line were used for PEDV isolation. Vero cells were grown in Minimum Essential Medium Eagle (Sigma) supplemented with antibiotics (10 units/mL of penicillin, 100 µg/mL streptomycin, and 0,25 µg/mL Amphotericin B) (Gibco) and 10% FBS - heat inactivated fetal bovine serum (Gibco) at 37°C with 5% CO_2.

The tissue culture infection dose of PEDV used for this experiment was 1 x $10^{4,5}$ $TCID_{50}$/1ml. One-day-old Vero cells monolayers were used for virus inoculation. Before inoculation cells were washed 2 times with PBS (–). Then they were incubated for 2 hours with growth medium (MEM) supplemented with 5 µg/ml trypsin and 1% A/A, 0,3% tryptose phosphate broth (TPB, Sigma). Samples and positive control were tested at various dilutions: 1:2, 1:3 and titrated from dilution

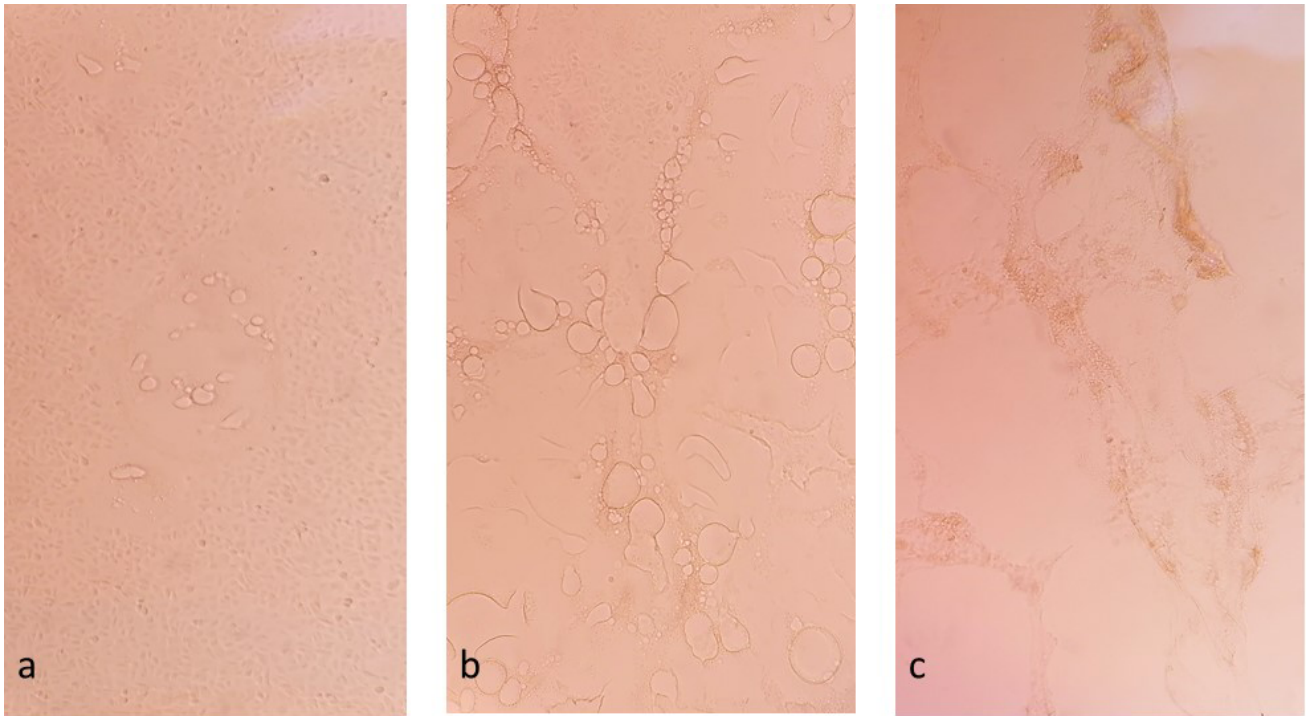

Fig. 1. Characteristic cytopathic effect in Vero-81 cells after PEDV infection observed at various intervals of time: a - 17 hours post inoculation, b - 36 hours post inoculation, c - 6 days post inoculation.

Table 1. Results obtained in the first experiment (+ - CPE observed, CTX - cytotoxic effect).

	20 %	40%	60%	80 %	100%
10 min	+	-	-	CTX	CTX
20 min	+	-	CTX	CTX	CTX
30 min	-	-	CTX	CTX	CTX

10^{-1} to 10^{-6}. Sample and control dilutions were made in growth medium (MEM) supplemented with 5 µg/ml trypsin and 1% A/A, 0,3% tryptose phosphate broth (TPB, Sigma) and incubated at 37°C with 5% CO_2.

The humidifier was used for dispersion with the addition of a disinfectant that contained 0.57 g/L of sodium hypochlorite in 5 different concentrations: 20% (0.114 g/L), 40% (0.228 g/L), 60% (0.342 g/L), 80% (0.456 g/L) and 100% (0.57 g/L) to assess virucidal efficacy and select the appropriate dilution.

The carrier of the virus, which was later treated with steam from a humidifier with the addition of a virucidal substance, were disposable surgical masks. The virucidal efficacy study was performed twice using two other techniques described below and the same results were obtained in both experiments.

Five surgical masks were used for the first stage of the study. A circle with a diameter of 8 cm was marked at the center of each mask. The virus was added to the atomizer, which was applied to the virus at a distance of 10 cm from the mask using 3 sprays for each mask. The masks were subjected to sodium hypochlorite dispersion in five different dilutions (20%, 40%, 60%, 80%, 100%) for various time intervals (10 min, 20 min, 30 min). After dispersion, 3 swabs were taken from each mask. The first swab was taken after 10 min dispersion, the second swab after 20 min and the third swab after 30 min. The same was done with each dilution of sodium hypochlorite. In total, after the end of first experiment, 15 swabs were collected and diluted in 0.5 ml MEM in 2 ml Eppendorf tubes, vortexed for 2 min and centrifuged at 4,000 × g for 1 minute. The supernatant served as the inoculum of Vero cells. The positive control was a swab taken from the mask before using the disinfectant.

Three surgical masks were used during the second stage of the experiment. A 1 cm x 1 cm point was determined on each mask and flooded with 100 µl of virus suspension so that it penetrated all layers of the mask. The masks were fogged with a 40% sodium hypochlorite solution. Then the marked fragment and the extreme fragment of the mask were cut out to check if the mask was completely disinfected. Samples of 6 mask slices were obtained: two slices from the first mask after 10 minutes of dispersing, two from the second mask after 20 minutes and two of the third mask after 30 minutes.

The mask fragments were soaked in 0.5 ml MEM in 2 ml Eppendorf tubes, vortexed for 2 min and centrifuged at 4,000 × g for 1 minute. The supernatant served as the inoculum of Vero cells. As a positive control, a virus strain that was applied to the masks was used.

The samples and virus control at various dilutions were applied to the Vero-81 (ATCC No. CCL-81) cell monolayer cultured in 96-well plates and the appearance of cytopathic effect (CPE) (Fig.1) was observed 2, 4 and 6 days post inoculation. If no cytopathic effect was observed 6 days post inoculation, the samples were considered negative and the dilution of the disinfectant used was regarded effective.

Results and Discussion

According to the German Guidelines of the Federal Health Office (Bundesgesundheitsamt Ľ BGA, now Robert Koch-Institute, Berlin, Germany) and of the German Association for the Control of Virus Diseases e.V. (DVV), a titre reduction of 10^4 fold is necessary for demonstrating virucidal efficacy of the disinfectant. The European draft of a guideline also requires the same reduction to confirmed the virus-inactivating properties of a disinfectant (Steinmann 2004).

Dilution of the disinfectant from 60%, 80% and 100% sodium hypochlorite has been excluded due to its cytotoxic effect on Vero cells. At a 20% dilution, CPE was observed and therefore considered ineffective. The results are presented in Table 1.

Forty percent dilution proved to be an effective dilution of sodium hypochlorite (0.228 g/L). The results showed that the virus was not isolated from any of the samples taken in 3 time intervals (10 min, 20 min and 30 min) using a 40% dilution disinfectant while the virus control reached 1 x 10^4 $TCID_{50}$/1ml. This result corresponds to a reduction of the titre by 4 logarithms, therefore the agent can be considered effective in virucidity in relation to the surrogate virus model, which also indicates the virucidal effect for SARS-CoV-2. Decontamination with sodium hypochlorite dispersion may have many implementations, e.g. for decontamination of various objects, rooms and vehicles and even hard to reach places. Another advantage is the low cost of sodium hypochlorite production and its effectiveness in small doses.

Since no special therapies are available for SARS-CoV-2, early prevention of further spread of the virus is critical to stop an ongoing outbreak and control the pandemics.

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