Coronaviruses with Special Emphasis on First Insights Concerning SARS
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Environmental survival and microbicide inactivation of coronaviruses

Manfred H. Wolff,[1] Syed A. Sattar,[2] Olusola Adegbunrin[2] and Jason Tetro[2]

[1] *Faculty of Bioscience, Institute of Microbiology and Virology, Stockumer Str. 10, D-58453 Witten, Germany*
[2] *Centre for Research on Environmental Microbiology (CREM), Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5*

Introduction

Since their first isolation from chickens in 1937 [1], coronaviruses have proven to be significant pathogens of many types of wild as well as economically important domesticated animals. Though coronaviruses were first identified as human respiratory pathogens in 1965 [2], only recently, with their established link with the severe acute respiratory syndrome (SARS), has there been a sudden upsurge of interest in this group of viruses.

Taxonomically, these enveloped, positive-sense RNA viruses [1] belong in the genus *Coronavirus* of the family *Coronaviridae* in the order *Nidovirales* [3]. To date, the genus contains some 14 members. Birds and mammals are the known hosts with a wide variety of species affected. In mammals, coronaviruses have been isolated from pigs, cattle, mice, rats, dogs, horses, cats, and humans [1], and in birds mainly from chickens [4] and turkeys [5].

Coronaviruses 229E and OC43 are recognized respiratory pathogens of humans. The causative agent of SARS (SARS-CoV), which has now been fully characterized [6], awaits its formal inclusion in the genus. Genomic studies show SARS-CoV to be unique as it contains elements of both mammalian and avian ancestry [7] and the effect of this recombination has been disastrous for humans. In the first recorded outbreak in 2003, the virus caused 8,461 clinical cases and 804 recorded deaths globally [8]. Fortunately, and in spite of its seemingly high mutation rates [9], the spread of the virus was effectively controlled, mainly through general public health measures and basic infection control practices. Nevertheless, the SARS incident has had a significant impact on human health and the global economy [10] and thus highlighted the need to better understand the modes and vehicles for its spread and proper means to interrupt its environmental transmission.

Pathogenesis and shedding of infectious virus

Depending on the type of coronavirus and the animal host species, virus shedding can occur from the respiratory and/or gastrointestinal tract [11] and the two main portals of virus entry into susceptible animals are the nose and the mouth [12]. In humans, coronaviruses normally cause the common cold, SARS being a notable exception because it causes severe pneumonia as well as acute gastroenteritis. The risk of environmental contamination with the SARS-CoV is thus much higher. The evidence available thus far from outbreak investigations is strongly suggestive of droplet transmission [13] and to a much limited degree by aerosols [14]. It is not known if the SARS-CoV can also be acquired through the mouth or the conjunctivae, nor do we know if inhaled virus deposited in the throat can be directly translocated to the gut.

In SARS, massive spiking of the immunological response leads to hypoxia and eventual respiratory distress syndrome [15]. Infection of the gastrointestinal tract causes acute diarrhea and may also result in peritonitis and necrotizing colitis [16]. The simultaneous involvement of the respiratory as well as the gastrointestinal tracts in SARS [11] is highly reminiscent of coronavirus infections in cattle [17] and other animals.

Based on the pig model, the infectious period for coronaviruses is approximately 6 days post-infection [12, 18] with a peak at day 4 post-infection. This holds true for bovine [17, 19] and human infections [20, 21] as well. In pigs, virus release from the nose peaks on the fourth day of infection with yields of >5.5 $\log_{10}$ $TCID_{50}$/g of nasal discharge, with the air around such animals containing nearly 2 $\log_{10}$ of infectious virus/m^3 [12]. Similarly, infectious virus has been detected in the nasal discharge and feces of SARS patients [11].

Environmental survival and spread of coronaviruses

In general, a given pathogen must remain viable outside the host to allow for environmental spread, and the combined effect of many biotic and abiotic factors determines how long such viability can be retained. While environmental survival of coronaviruses has been studied to some degree under experimental conditions [22, 23] including SARS (Tab. 1), we know much less about the types and relative significance of vehicles in the in-nature spread of coronaviruses, in particular those that can infect humans. For example, infectious virus has been recovered from both droplets and air in the vicinity of pigs experimentally infected with the porcine respiratory coronavirus [12], but the potential, if any, of droplets and aerosols in the spread of the virus to susceptible animals in the vicinity is unknown.

The following is a summary of the available information on the environmental survival and spread of coronaviruses.

Table 1. Environmental survival of SARS-CoV (modified from [20]

Environment	Number of hours virus infectivity remained detectable
Fluid	
Cell culture fluid at 4°C	>120
Cell culture fluid at room temperature	60
Autoclaved water	72
Serum	72
Sputum	96
Feces	96
Urine	<6
Non-porous environmental surface	
Glass	60
Mosaic	60
Metal	72
Plastic	60
Porous materials	
Cloth	72
Filter paper	72
Autoclaved soil (Beijing, China)	<6

Air

The effect of relative humidity (RH) and air temperature has been studied on the airborne survival of experimentally aerosolized human coronavirus 229E [22]. As is true for enveloped viruses in general, 229E survived better at 30-50% RH than at 80% RH when the air temperature was about 20°C. Under these conditions, the half-lives of the virus at 30%, 50% and 80% RH were 27, 67 and 3 hours, respectively. Lowering the air temperature to 6°C increased the half-lives of the virus at 30% and 50% RH to 34 and 103 hours, respectively. But the lower air temperature produced the most dramatic effect on virus survival at 80% RH and changed its half-life from 3 to over 86 hours.

The available epidemiological evidence strongly suggests that SARS spreads through droplets [13] and such spread is much easier to control than that through aerosols. However, the pattern of spread of SARS-CoV in at least two instances is highly suggestive of airborne spread.

A cluster of 329 SARS cases was recorded in one apartment complex in Hong Kong with the majority of them occurring on several floors in one wing [24]. This pattern of spread is highly suggestive of virus dissemination by air, and the aerosolization of the virus was speculated to have occurred

from malfunctioning sewers in the building [24]. Rodent pests in the building have been hypothesized as possible amplifiers and disseminators of the virus [25]; the virus, most likely acquired from infected residents, may have multiplied in the rodents, being released in their excreta and then possibly becoming airborne. While hantaviruses, for example, can infect humans from aerosolized rodent excreta [26], the role of air in the transmission of SARS-CoV in this outbreak remains speculative at this stage. However, a more detailed analysis of the outbreak of SARS at that apartment complex suggests that the virus released by the patients themselves may have spread through air [27].

Limited airborne spread of SARS may have occurred on board commercial aircraft. In one such instance, a symptomatic index case infected at least 22 of 120 (18.3%) passengers and crew during a three-hour flight [14]. Some of those infected were seated over 2 m (90 inches) away from the index case, a distance much longer than the 0.9 m (36 inches) generally believed to be the limit for droplet transmission. In such retrospective investigations it is virtually impossible to rule out the role of other possible means of virus spread. Also, the inside of an aircraft combines features which may be more conducive to airborne spread of pathogens.

A recent report from Canada suggests that oxygen delivery masks with open vents could promote the dispersal of respiratory pathogens such as SARS-CoV through their enhanced release in mists of exhaled pulmonary gases [28]. The exhaled moist air ejected from such oxygen masks is believed to carry pathogen-laden droplets over longer distances and possibly contribute to an increased risk of spread of respiratory infections in nosocomial settings. Additional investigations are needed to first prove that viruses such SARS-CoV can retain their infectivity better in the warm, moisture-laden air exhaled from oxygen masks. The findings on the influence of RH and air temperature on the airborne survival of coronavirus 229E would tend to suggest otherwise [22].

Sewage and biosolids

SARS-CoV can survive for up to 96 hours in body fluids such as sputum, feces and serum, but is less stable in urine [20]. The fecal excretion of SARS-CoV generated much concern on the safety of handling and spreading municipal solids on lands [29]. There are no reports of the recovery of infectious SARS-CoV from raw sewage or sludge and if the behavior of other enveloped viruses such as HIV is an indication, it would be highly unlikely that the virus can survive in such wastes long enough to pose any risks to human health. In view of this, it is even less likely that the virus would survive the conventional methods of sewage and biosolids treatment. Therefore, any suggestion of health risk from handling of municipal wastes or their proper disposal would be difficult to justify [29].

Skin

Since safety and ethical considerations would not permit the experimental contamination of the hands of human subjects with SARS-CoV, we have used 229E as a surrogate to study the potential of coronaviruses to survive on hands. Preliminary findings from such experiments indicate that nearly 45% of infectious virus remains viable on the hands of adult subjects after 1 hour. This is in contrast to other enveloped respiratory viruses (e.g. parainfluenzavirus) which become essentially undetectable in about 10 minutes on human skin [30].

Food and water

We are not aware of any published information on the survival of coronaviruses in food. Recent studies have shown SARS-CoV to survive in water to a very limited degree [20]. There is no evidence to suggest the spread of coronaviruses through food or water.

Environmental surfaces

SARS-CoV [20] and 229E and OC43 [23] can retain their infectivity for several hours on porous and non-porous environmental surfaces. While such survival is better than that of other enveloped human pathogens [30], there is no evidence to suggest that environmental surfaces play any direct or indirect role in the spread of coronaviruses.

Activity of microbicides against coronaviruses

Because of their enveloped nature coronaviruses are more susceptible to microbicides than non-enveloped viruses. In a comparative study, an animal parvovirus (non-enveloped) required 20- to 500-fold higher concentrations of the tested microbicides than were needed to inactivate an animal coronavirus [31].

Even before the advent of SARS considerable evaluation of the activity of microbicides against this virus group had been carried out. The following is a summary of this information.

Virucidal activity of several microbicides was tested against the mouse hepatitis virus (MHV) and the canine coronavirus (CCV), Kilham rat virus and canine parvovirus [32]. Both coronaviruses were readily inactivated by ethanol, isopropanol, benzalkonium chloride, iodophor, sodium hypochlorite, sodium chlorite, cresol soap and formaldehyde whereas the two parvoviruses proved to be considerably more resistant.

The relative resistance of 229E to microbicides was compared to that of coxsackievirus B3, adenovirus type 5, and parainfluenzavirus type 3 using the second tier of a quantitative carrier test (QCT-2) [33]. Stainless steel disks were used as carriers and each one received 10 µl of the test virus, suspended in either faeces or mucin and the inoculum allowed to dry for 1 h under ambient conditions. After 1 min exposure to 20 µl of the disinfectant, the virus from the disks was immediately eluted and plaque assayed. The efficacy criterion of a ≥ 3 $\log_{10}$ reduction in virus infectivity was used. As expected, the coronavirus proved to be generally less resistant to microbicides than the two non-enveloped viruses.

In a more recent study, Wood and Payne [34] used a suspension test to assess the activity of chloroxylenol, benzalkonium chloride and cetrimide/chlorhexidine against three types of enveloped viruses (herpesvirus type 1, HIV-1 and a human coronavirus). The coronavirus was found to be generally more resistant than the other two enveloped viruses tested. While this observation is of interest, the findings have limited practical significance because the testing was based on a suspension test which presents the test microbicide with a weaker challenge than a carrier test protocol. A summary of all findings can be found in Table 2.

Alcohols

Most alcohols used in disinfection are ethanol and isopropyl alcohol, both usually at a concentration of 70% [32, 34]. Without soil load, the transmissible gastroenteritis virus (TGEV) was reduced 4.5 $\log_{10}$ by ethanol in the suspension test over 5 minutes [35]. Similarly, using QCT-2 and organic load, ethanol reduced 229E [33] by at least 3 log10 over the course of 5 minutes.

Chlorine and other halides

In tests using varying concentrations of sodium hypochlorite, 1000 ppm was effective against TGEV [34], bovine coronavirus [31] and 229E [33] irrespective of test conditions. Chloramine T ($C_7H_7SO_2NNaCl$) had similar results against bovine coronavirus and 229E. Povidone-iodine at a concentration of 1% reduced 229E by >3 $\log_{10}$ with the QCT-2 test.

Aldehydes

Formaldehyde at 4% is used as an overall disinfecting and sterilizing solution albeit its use as a general disinfectant is not recommended. It is effective against TGEV, reducing it over 5 $\log_{10}$ in under 5 minutes in suspension. At 2%, glutaraldehyde can inactivate 229E over 3 $\log_{10}$ [33] in the QCT-2 test.

Table 2. Effect of microbicides on coronaviruses

Active ingredient(s)	Virus	Test condition	Time	≥3 Log_{10} reduction	Refs.
		Ethanol			
70%	229E	QCT-2 + SL	5 min	Yes	[32]
70%	TGEV	Suspension	5 min	Yes	[34]
		Chlorine			
Sodium hypochlorite (100 ppm)	229E	QCT-2 + SL	5 min	No	[32]
Sodium hypochlorite (1000 ppm)	229E	QCT-2 + SL	5 min	Yes	[32]
Sodium hypochlorite (1500 ppm)	TGEV	Suspension	5 min	Yes	[34]
Chloramine T (1500 ppm)	229E	QCT-2 + SL	5 min		[32]
		Iodine			
1%	229E	QCT-2 + SL	5 min	Yes	[32]
1%	TGEV	Suspension	5 min	Yes	[34]
0.5%	Bovine	Suspension + SL	60 min	Yes	[30]
		Formaldehyde			
0.3%	Bovine	Suspension + SL	60 min	Yes	[30]
		Glutaraldehyde			
2%	229E	QCT-2 + SL	5 min		[32]
2%	TGEV	Suspension	5 min	Yes	[34]
		Quaternary ammonium compounds – benzalkonium chloride			
100 ppm	TGEV	Suspension	5 min	Yes	[34]
		Chlorhexidene gluconate			
80 ppm	229E	QCT-2 + SL	5 min	No	[32]
80 ppm + 70% ethanol	229E	QCT-2 + SL	5 min	Yes	[32]
		Phenolics			
O-phenylphenol (200 ppm)	229E	QCT-2 + SL	5 min	No	[32]
O-phenylphenol + sodium lauryl sulfate (0.6%)	229E	QCT-2 + SL	5 min	Yes	[32]
O-phenylphenol + 70% ethanol	229E	QCT-2 + SL	5 min	Yes	[32]
O-phenylphenol + 5% isopropyl alcohol	TGEV	QCT-2 + SL	5 min	Yes	[34]

SL, soil load; QCT-2, quantitative carrier test – Tier 2; TGEV, transmissible gastroenteritis virus

Quaternary ammonium compounds

A quaternary ammonium disinfectant requires the presence of an ammonium ion and at least one of its hydrogen atoms substituted by an organic radical. As a result, the list of quaternary ammonium compounds is rather extensive. This type of disinfectant is usually cationic and affects a

Table 3. Data on stability and resistance of SARS-CoV compiled by members of WHO laboratory network (modified from [35])

Substrate	Initial viral count log_{10} PFU	Condition	Survival time	Method of testing viability
Government Virus Unit, Dept. of Health, Hong Kong, SAR China				
Virus spiked in baby stool	1.00E+03	pH 6–7	3 hr	Virus isolation in cell culture
Virus spiked in normal stool	7.50E+03	pH 8	6 hr	Virus isolation in cell culture
Virus in diarrheal stool	7.50E+03	pH 9	4 days	Virus isolation in cell culture
Queen Mary Hospital, The University of Hong Kong, Hong Kong, SAR China				
Stool	1.00E+03	Room temperature	at least 2 days	Virus isolation in cell culture
Urine	1.00E+03	Room temperature	at least 24 hr	Virus isolation in cell culture
Virus culture medium + 1% bovine serum	1.00E+03	On plastic surface in room temperature	at least 2 days	Virus isolation in cell culture
Virus culture medium + 1% bovine serum	1.00E+04	30–37°C	at least 1hr	Virus isolation in cell culture
Virus culture medium + 1% fetal calf	1.00E+04	56°C	degradation of serum titre over time (10000 infectious virus units in 15 min)	Virus isolation in cell culture
Virus in acetone, 10% formaldehyde and paraformaldehyde, 10% clorox, 75%ethanol, 2% phenol	1.00E+06	Room temperature	less than 5 min	Virus isolation in cell culture
National Institute of infectious Diseases, Tokyo, Japan				
Virus culture + 2% bovine serum	1.00E+06	minus 80°C	at least 4 days	Virus isolation and RT-PCR
Virus culture + 2% fetal calf serum	1.00E+06	4°C	at least 4 days	Virus isolation and RT-PCR
Virus culture + 2% fetal calf serum	1.00E+06	37°C	less than 4 days	Virus isolation and RT-PCR
Virus culture + 2% fetal calf serum	1.00E+05	56°C	less than 30 min	
University of Marburg, Germany				
Virus culture	1.00E+06	4°C	at least 21 days	Virus isolation
Virus culture	1.00E+06	minus 80°C	at least 21 days	Virus isolation
Chinese University, Hong Kong				
Virus in phosphate buffered saline (PBS)	9.00E+04	Room temperature on	PBS	Stool	Virus isolation in cell culture
Virus in sterilized		Plastered wall	24h	36 h	
		Plastic surface	36h	72 h	
		Formica surface	36h	36 h	
		Stainless steel	36h	72 h	
		Wood	12h	24 h	
		Cotton cloth	12h	24 h	
		Pig skin	?24h	?24 h	
		Glass slide	72h	96 h	
		Paper file cover	24h	36 h	

cell by adhering to its negatively charged membrane and disrupting the ionic potential of the cell. In the case of viruses, this adherence can disrupt the envelope, rendering the virus noninfectious. The quaternary ammonium compounds are best represented by chlorhexidine gluconate, which is widely used in health care institutions and in consumer disinfectants and antiseptics. In both suspension and QCT-2 tests, chlorhexidine gluconate faired well against TGEV [35] and 229E [33, 34]. Concentrations ranging from 500 to 15,000 ppm were effective in reducing the virus by at least 3 $\log_{10}$.

Phenolics

The phenolic compounds have a long history as their ancestry dates back to the use of coal tar soaps, whose active ingredients were creosol-based. Although some disinfectants continue to use the same historical active ingredient, creosol, most have developed over time to become more active against various microbes. For example, *o*-phenylphenol at 200 ppm is highly ineffective against most viruses, including 229E in the QCT-2 test [33]. However, the addition of either a detergent, such as sodium lauryl sulphate, or ethanol, proves highly effective against the virus over 3 $\log_{10}$ reduction is seen after 5 minutes. In the same way, many phenolic compounds rely on the helper effect of other chemicals to work effectively.

Survival and inactivation of the SARS coronavirus

The World Health Organization Laboratory Network has summarized the available data on SARS-CoV survival and inactivation [36], which are redrawn for this chapter as Table 3. Tables 4 and 5 summarize more recent studies on the inactivation of 229E by environmental surface disinfectants and antiseptics, respectively.

Concluding remarks

Despite the enhanced awareness of the potential of coronaviruses as animal and human pathogens, our understanding of their environmental survival and the exact means of their spread remains weak. Such information would be essential to design and implement more rational approaches to prevention and control of outbreaks of coronaviral infections. This is particularly relevant for the recently discovered SARS-CoV. However, the limited data available indicate that coronaviruses as a group are more stable in the environment that other enveloped viruses.

Table 4. Activity of microbicides against coronavirus 229E (Sattar et al., unpublished data)

Microbicide	Dilution tested (ppm)	Contact time (min)	Log_{10} reduction
Accelerated H_2O_2	Undiluted (5000)	3	4.0
Spray (26 g ethanol +	Undiluted	3	4.4
11.5 g 2-propanol +		1	4.4
0.054 g polyhexanide/100 g)			
Ethanol	75% (v/v)	5	4.0
Potassium monopersulfate	1% (w/v) solution	0.5	2.3
23.0% + sodium dichloro-s-	in water	1	4.1
triazinetrione 5.0%			
5.25% sodium hypochlorite	1:50 (1000)	3	4.0
(bleach)			
5.25% sodium hypochlorite	2 ml 5% acid	0.5	2.2
+ 5% acetic acid (vinegar)	+ 8 ml water (5000)		
Spray (ethanol 79.646%	Undiluted	3	3.3
+ a quat.)		10	3.1
4.8% chloroxylenol	1:40 (1200)	10	4.1
A mixture of two quat. ammoniums	1:128 (660)	10	4.0
A mixture of two phenolics	1:256 (820)	10	4.1

The second tier of the quantitative carrier test (QCT-2) with stainless steel disks was used. Ten μl of the virus suspension with a soil load was dried on each carrier and the inoculum exposed to 50 μl of the test microbicide at 23±2 °C.

Table 5. Activity of an ethanol-based hand rub against coronavirus 229E (Sattar et al., unpublished data)

Treatment	Mean ± SD	% Reduction
Baseline	$1.32 \times 10^4 \pm 0.4$	–
Hard water (200 PPM $CaCO_3$)	$3.02 \times 10^3 \pm 2.0$	77.0
75% (v/v) ethanol	0	>99.99
Ethanol (60%) containing gel	0	>99.99

The fingerpad method (ASTM 1838) was used. Ten μl of virus in soil load was placed on each fingerpad & dried. The dried inoculum was exposed to 1 ml of control or test solution for 30 seconds. The fingerpads were eluted & eluates plaque assayed.

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