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Stability of SARS Coronavirus in Human Specimens and Environment and Its Sensitivity to Heating and UV Irradiation[1]

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Objective The causal agent for SARS is considered as a novel coronavirus that has never been described both in human and animals previously. The stability of SARS coronavirus in human specimens and in environments was studied. **Methods** Using a SARS coronavirus strain CoV-P9, which was isolated from pharyngeal swab of a probable SARS case in Beijing, its stability in mimic human specimens and in mimic environment including surfaces of commonly used materials or in household conditions, as well as its resistances to temperature and UV irradiation were analyzed. A total of 10^6 $TCID_{50}$ viruses were placed in each tested condition, and changes of the viral infectivity in samples after treatments were measured by evaluating cytopathic effect (CPE) in cell line Vero-E6 at 48 h after infectionn. **Results** The results showed that SARS coronavirus in the testing condition could survive in serum, 1:20 diluted sputum and feces for at least 96 h, whereas it could remain alive in urine for at least 72 h with a low level of infectivity. The survival abilities on the surfaces of eight different materials and in water were quite comparable, revealing reduction of infectivity after 72 to 96 h exposure. Viruses stayed stable at 4℃, at room temperature (20℃) and at 37℃ for at least 2 h without remarkable change in the infectious ability in cells, but were converted to be non-infectious after 90-, 60- and 30-min exposure at 56℃, at 67℃ and at 75℃, respectively. Irradiation of UV for 60 min on the virus in culture medium resulted in the destruction of viral infectivity at an undetectable level. **Conclusion** The survival ability of SARS coronavirus in human specimens and in environments seems to be relatively strong. Heating and UV irradiation can efficiently eliminate the viral infectivity.

Key words: Severe acute respiratory syndrome; Coronavirus; Stability; Environment; Specimen

INTRODUCTION

Severe acute respiratory syndrome (SARS) is a worldwide infectious disease that involves more than 30 countries and areas so far, affecting 5 127 individuals and resulting in 252 deaths by May 20, 2003[1]. Since the first cases were retrospectively identified in

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November 2002, numerous virus strains have been isolated from oropharyngeal and respiratory tract excretions, blood, stool, as well as from various kinds of tissues by postmortem examination and biopsies. It is believed that a novel coronavirus, with roughly 29 700 bp nucleotides in the context of the whole genome[2], is the causal agent for SARS, based on the evidences obtained in morphological, epidemiological, serological, molecular biological studies[3-6]. More recently, Osterhaus and his co-workers reported a successful animal model of SARS on non-human primate, *marcaque*, challenged by SARS coronavirus isolated from intranasal tissues[7]. Collectively, more and more data strongly suggest that the new coronavirus might be the pathogen of SARS.

Based on the data of genome and bio-informatic studies, SARS coronavirus belongs to the Coronoviridea family, which is an enveloped and positive-strand RNA virus[2,6]. The members of the Coronaviridea family usually have wide host ranges, which cause various respiratory infections in human, cattle and birds, as well as gastric-intestinal infections in rodents, cats, pigs, *etc.*[8-10]. Although coronaviruses are thought to be sensitive to heat and acids, their viral stability and survival ability in host specimens and in environments remain unsettled.

SARS epidemiological data implicate two main transmission clusters, family-cluster and hospital-cluster[11,12], in which droplets of respiratory secretions of SARS patients are believed to be a major concern[13]. Recently, more evidences have demonstrated that SARS coronavirus or SARS coronavirus related nucleotide segments could be detected from other specimens including blood, stool and urine, in addition to respiratory secretions[6]. Repeatedly isolating SARS viruses from non-respiratory specimens with typical cytopathic effect (CPE) in cultured cells and enteric transmission of SARS in Amoy Garden in Hong Kong, SARS viruses, imply multiple transmission pathways for SARS[14].

In this study, the stability of SARS virus in human specimens and environments was analyzed *in vitro*. Survival durations of SARS virus on or in some materials commonly used in household were evaluated mimically. Additionally, inactivation effect of irradiation of ultraviolet (UV) on SARS coronavirus was determined in cell cultures.

MATERIALS AND METHODS

Virus

SARS coronavirus strain CoV-P9 was isolated from pharyngeal swabs of a probable SARS patient as described previously[15]. The properties of CoV-P9 were confirmed by various tests including viral morphology, gene analysis and serological assays[15].

Cell Culture

Vero-E6 maintained in Eagle medium (Gibco) containing 10% fetal cattle serum 50 U/mL penicillin and 50 mg/mL streptomycin. Vero-E6 cells were harvested from 75 cm^2 flasks and diluted into 400 000 cells/mL, and spread into 96-well plates and cultured at 37℃ for 24 h before virus infection.

Stability in Clinical Specimens

All tested specimens were collected from healthy persons, including serum, sputum, urine and feces. Sputum and feces were diluted into 1:20 with PBS and the debris was removed by 5 000 g centrifuge. An aliquot of diluted specimen was filtrated with filter paper. Undiluted urine and serum were stored at 4℃ till use. Previously titrated viruses, in a final concentration of 10^6 $TCID_{50}$, were added into undiluted urine and serum, as well as into 1:20

diluted filtrated and unfiltrated sputum and feces. The testing samples were maintained at room temperature (RT), and a 100 μL aliquot of each was taken out at different points of time and inoculated into the previously cultured cells in 96-well plates. Each assay was tested in two separated wells in parallel.

Materials in Household and in Nature

Commonly used materials in household including wood board, glass, mosaic, metal, cloth, press paper, filter paper and plastic were collected for analysis of stability of SARS coronavirus. All the materials were cut to roughly 1.5 cm^2 square and autoclaved before usage. One mL water and 1 g soil were collected in Eppendorf tubes and autoclaved. Final concentration of 10^6 $TCID_{50}$ viruses in 300 μL was dropped onto the surfaces of the testing materials which were maintained at room temperature in a BSL-3 carbine. At each time check-point, one copy of each material was taken out and washed with 300 μL Eagle medium. One hundred μL washed medium was inoculated into the previously cultured cells in 96-well plates. Each assay was tested in two separated wells in parallel.

Temperature Stability

A total of 10^6 $TCID_{50}$ viruses were maintained in culture medium with a volume of 100 μL and exposed at various temperatures for different time-lengths. The treated media were transferred onto the cultured cells in 96-well plates. Each assay was tested in two separated wells in parallel.

UV Inactivation

A total of 10^6 $TCID_{50}$ viruses maintained in culture medium with a volume of 100 μL were added in 96-well plates and exposed to irradiation of UV. The intensity of UV was >90 uw/cm^2, and the irradiating distance was 80 cm. At various time-points, the exposed viruses were collected to infect the cultured cells in 96-well plates. Each assay was tested in six separated wells in parallel.

Stability in Dry Environment

A total of 10^6 $TCID_{50}$ viruses in 300 μL were added onto cotton cloths in separating autoclaved plates, and exposed in a sealed vat with $CaCl_2$ inside. The viruses were washed with 300 μL Eagle medium at various time-points and infected into the cultured cells in 96-well plates. Each assay was tested in six separated wells in parallel.

RESULTS

To determine the stability of SARS coronavirus in various specimens and environments, SARS coronavirus strain CoV-P9[15] was used in this study. Viral challenges in Vero-E6 cells showed that strain CoV-P9 induced a typical cytopathic effect (CPE) 2 to 3 days after infection. CPE started to emerge 16 to 24 h after infection, with spots of cell becoming rounded and more particles appearing in cytoplasma. 24 to 48 h after infection, more cells changed to round and multi-shapes, some of them started to aggregate to clusters and reticulation (Fig. 1B). After 72 h infection, nearly all cells aggregated to reticulation, with more cells falling off from the flask (Fig. 1C). For analysis of the stability of coronavirus, CPE in all tests was observed for 48 h after infection.

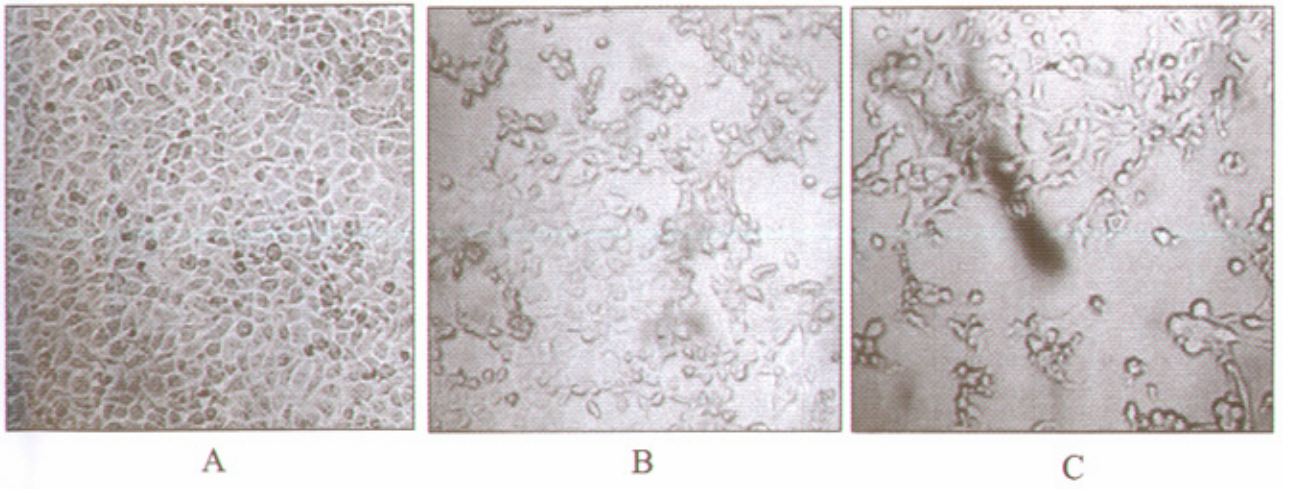

FIG. 1. Cytopathic effect (CPE) on Vero-E6 cells after SARS coronavirus infection. A. Uninfected Vero-E6 cells; B. Vero-E6 cells 48 h postinfection; C. Vero-E6 cells 72 h postinfection.

Stability in Human Specimens

To observe the stability of SARS coronavirus in different human specimens, healthy human blood, sputum, urine and feces were collected and treated as described above. An aliquot of tested samples was taken for determining viral infectivity on cells in every each 12 h. Table 1 shows that the infectivity in undiluted serum started to drop down at 60 h after incubation and became undetectably after having stayed at RT for 120 h, whereas the infectivity in undiluted urine was kept in lower titer in all experiment days, and dropped slightly down after 120 h incubation. The infectivity in filtrated sputum dropped down slightly quicker than that in unfiltrated one. However, it revealed no difference between filtrated and unfiltrated feces (Table 1).

Stability on Some Commonly Used Materials in Household

To mimic the survival duration of SARS virus in household, CoV-P9 was dropped on the surfaces of some commonly used materials, as well as in water and soil. Table 2 shows that the infectivity on or in the tested materials dropped to an almost undetectable level after having stayed at RT for 5 days. Compared among the different materials, the infectivity of virus on wood board and in soil started at low level (less than 25% infected cells with CPE) even after only 12 h incubation at RT, but remained so till 96 h exposure. The tests on the rest materials and in water revealed a similar viral infectivity (mostly with ++ CPE) and a declining trend, except for the surface of glass with a higher infectivity (with +++ CPE within 2 days exposure). The virus seemed to be alive a little longer on filter paper than on print paper (Table 1).

Temperature and Viral Infectivity

To observe the survival ability of SARS virus according to temperature, CoV-P9 in culture mediums was incubated at six different temperature situations from 15 to 120 min. The infectivity remained unchanged under the conditions of 4℃, RT (roughly 20℃) and 37℃, even for 120 min incubation (Table 3). Along with increasing temperature in environment and prolonged incubation, the CPE inducing ability became weaker and ultimately undetectable. The infectivity was virtually eliminated after exposure at 56℃ for 90 min, at 67℃ for 60 min and at 75℃ for 30 min (Table 3). Meanwhile, it showed that even a short incubation time in a higher temperature (67℃ and 75℃) could reduce virus infectivity distinctly, with ++ CPE at 67℃ and + CPE at 75℃ for 15 min.

Viral Infectivity Inactivated by UV Irradiation

To address the inactive possibility of SARS virus under irradiation of UV, CoV-P9 in culture medium was exposed to a 260 nm-length UV from 15 to 150 min, with a fixed intensity and distance. Compared with the results without irradiation of UV (+++ CPE), the infectivity went down to + CPE after only 15 min exposure and dropped to an undetectable level for 60 min irradiation (Table 4).

Little Effect of Dry Environment on Viral Stability

To mimic a dry environment, pieces of autoclaved cotton cloths were dipped with viruses and placed in a sealed vat containing $CaCl_2$ from 12 h to 72 h. The virus infectivity was successively evaluated each 12 h *in vitro*. In this environment, the infectivity showed no detectable reduction with a prolongation of the exposing time, even after 72 h (Table 4), it was similar to that done in general laboratory environment (Table 2).

TABLE 1

Stability of SARS Coronavirus in Human Specimens and in Environments at Room Temperature

	12 h		24 h		36 h		48 h		60 h		72 h		96 h		120 h	
Serum	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+	-	-
Sputum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
Filtrated Sputum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	-	-
Feces	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
Filtrated Feces	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
Urine	+	+	+	+	+	+	+	+	+	+	+	+	+	±	±	±
Wood Board	+	+	+	+	+	+	+	+	+	+	±	±	±	-	-	-
Glass	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	±	±	-	-
Mosaic	++	++	++	++	++	++	++	++	++	++	+	+	-	-	-	-
Metal	++	++	++	++	++	++	++	++	++	++	++	++	+	+	±	±
Cloth	++	++	++	++	++	++	++	++	++	++	++	++	+	+	±	±
Press Paper	++	++	++	++	++	++	++	++	++	++	+	+	±	±	-	-
Filter Paper	++	++	++	++	++	++	++	++	++	++	++	++	+	+	±	±
Plastic	++	++	++	++	++	++	++	++	++	++	+	+	±	±	-	-
Water	++	++	++	++	++	++	++	++	++	++	++	++	+	+	-	-
Soil	+	+	+	+	+	+	+	+	+	+	+	+	±	±	-	-
Cell Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Virus Control (4℃)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Virus Control (RT)	++	++	++	++	++	++	++	++	++	++	++	++	+	+	-	-

Note. CPE of infected cells was determined 48 h postinfection. +: less than 25% cells with CPE, ++: 26%-50% cells with CPE, +++: 51-75% cells with CPE, ±: only few cells with CPE, -: without detectable CPE.

TABLE 2

Stability of SARS Coronavirus in Various Temperature Environments

	4℃		20℃		37℃		56℃		67℃		75℃	
15 min	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
30 min	+++	+++	+++	+++	+++	+++	+++	+++	+	+	-	-
60 min	+++	+++	+++	+++	+++	+++	++	++	-	-	-	-
90 min	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-
120 min	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-
Cell Control	-	-	-	-	-	-	-	-	-	-	-	-
Virus Control	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Note. CPE of infected cells was determined 48 h postinfection. +: less than 25% cells with CPE, ++: 26%-50% cells with CPE, +++: 51%-75% cells with CPE, ±: only few cells with CPE, -: without detectable CPE.

TABLE 3

Stability of SARS Coronavirus Under Different Exposure Times of UV

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6
15 min	+	+	+	+	+	+
30 min	±	±	±	±	±	±
60 min	-	-	-	-	-	-
90 min	-	-	-	-	-	-
120 min	-	-	-	-	-	-
150 min	-	-	-	-	-	-
Cell Control	-	-	-	-	-	-
Virus Control	+++	+++	+++	+++	+++	+++

Note. CPE of infected cells was determined 48 h postinfection. +: less than 25% cells with CPE, ++: 26%-50% cells with CPE, +++: 51%-75% cells with CPE, ±: only few cells with CPE, -: without detectable CPE.

TABLE 4

Survival Ability of SARS Coronavirus in Mimic Dry Condition

	12 h		24 h		36 h		48 h		60 h		72 h	
On Cotton Cloth	++	++	++	++	++	++	++	++	++	++	++	++
Cell Control	-	-	-	-	-	-	-	-	-	-	-	-
Virus Control (RT)	++	++	++	++	++	++	++	++	++	++	±	±
Virus Control (4℃)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Note. CPE of infected cells was determined 48 h postinfection. +: less than 25% cells with CPE, ++: 26%-50% cells with CPE, +++: 51%-75% cells with CPE, ±: only few cells with CPE; -: without detectable CPE.

DISCUSSION

The presence of SARS virus in respiratory secretions and in enteric discharge and its implications for infections among human population have been well understood[13]. Although the implications of the presence of SARS virus and/or SARS virus specific sequences in blood and urine for SARS transmission remain unclear, repeated isolation of virus and identification of viral sequences in blood and urine strongly suggest potential infectious sources. The data in this study demonstrated that the infectivity of SARS virus could maintain for at least 4 days in human serum at room temperature, though the infectivity started to decline 60 h after exposure and the concentration of loading virus might be higher than that in nature. Despite the fact that blood transmission may not be possible in the outbreak of SARS naturally, the presence of SARS virus in blood and its long survival duration in blood *in vitro* imply that the blood donor may arouse a meaningful concern. Special emphasis should be placed on the blood which is usually stored at 4℃ till clinical use, and a much longer survival duration of virus infectivity would be expected. A similar duration of virus infection has been observed in mimic sputum and feces samples, strongly indicating potential infectious sources. One might argue that 1:20 diluted samples may help virus to live longer, howbeit, a high incidence of SARS among close contacts, both in family members and medical staff, and internal transmission in Amoy Garden propose that SARS coronavirus be able to live long enough in environment to induce the next generation of infection. It is interesting that the virus infectivity in undiluted urine dropped to a low level after a short exposure (12 h), but was maintained till 4 days. The low level of virus infectivity when mixed with urine indicates that there may be some inhibitor(s) for SARS coronavirus in urine. Prolonged persistence of viral infectivity may be resulted from a high dosage of the input virus.

Analyses of the survival durations of SARS coronavirus on or in the commonly used materials, as well as in water and soil showed that the viral infectivity persisted for 60 h after exposure, started to drop after 72 h and became almost undetectable after 120 h. These preliminary results indicate that SARS coronavirus has a relatively strong resistibility in household environment. A lower level, but persistent infectivity when exposed on wood board and in soil might not implicate a direct inhibiting activity. Other chemical contaminations in wood board may not be possible, since only rude wood was used in this study. It is reasonable to speculate that the low level infectivity is more likely due to the absorbant effects of board and soil, as only 300 μL culture medium was used after exposure. The strong stability of SARS coronavirus in household environments and in nature implies the essentiality of disinfection both in household and in health care facilities.

Like most other viruses, SARS coronavirus seems to be relatively sensitive to high temperatures. However, it is worth stressing that a short time exposure under the usual temperature for inactivation of complement (56℃) and antibody (67℃) is unable to eliminate SARS virus infectivity. A more safe threshold for inactivation should be set above 75℃ for at least 30 min, based on this study. Moreover, a mimic dry environment in laboratory conditions seems not to influence viral infectivity. Certainly, it is not comparable with the situation of natural environment, since the exact humidity in a sealed vat has not been determined. More relevantly designed experiments are needed to address this problem. Data collected from other coronaviruses showed that the half-life period of virus in aerosol was raised to 86 h when the humidity in environment reached to 80%, versus only 26 h in usual situation at 30℃, it is reasonable to think that a dry or lower humid environment may

not be suitable for virus survival.

Contrast to enteric coronaviruses, SARS coronavirus is likely to be sensitive to irradiation of UV. In this study, viruses were suspended in the culture medium and placed in 96-well plates. The distance from liquid surface to bottom was around 0.25 to 0.3 cm. Although UV is believed to be poorly penetrative, the viral infectivity in such a condition can be reduced to a very low level and even to an undetectable level after 30 min and 60 min irradiation, respectively. The irradiation dosage after 30 min exposure was measured around 162 000 $\mu W \cdot S/cm^2$. One can speculate that SARS virus absorbing or existing on the surfaces of objects in household, laboratory and hospital settings may be more likely inactivated when exposed to irradiation of UV.

Members of the SARS Research Team

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