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## STUDIES ON THE SURVIVAL OF AEROSOLIZED BOVINE ROTAVIRUS (UK) AND A MURINE ROTAVIRUS

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**Abstract**—The effect of relative humidity (RH) and temperature on the survival of airborne bovine rotavirus UK isolate (BRV-UK) and a murine rotavirus (MRV) was studied. In any one experiment, the virus under test was suspended in tryptose phosphate broth (TPB) supplemented with uranine (physical tracer) and an antifoam, was aerosolized using a Collison nebulizer into the rotating drum with the RH at either low ( $30 \pm 5\%$ ), medium ( $50 \pm 5\%$ ) or high ( $80 \pm 5\%$ ) level at  $20 \pm 1^\circ\text{C}$ . Following a 15-min period of viral aerosol stabilization, sequential samples of drum air were collected using an All-Glass Impinger (AGI) for 24 h post-aerosolization. Both of the rotavirus isolates were found to survive best at medium RH level and high RH was found least favorable for the survival of these aerosolized rotaviruses. The survival pattern of aerosolized MRV was found to be the best when compared with survival pattern of all animal and human rotavirus isolates studies performed under aerosolized conditions in our laboratory. The findings of these experiments confirm and extend our previous reports on the survival of other animal and human aerosolized rotaviruses and emphasize the fact that air may be one of the vehicles for their dissemination and could explain why it is difficult to control nosocomial outbreaks of rotavirus gastroenteritis and to keep animal colonies rotavirus-free.

**Key words:** Rotaviruses, viral aerosols, rotavirus transmission, bovine rotavirus, murine rotavirus.

**Résumé**—On a étudié ici les effets de l'humidité relative (RH) et de la température sur la survie du rotavirus bovin isolé, dans l'air ambiant, au Royaume-Uni (BRV-UK) et d'un rotavirus murin (MRV). Pour chaque expérience, le virus testé était placé dans un bouillon de culture de tryptose phosphate (TPB) supplémenté en uranine (marqueur physique) et en agent antimoussant, puis était mis en suspension aérosole à l'intérieur du tambour rotateur grâce au nébuliseur de Collinson, à une humidité relative, soit faible ( $30 \pm 5\%$ ), soit moyenne ( $50 \pm 5\%$ ), soit forte ( $80 \pm 5\%$ ) et à une température de  $20 \pm 1^\circ\text{C}$ . Une fois la suspension aérosole virale stabilisée au bout de 15 min, et pendant les 24 h suivant la nébulisation, des prélèvements d'air à l'intérieur du tambour étaient régulièrement effectués, grâce à un "All Glass Impinger" (AGI). On a alors observé que les deux virus isolés survivaient mieux à une humidité relative moyenne alors qu'une forte RH était la moins favorable à la survie en milieu aérosole des rotavirus. On a remarqué que le type de MRV ayant survécu en condition aérosole était celui correspondant le mieux aux rotavirus des animaux et de l'homme, déjà isolé dans notre laboratoire aux cours d'études en milieu aérosole. Les résultats de ces expériences confirment et complètent nos compte-rendus précédents sur la survie en suspension dans l'air des rotavirus d'autres animaux et de l'Homme; cela renforce l'idée que l'air pourrait être un des agents de leur dissémination, et pourrait expliquer pourquoi il est difficile d'endiguer

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l'apparition nosocomiale de gastro-entérites à rotavirus et de protéger de ces rotavirus les allotements de'animaux.

*Mots-clefs:* Rotavirus, aerosols viraux, transmission, rotavirus bovin, rotavirus de muridés.

## INTRODUCTION

Rotaviruses are well recognized as important agents of neonatal enteritis of humans and animals [1–3]. It has also been shown to be involved in cases of adult diarrhea [4, 5]. Different modes and vehicles, like food, water and surfaces [6] have been implicated in their transmission. The seasonal nature of rotavirus-induced gastroenteritis outbreaks and other epidemiological evidence available suggest that air may play a role in the direct or indirect dissemination of rotaviruses [7].

We had reported earlier that a number of animal and human rotavirus isolates could survive well in the airborne state and that the medium RH was found to be the most favorable in this regard. In contrast to our work with several isolates of rotavirus, Moe and Harper [8] reported that the low (20%) and high (90%) RH levels were more conducive to the airborne survival of BRV-UK. We had compared our bovine (Canadian isolate C-486), simian (SA-11 strain), and human rotaviruses (Wa strain) with other enveloped and non-enveloped viruses in our experimental setup and confirmed the reported data on both enveloped and non-enveloped viruses [6, 7, 9–17].

In order to further investigate if there is any difference in the behavior of airborne Canadian and UK isolates of bovine rotaviruses, we have studied the survival of UK isolate of BRV in the airborne state under the same set of experimental conditions as reported earlier for other animal and human rotavirus isolates [7, 9–13, 17]. In this communication we confirm and extend our investigations on the survival of BRV-UK isolate and an MRV which has been found to be highly virulent compared to all known animal and human rotavirus isolates studied to date [18–20].

## MATERIALS AND METHODS

### *Cells and viruses*

The MA-104 cell line was used throughout the study for cultivation and quantitation of both BRV-UK and MRV. The procedure for cultivation, maintenance and passage has been described previously [21]. The MRV was obtained from Dr A. Cepica (Atlantic Veterinary College, University of Prince Edward Island, Charlottown, Canada). We obtained BRV-UK from Dr M. A. McCrae (University of Warwick, Coventry, UK).

### *Virus propagation, purification and quantitation*

The virus isolates used in this study were plaque purified in MA-104 cells and same cell line was used for preparing virus stock. In order to purify the virus, the following steps were taken. First, the cellular debris was removed from cell culture supernatant via low speed centrifugation. The virus was then concentrated by pelleting for 3 hr at 100,000 *g* through a 40% sucrose cushion. The resulting pellet was then layered onto 11.5 ml of CsCl solution (analytical grade, density 1.3688 g/ml; Sigma Chemical Co., St Louis, Mo., U.S.A.) and centrifuged at 38,000 rpm in an SW41 rotor (Beckman Model L5-65) for 17 hr

at 15°C. Complete double-shelled particles containing RNA banded at a density of 1.3692 g/ml and particles devoid of RNA band at 1.3640 g/ml. The procedure used for the cultivation and quantitation of both rotavirus isolates has been the same as reported previously [10, 19].

#### *Experimental procedure*

The procedure used for the generation, storage and collection of viral aerosols have been described earlier [13]. Briefly, the two virus isolates under test were used in separate sets of experiments. The virus under test and dye (physical tracer) was suspended in TPB and using the Collision nebulizer [22] aerosolized into a 300-l rotating drum [23] and virus-containing aerosols were held at  $20 \pm 1^\circ\text{C}$  with the desired RH level. The RH levels were adjusted with the help of a dial type hygrometer (Airguide Instruments Co., Il., U.S.A) as reported earlier [13]. The RH level was kept at either high ( $80 \pm 5\%$ ), medium ( $50 \pm 5\%$ ) or low ( $30 \pm 5\%$ ) depending upon the experiment being conducted. After rotaviral aerosolization was complete, a 15-min period was allowed for the stabilization of the viral aerosols and its even distribution within the drum. Using an AGI [24], the first air sample (time zero) from the drum was collected at the end of this aerosol stabilization period. Additional samples of air from the drum were obtained at 2, 4, 8 and 24 hr of aerosol age. The impinger fluid was divided into two portions. One of these was used for the quantitation of the dye and the other for determination of infectious virus using a plaque assay. The results described below represent the mean values taken from at least three separate sets of experiments. The formula used to calculate the pattern of biological decay of the virus in the airborne state has been reported elsewhere [13].

## RESULTS

### *Survival of aerosolized BRV-UK and MRV during the process of aerosol generation and stabilization*

It has been demonstrated that during the process of experimental aerosol generation, a certain portion of infectious virus particles may become inactivated immediately following aerosolization. The extent of such a loss of virus infectivity depends on the nature of the spray fluid as well as temperature and RH of the air [7, 13]. In order to determine these losses at the three levels of RH, the amount of infectious virus recovered in the first air sample (15-min) from the drum was compared with the quantity of infectious virus aerosolized into the drum from the pre-aerosolized infectious virus present in the spray fluid. The results of these experiments and for comparison, our previous data on other aerosolized rotaviruses are summarized in Table 1. The "initial loss" (loss in infectious virus that occurs during the process of aerosol generation and stabilization) in infectivity of both BRV-UK and MRV was highest at high level ( $80 \pm 5\%$ ) and like other animal and human rotavirus isolates they appeared to survive best during the aerosol stabilization period when the RH was at the medium level ( $50 \pm 5\%$ ).

### *Effect of RH on the survival of aerosolized BRV-UK and MRV*

The two rotavirus isolates under study were sprayed into the drum in separate experiments to compare their survival at three levels of RH. At least three experiments were conducted at each of the three RH levels. The pattern of biological decay of BRV-UK and MRV has been shown in Figs 1, 2 and Table 2. In both cases, when viral aerosols were

Table 1. Percentage recovery of rotaviruses following the process of aerosol generation and stabilization held at  $20 \pm 1^\circ\text{C}$ 

Viruses	Relative humidity			Reference
	High ( $80 \pm 5\%$ )	Medium ( $50 \pm 5\%$ )	Low ( $30 \pm 5\%$ )	
Bovine rotavirus (UK)	$29 \pm 2$	$74 \pm 3$	$39 \pm 3$	Ijaz <i>et al.</i> (Present study)
Bovine rotavirus (C-486)	$27 \pm 2$	$70 \pm 2$	$37 \pm 5$	Ijaz <i>et al.</i> [10]
Bovine rotavirus (UK)	NA	NA	NA	Moe and Harper [8]
Simian rotavirus (SA-11)	$25 \pm 5$	$78 \pm 2$	$42 \pm 3$	Sattar <i>et al.</i> [17]
Human rotavirus	$34 \pm 4$	$87 \pm 5$	$46 \pm 4$	Ijaz <i>et al.</i> [11]
Mouse rotavirus	$25 \pm 4$	$94 \pm 2$	$59 \pm 5$	Ijaz <i>et al.</i> (Present study)

In all experiments (except Moe and Harper [8]) a 15-min period was allowed for aerosol stabilization and the first air sample was collected at the end of this period. To determine the extent of virus survival during this period, the titer of the air sample was compared with the amount of virus aerosolized. At least 3 separate experiments were conducted with both rotavirus isolates and the results are presented as the mean  $\pm$  SD.

NA: Not available.

maintained in the drum at medium RH, the survival of both of these viruses was found to be the best with half-life of 18 hr (BRV-UK) and  $>24$  hr (MRV). The half-life of these viruses at low RH level was 9 hr (BRV-UK) and approximately 15 h (MRV). The high RH was found to be least favorable, where the half-life for both virus isolates was approximately 2–3 hr.

The influence of relative humidity, air temperature, and suspending medium on the survival of rotaviruses in air has been studied in our laboratory and the available information generated in our laboratory on the half-life of aerosolized enveloped and non-enveloped viruses including various rotavirus isolates has been summarized in Table 2. When the pattern of biological decay of both BRV-UK and MRV at three levels of RH was compared with other rotavirus isolates, their behavior in aerosolized form was found to be similar at all three levels of RH.

## DISCUSSION

The survival of viruses in the environment is influenced by a number of factors e.g. RH, temperature and suspending medium etc. [6, 7]. We have observed over the past several years that survival of different rotavirus isolates in air [7, 9–13, 16, 17], and on surfaces [6] is influenced drastically by the level of RH used in experimental conditions. The experimental observations made in the present study on the survival of aerosolized

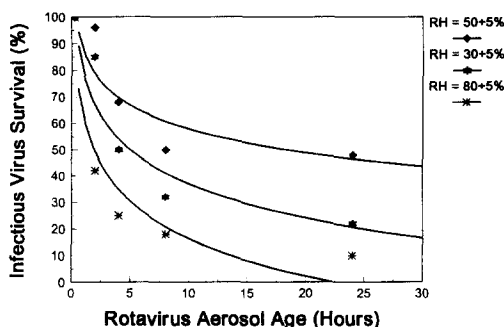


Fig. 1. Effect of relative humidity (RH) on the survival of aerosols of BRV-UK stored at  $20 \pm 1^\circ\text{C}$  for 24 hr.

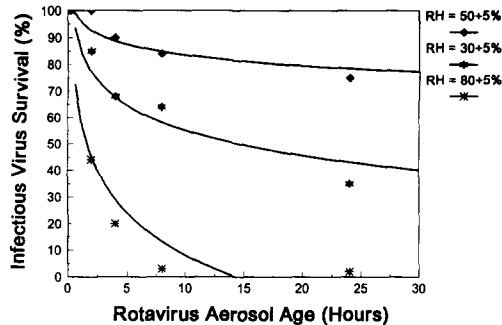


Fig. 2. Effect of relative humidity (RH) on the survival of aerosols of MRV stored at  $20 \pm 1^\circ\text{C}$  for 24 hr.

BRV-UK and MRV confirms and extends our previous reports on a number of aerosolized animal and human rotaviruses (Tables 1 and 2).

Regarding BRV-UK this report also nullifies any possible discrepancy which could have occurred due to the difference in BRV-C486 isolate (Canadian) used by us in our previous studies [10] as compared with the one (BRV-UK) reported by Moe and Harper [8]. Here it should be noted that the findings of Moe and Harper were based on a single set of experiments. In addition they ran their experiment for only 2 hr as opposed to our studies involving aerosolized rotavirus isolates where samples of viral aerosols were collected for a minimum period of 24 hr. Furthermore, in order to discount for the "initial loss" which takes place during the period of aerosol generation and stabilization [13], we started sampling drum air 15-min following virus aerosolization and stabilization of rotavirus-containing aerosol cloud where as Moe and Harper started taking air samples immediately following virus aerosolization.

The aerosolized MRV was found to survive much better compared to the survival pattern of any other aerosolized rotavirus isolate studied so far in our laboratory (Tables 1 and 2). *In vitro* morphological and biochemical studies carried using MRV also revealed that MRV being highly stable as compared to other animal rotaviruses [20, 25, 27]. EDTA has been reported to convert double-shelled BRV into single-shelled particles at pH 7.1 [28]. However, we found that higher pH levels were required to solubilize MRV glycoprotein and that, even at pH 8.0, fewer particles were converted to the single-shelled form, as compared to BRV. Furthermore, under *in vivo* conditions the MRV was found

Table 2. Half-life in hours of aerosolized viruses under different conditions of relative humidity at  $20 \pm 1^\circ\text{C}$

Viruses	Relative humidity			Reference
	High ( $80 \pm 5\%$ )	Medium ( $50 \pm 5\%$ )	Low ( $30 \pm 5\%$ )	
Bovine rotavirus (UK)	NA	NA	NA	Moe and Harper [8]
Bovine rotavirus (UK)	$3 \pm 0.2$	$18 \pm 2$	$9 \pm 0.5$	Ijaz <i>et al.</i> (Present study)
Bovine rotavirus (C486)	**	$25 \pm 3$	$19 \pm 4$	Ijaz <i>et al.</i> (Present study)
Mouse rotavirus	$2 \pm 0.1$	$24 \pm 2$	$15 \pm 1$	Ijaz <i>et al.</i> (Present study)
Rotavirus SA-11	$5 \pm 0.32$	$64 \pm 16$	$22 \pm 5$	Sattar <i>et al.</i> [17]
Human rotavirus (Wa)	$9 \pm 2$	$28 \pm 10$	$19 \pm 4$	Ijaz <i>et al.</i> [11]
Human coronavirus	$3 \pm 0.16$	$67 \pm 8$	$27 \pm 6$	Ijaz <i>et al.</i> [12]
Poliovirus type 1 (Sabin)	$9 \pm 2$	NR	NR	Ijaz <i>et al.</i> [10]

\*\*Drop in virus titer was too rapid to make any possible statistical calculations.

NA: Not available from Moe and Harper [8] as they studied the survival for only 2 hr.

NR: No infectious virus was recovered.

to be more virulent than BRV not only in homologous host but in the heterologous host as well. Taken together our aerovirological data on MRV along with other *in vitro* and *in vivo* studies explain the general difficulty of the scientific community in keeping rodent's colonies rotavirus-free [7, 19, 29].

It is therefore suggested that rotaviruses in general survive well in an aerosol state and medium-range of RH is highly conducive for the survival of different animal and human rotaviruses [7, 9–13, 16, 17]. This information along with the findings of Yolken's group [34] that mice can be infected with rotavirus via aerosol challenge could explain the outbreaks of rotavirus enteritis in the hospital setting and under temperate environmental conditions [21] where the RH is medium and temperature is low. This further emphasizes the need of keeping rotavirus patients in isolation units in order to control nosocomial outbreaks involving rotavirus-enteritis and hence help to reduce the hospitalization cost [31].

During nosocomial and community outbreaks of rotaviral diarrhea, it has been commonly observed that the appearance of respiratory symptom precedes diarrheal phase [1, 2, 32]. However attempts to recover rotavirus in respiratory mucous specimens have met only a limited success [33–35]. Here it should be noted that replication of rotavirus in the respiratory tract may not be necessary before producing gastroenteritis because rotavirus-containing aerosols collected in the respiratory tract can simply be translocated by mucociliary activity and ingested [7]. Such a mechanism appears to be operating specially when one examines the data in the light of several previous observations on the distribution of inhaled protein antigens following aerosol administration under *in vivo* conditions. It has been reported that the bulk (70%) of inhaled protein antigens are rapidly cleared into the gastrointestinal tract following both short-term and prolonged aerosol exposure [36, 37]. It is quite conceivable that such mechanisms may not be only operative in cases of rotaviruses but also following respiratory exposure to other enteric viruses. Additional *in vitro* and *in vivo* aerovirological studies on such viruses might help to elucidate further the mechanisms of enteric viral infections.

Although the technology is now available to sample large volumes of air for recovery of viruses from the air [7, 14], no attempt has yet been made to isolate infectious rotavirus from naturally contaminated air. If naturally aerosolized rotavirus survive to the same extent as laboratory-adapted viruses, air should readily disseminate rotaviruses in settings such as hospital wards, nursing homes, day-care centers, animal houses, municipal waste treatment plants, and research and clinical laboratories.

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