Comparative studies to assess the inactivation patterns of chlorine and ethanol against MNV and FCV

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Objective

A study was performed aiming to assess the inactivation patterns of two surrogates' models of human norovirus - murine norovirus (MNV) and feline calicivirus (FCV) - to two common disinfectants, ethanol and bleach. The inactivation profiles of MNV and FCV after different disinfectant treatment conditions were compared by measuring reduction of infectivity by viral plaque assay and Tissue Culture 50% Infectivity Dose ($TCID_{50}$) and reduction of viral RNA by TaqMan real-time RT-PCR (qRT-PCR).

Methodology

VIRUSES AND CELL LINES: MNV (strain CW3) was propagated in RAW 264.7 cells. FCV strain F9 was propagated in CRFK (Crandell Reese Feline Kidney cell line).

TESTING PROCEDURE:
Surface test **: ASTM E1052-96**
Suspension test **: ASTM E1053-97**

TREATMENT CONDITIONS:

Ethanol:
- Concentrations: 50 , 70 and 90%
- Surface and suspension tests
- Contact times: 1, 5 and 10 minutes at room temperature
- Neutralizer solution: 10% fetal bovine serum (FBS)

Sodium hypochlorite
- Free chlorine concentrations: 1000 , 2500, 5000 ppm
- Surface test
- Contact times: 1 minute at room temperature
- Neutralizer solution: 0.5% $Na_2S_2O_3$ in 10%FBS

Effect of pH and organic load on virucidal activity of 70% ethanol
- pH range: 5, 7 and 8
- Organic load: 25% FBS for bleach experiment
- Test condition: 1 minute at room temperature
- Neutralizer solution: 10% FBS

VIRAL PLAQUE ASSAY

- Cell culture dishes (60 mm) seeded with 10^5 viable cells
- Confluent monolayer formation (37°C, 5% CO_2, 4-5 days)
- Inoculation with neutralized viral treated suspension
- Viral adsorption during 1h (37°C, 5% CO_2)
- First overlay - complete MEM with 0.5% agarose
- Incubation for 48 h (37°C, 5% CO_2)
- Second overlay – complete MEM with 0.5% agarose plus 0.6% neutral red

TISSUE CULTURE 50% INFECTIVITY DOSE ($TCID_{50}$)

- Cell culture plates (24-wellls) seeded with 10^5 viable cells
- Confluent monolayer formation (37°C, 5% CO_2, 4-5 days)
- Inoculation with 10-fold dilutions of neutralized viral treated suspension (four replicates)
- Viral adsorption during 1h (37°C, 5% CO_2)
- Maintenance media (2 ml)
- Incubation for 7 days (37°C, 5% CO_2)
- Monitor cytophatic effect for 1 week

TAQMAN REAL-TIME RT-PCR (RT-qPCR)

- PCR amplication conditons:
  (i) RT 30 min at 50°C
  (ii) 15 min at 95°C
  (iii) 45 cycles of 10s at 95°C, 32 s at 55°C and 15s at 72°C

QuantiTect RT-PCR kit (Qiagen) and MNV/FCV oligonucleotide primers and probes (Park *et al*., 2010 *J Food Protection*) were used.

Results

Disinfectant	Concentration	Virus Suspension	Test type	Time (min)	Reduction in titer: Plaque assay (log PFU/ml) MNV	Plaque assay (log PFU/ml) FCV	qRT-PCR (log copy no./ml) MNV	qRT-PCR (log copy no./ml) FCV	$TCID_{50}$ (log TCID50/ml) MNV	$TCID_{50}$ (log TCID50/ml) FCV
Ethanol	50%	individual	Surface	1	1	>5	2	1	n.d.	n.d.
				5	2	4	2	1	n.d.	n.d.
				10	1	>5	2	1	n.d.	n.d.
			Suspension	1	1	<1	1	1	n.d.	n.d.
				5	1	1	1	1	n.d.	n.d.
				10	1	2	1	1	n.d.	n.d.
	90%		Surface	1	3	3	4	2	n.d.	n.d.
				5	3	3	4	2	n.d.	n.d.
				10	2	3	3	2	n.d.	n.d.
			Suspension	1	3	1	4	1	n.d.	n.d.
				5	3	1	5	1	n.d.	n.d.
				10	3	2	4	1	>4	3
	70%; pH 5	individual	Suspension	1	3	3	6	1	n.d.	n.d.
	70%; pH 7				5	2	5	1	n.d.	n.d.
	70%; pH 8 [a]				5	2	5	1	n.d.	n.d.
	70%; pH 5	Pooled 2MNV:FCV			2	1	5	1	n.d.	n.d.
	70%; pH 7				3	1	5	1	>4	n.d.
	70%; pH 8 [a]				4	2	>6	1	n.d.	n.d.
Bleach	1000 ppm	individual in 25% FBS	Suspension	1	1	>4	<1	<1	>3	n.d.
	2500 ppm				1	>4	<1	<1	n.d.	n.d.
	5000 ppm				2	>4	<1	<1	n.d.	>2

n.d. not determined
[a] natural pH 0f 70% Ethanol solution

Different inactivation responses were obtained by MNV and FCV for the same disinfection assays conditions :

- **50% ethanol was effective against FCV with ≥ 4 log10 PFU/ml reduction after 1 minute of exposure using the surface test, while ethanol at ≥ 70% showed reduced efficacy against FCV.**
- **MNV was very susceptible to ethanol at ≥ 70%**
- **Compared to the suspension test, the surface test showed a higher reduction of both surrogate viruses for 50% ethanol.**
- **Overall, the use of pooled surrogate virus suspensions yielded reduced infectivity compared to individual surrogate viruses.**
- **Sodium hypochlorite at concentrations of 1000, 2500 and 5000 ppm inactivated FCV by > 4 log10 PFU/ml after 1 minute, whereas MNV infectivity was reduced by 2 log10 after exposure to 5000 ppm of sodium chlorite for 1 minute.**
- **Both ethanol and sodium hypochlorite demonstrated that no strong correlation between viral RNA loss and infectivity reduction exist.**

Concluding Remarks

- **FCV and MNV displayed different inactivation profiles against ethanol. FCV is more sensitive to low concentrations of ethanol, and MNV is more sensitive to high ethanol concentration.**
- **Present data imply that MNV is more resistant to sodium hypochlorite than FCV.**
- **Pooling MNV and FCV was less sensitive than testing the individual viruses.**
- **No consistent correlation was found between infectivity assay and RT-qPCR assay.**
- **Data of susceptibility to disinfectants obtained using surrogate viruses should be extrapolated to human norovirus with caution.**

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