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CHEMICAL INACTIVATION OF CALICIVIRUSES: A COMPARATIVE STUDY

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Introduction

Members of the family *Caliciviridae* are non-enveloped small viruses, with a single stranded positive-sense genomic RNA grouped into four different genera: Norovirus, Sapovirus, Vesivirus and Lagovirus.

Out of these genera Noroviruses (NV) have emerged as one of the most common causes of acute gastroenteritis in humans with sporadic cases as well as large outbreaks in various community settings (6, 9). Although the course of disease is usually mild and self-limiting, the combination of high viral load in vomit and feces together with high environmental stability of the viral particles represents a public health risk (10). Residual virus after disinfection can result in prolonged or recurring outbreaks with considerable economic losses. Previous studies have revealed the potential role of environmental surfaces in the transmission of Caliciviruses (3, 7). Therefore, measures for prevention and control of Calicivirus-spread are necessary to assure public safety.

Since there are no known animal or mammalian cell culture systems that can determine infectivity of NV, a closely related virus, the feline Calicivirus (FCV) of the genus Vesivirus, has been suggested to be an adequate model for NV-inactivation studies because it has similar genome organization, capsid architecture and biochemical properties (3, 5, 7, 12, 15, 17). FCV, one of the most common agents of conjunctivitis and upper respiratory tract disease in cats, can be cultured and plaque assayed (5, 11, 12, 15, 16, 17).

According to the guidelines of the German Veterinary Association (DVG) disinfection tests with four commercially available disinfectants (an organic acid, an aldehyde, a halogen compound and peroxide) were carried out in this study to determine their potential to accomplish the claims which are made regarding their efficacy under realistic test conditions. In this context we evaluated the suitability of FCV as a model for NV using molecular methods (RT-PCR) to test both cultivable and non-cultivable viruses.

Materials and methods

Viruses

FCV, strain F9 (Viroagen-felis RC Virbac) was propagated in Crandell Reese feline Kidney (CRFK- CCL-94 American Type Culture Collection). The virus pool supernatant from infected monolayers was titrated for $TCID_{50}$/ml according to Kärber (8), and tested by RT-PCR estimating the endpoint of serial 10-fold dilutions resulting in positive amplification-products.

Stool specimens positive for NV (genogroup I or II), provided by the Landesgesundheitsamt Baden-Württemberg (Stuttgart, Germany), were prepared as 50% (wt/vol) suspension in water of standardized hardness (WSH) pH 7,2 and tested by RT-nested PCR.

Virus was finally stored in 1 ml aliquots at –80C°.

Disinfection tests

Suspension tests with known quantities of virus (FCV and NV) mixed, for defined contact periods (15', 30', 60', 120'), with the disinfectant in concentrations (disposed in WSH) recommended by the manufacturer were carried out with *Venno* ™ *FF super* (Menno Chemie Vertrieb GmbH), *Venno* ™ *Vet 1 super* (Menno Chemie Vertrieb GmbH), *Sodium hypochlorite solution* (Roth GmbH) and *Oxystrong FG* (Ausimont GmbH) according to the guidelines of the DVG (1, 2).

For FCV, disinfection tests were carried out both with and without protein-load (40% of FBS) and in a 25% suspension (wt/vol) with human fecal material negative for NV and free from FCV-contamination as tested by RT-(nested) PCR.

FCV-disinfectant mixture was assayed after each time of contact by calculating the $TCID_{50}$/ml in cell culture and tested by RT-PCR after RNA-extraction using the guanidinium isothiocyanate method previously described by Boom et al. (1990). For NV a RT-nested PCR was used alone as detection method.

The four highest 10-fold dilutions of the untreated virus (NV and FCV) tested positive in RT-(nested) PCR were then examined after disinfection tests.

RT-(nested) PCR

FCV RT-PCR (126-bp) and NV RT-nested PCR (338 bp) target conserved portions of the RNA-dependent-RNA polymerase gene respectively (13, 14).

Results

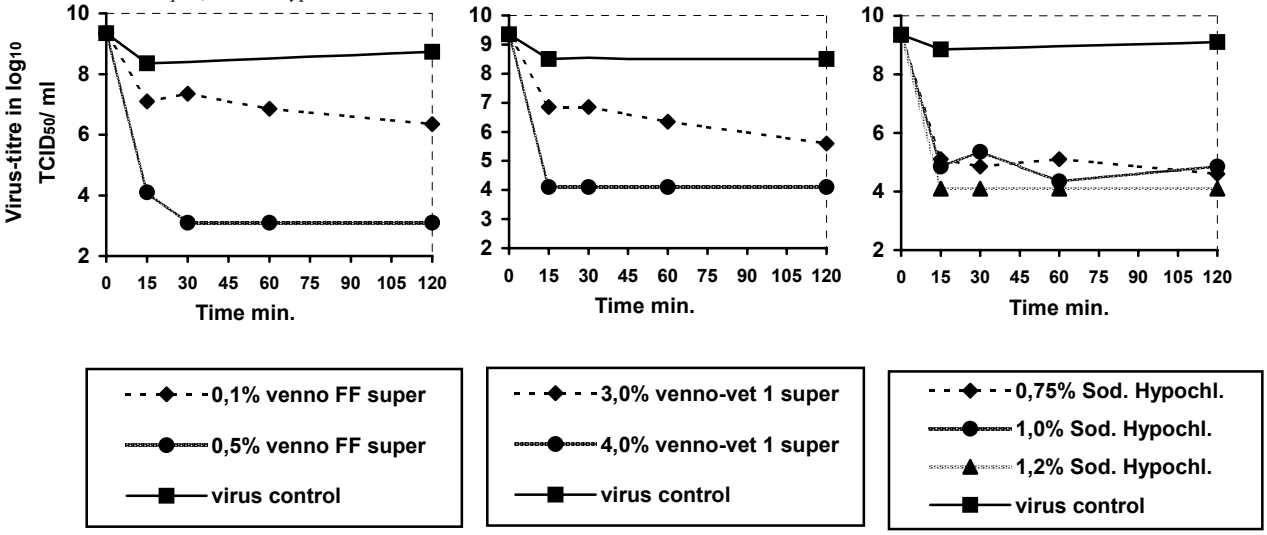
Suspension tests with FCV mixed with human fecal material showed for the aldehyde (*Venno*^{TM} *FF super*) a sufficient inactivation at a concentration of 0,5 % (Fig. 1) regardless of the presence of FBS (data not shown). However, by RT-PCR viral RNA could still be detected with a reduction of only 2 dilution steps (log10) at this concentration, depending on the term of contact (Tab. 1). In comparison, for NV, RT-PCR did not show any reduction. Only at concentrations of 1,0% and 2,0% intact RNA was reduced by 1 dilution step.

For the organic acid (*Venno*^{TM} *Vet 1 super*) a concentration of 3 to 4% was necessary (Fig.1) to remarkably reduce the infectivity of FCV mixed with human fecal material and FBS, while without FBS 0,5% of the disinfectant inactivated FCV by more than 3 dilution steps (data not shown). With fecal material RT-(nested)PCR showed no reduction of intact RNA until a concentration of 4% of the organic acid, where it was reduced by 3 dilution steps for FCV and 1 dilution step for NV. A reduction of 2 dilution steps in RT-nested PCR is provable in the NV disinfection test with 5% of the organic acid (Tab. 1).

Considering the halogen compound (*Sodium hypochlorite solution*), FCV is noticeably inactivated, in fecal suspension, with 0,75% of sodium hypochlorite and at least 5500 ppm of free-chlorine (Fig.1) but, in absence of impurities was enough a concentration of 0,1% and 900 ppm of free-chlorine (data not shown). A retention of nucleic acid, after disinfection test in feces, was still evident without (for FCV) or with only 1 step (for NV) reduction in RT-(nested) PCR, in comparison with the untreated virus-dilutions, even in correspondence of the highest hypochlorite concentrations (1%-1,2%) (Tab.1).

For the peroxide (*Oxystrong FG*), disinfection tests are still proceeding.

Fig. 1: Inactivation of FCV, in sunspension test with human fecal material, with reference to different concentrations of *Venno*^{TM} *FF super, Venno*^{TM} *Vet 1 super*, *Sodium hypochlorite solution* and to different terms of contact.



Tab. 1: PCR results of the FCV/NV disinfection tests with Venno™ FF super, Venno™ Vet 1 super, Sodium hypochlorite solution:
10^{-n} = 10 – fold dilutions ; + = positive result ; - = negative result; n.t. = not tested.

PCR RESULTS		SUSPENSION TEST WITH HUMAN FECAL MATERIAL									FCV/NV Virus Control
Disinfection middle concentration		Time of contact (min)	FCV				NV				
			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2} - 10^{-5}
VENNO FF SUPER	0,1%	15	+	+	+	+	n.t.				+
		30	+	+	+	-					+
		60	+	+	+	+					+
		120	+	+	+	+					+
	0,5%	15	+	+	+	-	+	+	+	+	+
		30	+	+	-	-	+	+	+	+	+
		60	+	+	-	-	+	+	+	+	+
		120	+	+	-	-	+	+	+	+	+
	1,0%	15	n.t.				+	+	+	-	+
		30					+	+	+	-	+
		60					+	+	+	-	+
		120					+	+	+	-	+
	2,0%	15	n.t.				+	+	+	-	+
		30					+	+	+	-	+
		60					+	+	+	-	+
		120					+	+	-	-	+
VENNO VET 1 SUPER	3,0%	15	+	+	+	+	+	+	+	+	+
		30	+	+	+	+	+	+	+	+	+
		60	+	+	+	+	+	+	+	+	+
		120	+	+	+	+	+	+	+	+	+
	4,0%	15	+	-	-	-	+	+	+	-	+
		30	+	-	-	-	+	+	+	-	+
		60	+	-	-	-	+	+	+	-	+
		120	+	-	-	-	+	+	+	-	+
	5,0%	15	n.t.				+	+	+	-	+
		30					+	+	-	-	+
		60					+	+	-	-	+
		120					+	+	-	-	+
SODIUM HYPOCL. SOLUTION	1,0%	15	+	+	+	+	+	+	+	-	+
		30	+	+	+	+	+	+	-	-	+
		60	+	+	+	+	+	+	-	-	+
		120	+	+	+	+	+	+	+	-	+
	1,2%	15	+	+	+	+	+	+	+	-	+
		30	+	+	+	+	+	+	+	+	+
		60	+	+	-	-	+	+	+	-	+
		120	+	+	+	+	+	+	+	-	+

Discussion

According to the results shown in the present study, for *Venno ™ FF super* even lower concentrations than those recommended by the DVG (2) are highly effective to inactivate FCV. This is not true for the organic acid (*Venno™ Vet 1 super*), that fails to inactivate FCV when additional protein or organic load is included. The activity of the *Sodium hypochlorite solution* is markedly reduced in the presence of proteins or organic matter and the results obtained in our studies on FCV are in agreement with previous reports (5, 7, 17).

The infectivity of viruses requires the functional integrity of the capsid and viral RNA. Disinfection treatment may produce conformational changes in the capsid which diminish viral stability or affect attachment to cell receptors reducing viral infectivity, but doesn't necessarily destroy the viral RNA (11). Therefore detection of viral RNA by RT-PCR does

not essentially represent infectious virus (15) and it cannot be assumed that detection of viral RNA by RT-PCR in samples negative on cell-culture was due to a greater sensitivity of the PCR over cell-culture assay.

Considering the differences in RT-PCR-protocols for NV and FCV, which base on different target sequences, as well as potentially different virus aggregation within cells and feces, the results of this study reveal analogies in FCV- and NV-reaction during chemical inactivation processes. Finally, for evaluating the discrepancies between the two RT-PCRs a quantitative real-time PCR, using LightCycler™ technique with corresponding software (Version 3.5) for analysis and SYBR Green reagents (Roche, Germany) will be performed.

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