Efficacy of disinfectants against porcine rotavirus in the presence and absence of organic matter

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Running Head: Efficiency of disinfectants against porcine rotavirus

Significance and impact of study:

Infection of rotavirus has a negative impact on the health and growth of pigs in production. Given that the virus is transmitted faecal orally, use of an effective disinfectant on farm, which works even in high organic matter, has the potential to save costs in terms of outbreaks of disease and viral contamination. Here we test a number of commercial disinfectants of which one a phenolic compound, Bio-OO-cyst, shows effectivity even in high organic matter, implying its use could have a huge impact in reducing viral contamination and preventing losses in production.

Abstract:

Rotavirus is an enteric pathogen that causes morbidity and mortality in young mammals, including pigs. Outbreaks of rotavirus on commercial farms have a significant economic impact in terms of losses in production. Effective cleaning and disinfection along with good farm management can reduce rotavirus contamination in the environment, and decrease the chance of outbreaks of disease. This study investigated the efficacy of six commercial disinfectants against MS2 bacteriophage and Group A porcine rotavirus, in the presence of high and low levels of organic matter to simulate the farm environment. A phenolic-based disinfectant (Bi-OO-cyst) was effective at all levels of organic matter concentrations. Iodophore based disinfectants did not have a significant virucidal effect against rotavirus under any conditions. For peroxygen compound-based disinfectants and glutaraldehyde-based disinfectants, organic matter load made a significant difference in reducing efficacy. This highlights the importance of thorough cleaning with detergent before disinfection to reduce viral contamination on the farm and decrease rotavirus disease incidence in pigs.

Keywords: Viruses, Bacteriophage, Disinfectant, Diseases, Veterinary

Introduction

Rotavirus is a common disease on pig farms worldwide (Svensmark et al. 1989b; Dewey et al. 2003; Katsuda et al. 2006; Miyazaki et al. 2012) and prevalent in the UK (Chandler-Bostock et al. 2014). It causes severe gastroenteritis in young pigs, leading to a loss in production (Svensmark et al. 1989a). It is transmitted by the faecal-oral route and mostly affects neonates (<7 days old) and pigs around the time of weaning (21–28 days old) (Estes and Kapikian 2007). There are porcine rotavirus vaccines but they do not have proven efficacy in pigs (Hoblet et al. 1986; Saif and Fernandez 1996; Kohler et al. 2012). The wide range of rotavirus genotypes that can affect pig herds adds to the complexity of producing an effective vaccine and leads to the chance of pigs being susceptible to being infected more than once with rotavirus. A pragmatic approach to prevent outbreaks of disease is to reduce the levels of rotavirus and other enteric viruses on a farm through cleaning and disinfection (Hancox et al. 2013).

Infectious rotavirus particles can survive over 9 months in a farm environment and over two years in faeces at 4°C (Ramos et al. 2000; Estes and Kapikian 2007). Thus without adequate cleaning and disinfection, rotavirus will potentially persist on a farm. Non-enveloped viruses such as rotavirus are likely to be more resistant to disinfection than other farm pathogens, as rotavirus has a double icosahedral shell, which is more robust than bacterial cell membranes or lipid enveloped viruses (Hansen et al. 2007).

Disinfectant testing

In the UK, the Department for Environment, Food and Rural Affairs (DEFRA) test disinfectants and approve their use on farms. They test disinfectants against food and mouth disease virus (FMDV), swine vesicular diseases (SVDV), avian influenza, tuberculosis and for general use but not against rotavirus (DEFRA 2014). A disinfectant is deemed effective if there is greater than 4 $\log_{10}$ reduction of viral titre by tissue culture assay and the approved dilution is recorded (DEFRA 2014). SVDV and FMDV

are non-enveloped viruses like rotavirus, so they should react similarly to rotavirus in terms of disinfectant efficacy. However, DEFRA do not routinely test disinfectants in the presence of a range of organic matter conditions, the viruses were tested without any organic matter except FMDV which was assessed in solutions with 1% FBS (DEFRA 2014). Although DEFRA do not test the full range of viruses that may be present on a farm in the presence of organic matter, it is likely that the disinfectant companies do, and this test is relevant to on farm application. However, disinfectant manufacturers may well use different protocols to test their disinfectants; therefore their results may not be directly comparable. Here, we compare disinfectants under identical conditions.

Results and Discussion

The effect of organic matter on disinfectant efficacy

Organic matter had a significant effect on reducing the efficacy of disinfectants tested against MS2 phage and porcine rotavirus (Vanodox, GPC8, and Virkon S), except when Bi-OO-cyst was used. Bi-OO-cyst was the only disinfectant that achieved more than 4 $\log_{10}$ reduction in MS2 titre at no, low or high organic matter concentrations (Fig. 1). Vanadox (peracetic acid based), GPC8 (glutaraldehyde based) and Virkon S (peroxygen compound based) disinfectants were only effective in low and no organic matter suspensions, all reducing the MS2 phage titre by more than 4 $\log_{10}$. However, these disinfectants had lower efficacy in the presence of high organic matter (5 % FBS, 10 % yeast extract). Both FAM30 and Biophen Plus failed to reach the 4 $\log_{10}$ titre reduction threshold in any conditions, neither of those disinfectants reduced the viral titre by more than 2.5 $\log_{10}$, so would not be considered effective against this virus (Fig. 1). In general, high levels of organic matter had an adverse effect on the efficacy of the disinfectants used in this study ($P > 0.001$ by two-factor ANOVA). There was a significant difference in viral inactivation between disinfectants ($P < 0.001$) and between MS2 when in suspension with different levels of organic matter ($P < 0.001$), but there was no significant interaction between the disinfectant and the level of organic matter ($P = 0.834$).

The high organic matter concentration (5% FBS, 10% yeast extract) represented a poorly cleaned pig farm whereas the low organic matter concentration (3% FBS) represented a "clean" pig farm with residual organic matter (Thompson et al. 2007). These levels of organic matter were higher than used in DEFRA tests (1% FBS or none). In general, there was a bigger difference in reduction in titre between high and low organic matter than between low and no organic matter solutions. This suggested that low levels organic matter left in the environment will not adversely affect the disinfectant, but without adequate cleaning to reduce organic matter to low levels before disinfection, the disinfectant will have little effect in reduction of viral titre

Disinfectant efficacy against porcine rotavirus

Similar to the MS2 phage results, Bi-OO-cyst reduced the viral titre by more than 4 $\log_{10}$ in all organic matter conditions. Vanadox and GPC8 reduced the viral titre by more than 4 $\log_{10}$ when there was no organic matter present in the solution; both disinfectants also gave about a 4 $\log_{10}$ reduction in titre with low levels of organic matter, but less than 1 $\log_{10}$ reduction in high levels of organic matter (Fig. 2). Virkon S behaved similarly to Bi-OO-cyst in no organic matter and low organic matter conditions, but the efficacy of the disinfectant dropped significantly with high levels of organic matter, to 1 $\log_{10}$ reduction in viral titre. Overall, the difference in viral inactivation in organic matter data was significant ($P \leq 0.001$), as was inactivation in the different disinfectants ($P \leq 0.01$) (Fig. 1, Fig. 2). The interaction between disinfectant and organic matter was also significant ($P \leq 0.05$) (Fig 1, Fig. 2).

MS2 phage as a model for porcine rotavirus

The disinfectants Vanodox, GPC8, Bi-OO-cyst and Virkon S were tested against MS2 phage and porcine rotavirus. The results from each test (Fig. 1 and Fig. 2) were compared statistically to determine the suitability of MS2 phage as a model for porcine rotavirus in these studies. A two-tailed Mann-Whitney test showed that there was no significant difference between porcine rotavirus and MS2 phage titre post-disinfection under a range of organic matter conditions: U = 61.0, n = 12, 12, $P = 0.551$. There was a significant relationship between rotavirus and MS2 page results in the disinfection studies;

Spearman's *rs* = 0.697, *n* = 12, *P* = 0.012. An *rs* value of 0.697 represented a reasonable model based on a weak-positive correlation (Fig. 3). The MS2 phage disinfection showed similar patterns to rotavirus disinfection, for all four disinfectants tested against both viruses. MS2 phage was a reasonable model for porcine rotavirus, in agreement with a previous study by Hansen et al. (2007).

Phenolic disinfectants

This study has shown both phenolic-based disinfectants were not affected by organic matter conditions, although their efficiencies at disinfection were different. Biophen plus had <2 $\log_{10}$ reduction in all conditions; Bi-OO-cyst had >5 $\log_{10}$ reduction in all conditions, making it the most effective disinfectant. Disinfectants cannot be judged solely on the primary active ingredient but also the delivery system, Bi-OO-cyst contains ether and Biophen contains isopropyl-alcohol, which may have altered efficacy of the disinfectant as well as the organic matter variable.

Iodophore disinfectants

This class of disinfectant can be effective against bacteria as it blocks electron transport in respiratory chain reactions and interacts with proteins of positive and neutral charge (Maris 1995). In this study the iodophore based disinfectant FAM30 had no significant effect on MS2 phage titre in any conditions (Fig. 1) so was not included in the tests against porcine rotavirus. Iodophore disinfectants have previously been shown to be ineffective against viruses (Sattar et al. 1983; Springthorpe et al. 1986; Martin et al. 2008). FAM30 is approved by DEFRA for use against non-enveloped picornaviruses, Foot and Mouth Disease Virus (FMDV) and Swine Vesicular Disease Virus (SVDV). However we would not recommend it to reduce levels of non-enveloped viruses such as porcine rotavirus on a farm.

Peroxygen compound and glutaraldehyde based disinfectants

Both Vanadox and Virkon S have peroxygen compounds as their primary active ingredient. Peroxygen compounds are oxidising agents, they denature the protein capsid of non-enveloped viruses, so they should have a high efficacy in this study (Kitis (2004). In low levels of organic matter both these disinfectants reduced MS2 phage titre by >4 $\log_{10}$. Virkon S showed higher efficacy than Vanodox in

low organic matter conditions with a 6 log_{10} reduction in porcine rotavirus titre compared to a 4 log_{10} reduction in MS2 phage titre. In high organic matter conditions, however, both disinfectants had limited efficacy against MS2 phage and porcine rotavirus with all results less than 1.5 log_{10} reduction in titre. GPC8 (a glutaraldehyde based disinfectant) showed similar efficacy to the peroxygen compounds. This disinfectant included quaternary ammonium compounds which denature proteins, as well as the glutaraldehyde which releases alkaline phosphatases affecting protein synthesis (Rutala et al. 2008). Again, these were effective in low levels of organic matter but efficacy was reduced in the presence of high organic matter. In an environment devoid of organic matter these disinfectants are effective, but in environments such as a pig farm efficacy is quickly reduced in the presence of organic matter.

Applications of study

This study used liquid suspension tests to accurately compare disinfectant efficacy in the presence and absence of organic matter. Disinfectants are more efficient in suspension at reducing viral titre than on a farm, where viruses are often surface associated or dried. However this study demonstrates how organic matter and disinfectant type have a significant impact of disinfectant efficacy. Bi-OO-cyst was the only disinfectant effective against porcine rotavirus in high organic matter conditions but peroxygen compound based disinfectants (Vanodox and Virkon S) and the glutaraldehyde-based disinfectant GPC8 all were effective in the presence of low organic matter. MS2 phage served as a model for porcine rotavirus and gave similar, although slightly lower, log_{10} reduction titres than porcine rotavirus.

This study highlights the importance of disinfectant choice to reduce porcine rotavirus contamination on a farm and the need for effective cleaning prior to disinfection to improve the efficacy of the disinfectant by removal of organic matter. In addition to cleaning and disinfecting livestock houses: regular cleaning and disinfection of personal protective equipment, such as footwear would reduce

the risk of viral transmission around the farm. A disinfectant such as Bi-OO-cyst would be effective or Vanodox, Virkon S or GPC8, assuming the organic matter was first removed with a detergent wash.

Materials and Methods

Disinfectant assay

Disinfectants (Table 1) were tested in suspensions with range of organic matter concentrations and against MS2 bacteriophage (MS2 phage) and rotavirus (OSU strain, a gift of Malcolm McRae, University of Warwick). Solutions containing organic matter were made to simulate the farm environment during disinfection (Springthorpe et al. 1986; Bellamy 1995; Thompson et al. 2007). Three assay conditions were tested; no organic matter, low organic matter (3% FBS) and high organic matter (10% FBS, 20% yeast extract). The disinfectant was diluted to the concentration recommended by the manufacturer, in accordance with DEFRA guidelines. The disinfectant solutions were made up as 10x concentration stocks with autoclaved tap water less than 1 hour before disinfection assay. Control solutions were made using the same organic matter suspensions, but without the disinfectant. Disinfectant suspensions were made up as described and incubated with MS2 phage ($1x10^6$ pfu ml^{-1}, from S. Hooton, University of Nottingham) or OSU rotavirus ($1x10^7$ pfu ml^{-1}) for 1 minute at 20°C.

To neutralise the disinfectant, the disinfectant solutions with MS2 phage were diluted 1:10 in a 10% sodium thiosulphate solution in SM buffer (50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 8 mM $MgSO_4$:$7H_20$, 0.01% gelatin, pH 7.5) for Virkon S and Vanodox, 1% tween in SM buffer for GPC8 and all disinfectant assays were diluted 100-fold in SM buffer. In porcine rotavirus assays, the disinfectant solutions were diluted 100-fold in MEM to neutralise the disinfectant action. Control experiments were carried out to ensure that diluted and neutralised disinfectants, and organic matter had no adverse effects on the *E.coli* lawn in the MS2 assays, or on the cell monolayer in the rotavirus assays (results not shown).

The titre of viable viral particles in the disinfectant solution was quantified by bacterial plaque assay for MS2 and cell plaque assay for porcine rotavirus (Arnold et al. 2009) using MA104 cells expressing PiV5-V protein (from R. Randall, University of St. Andrews). The efficacy of the disinfectant was defined as the $\log_{10}$ reduction of titre i.e. the difference between the titre of the viral control (incubated without disinfectant) and the post-disinfection viral titre.

Statistical analysis

Two-factor ANOVA tests were used to analyse the variance between disinfectants and organic matter in the disinfection studies. Two-tailed Mann Whitney test was used to determine whether there was a significant difference between the rotavirus and MS2 titres in assays using the same disinfectants and organic matter conditions. Spearman's rank correlation was used to calculate the correlation between the MS2 and rotavirus results. All statistics were calculated with Genstat (9^{th} Ed).

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Conflict of interest:

The authors have no conflicts of interest

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Table 1. Active ingredients of the disinfectants (supplier indicated) used in this study and the recommended general use dilutions for each disinfectant (* taken from company direction and DEFRA recommendations).

Disinfectant	Disinfectant Type	Active Ingredients	Recommended Dilution *
Vanodox (Evans Vanodine)	Peracetic Acid	Peracetic Acid Hydrogen Peroxide Acetic Acid Nonionic Surfactant	1:25
FAM 30 (Evans Vanodine)	Iodophore	Iodine Sulphuric Acid Phosphoric Acid	1:90
GPC 8 (Evans Vanodine)	Glutaraldehyde	Glutaraldehyde QAC Phosphoric Acid Nonionic Surfactant	1:35
Bi-OO-Cyst (Biolink)	Phenolic	Butyl Glycol Ether Acetic Acid N-alkylbenzene sulphonic acid 4-chloro-3-methylphenol	1:125
BioPhen Plus (Biolink)	Phenolic	Preventol GDA 50 2-benzyl-4-chlorophenol 4-chloro-3-methylphenol Dodecylbenzene sulphonic acid Isopropyl alcohol	1:10
Virkon S (DuPont)	Peroxygen Compounds	Peroxygen Compounds Surfactant Organic Acids Inorganic Buffer	1:100

Figure legends

Figure 1. The efficacy of Vanodox, Fam30, GPC8, Bio-OO-cyst, Biophen plus and Virkon S disinfectants in high (grey:10% FBS, 20% yeast extract), low (White: 3% FBS) and no organic matter, Black (OM) conditions against MS2 bacteriophage. The logarithmic values are based on the reduction in titre from control viral titre which followed the same neutralisation protocol as the disinfectants. The dotted line shows the threshold of 4 $\log_{10}$ reduction and the error bars show standard error for three replicates of each assay. The ❖ highlighted $P \leq 0.001$,

Figure 2. The efficacy of Vanadox, GPC8, Bi-OO-cyst and Virkon against porcine rotavirus in the presence of high (Grey), low (white) and no (black) organic matter (OM). The logarithmic values are based on the reduction in titre compared to control; the error bars show standard error for three replicates of each assay. The dotted line shows the threshold of 4 $\log_{10}$ reduction and the error bars show standard error for three replicates of each assay. The ❖ highlighted $P \leq 0.001$.

Figure 3. Comparison of MS2 and rotavirus titres post disinfection, using data from Fig. 1 and 2. The value is the mean $\log_{10}$ reduction in titre post disinfection with Vanodox, GPC8, Bi-OO-cyst and Virkon S in high, low and no organic matter solutions. There is a linear tend line shown on the graph.

Figure 1

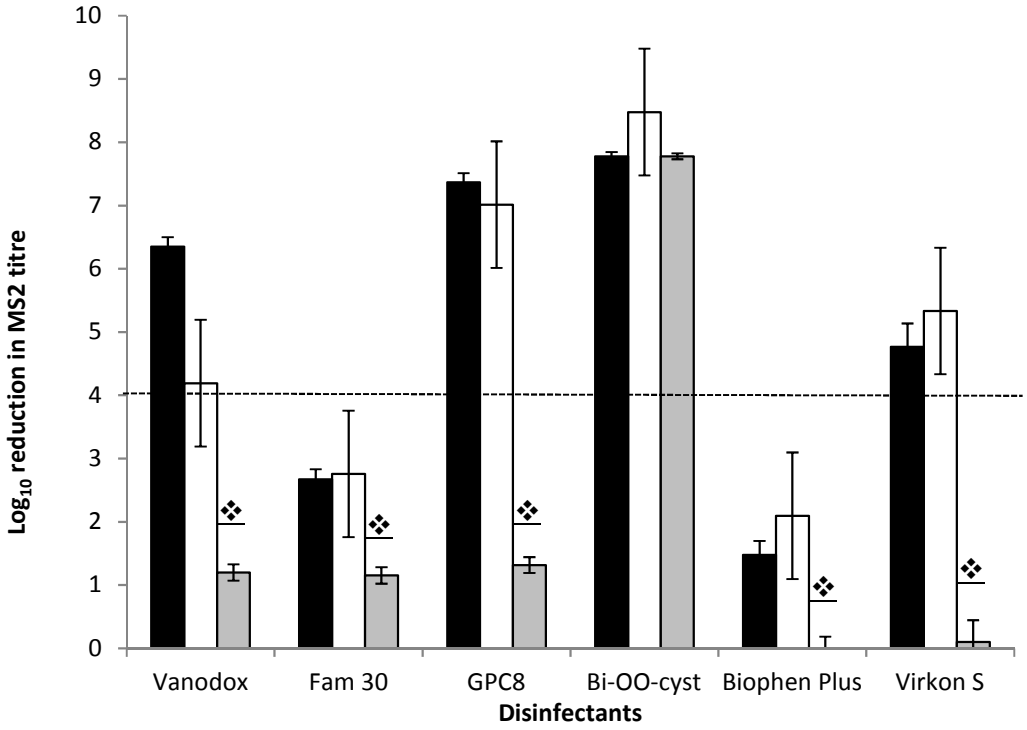
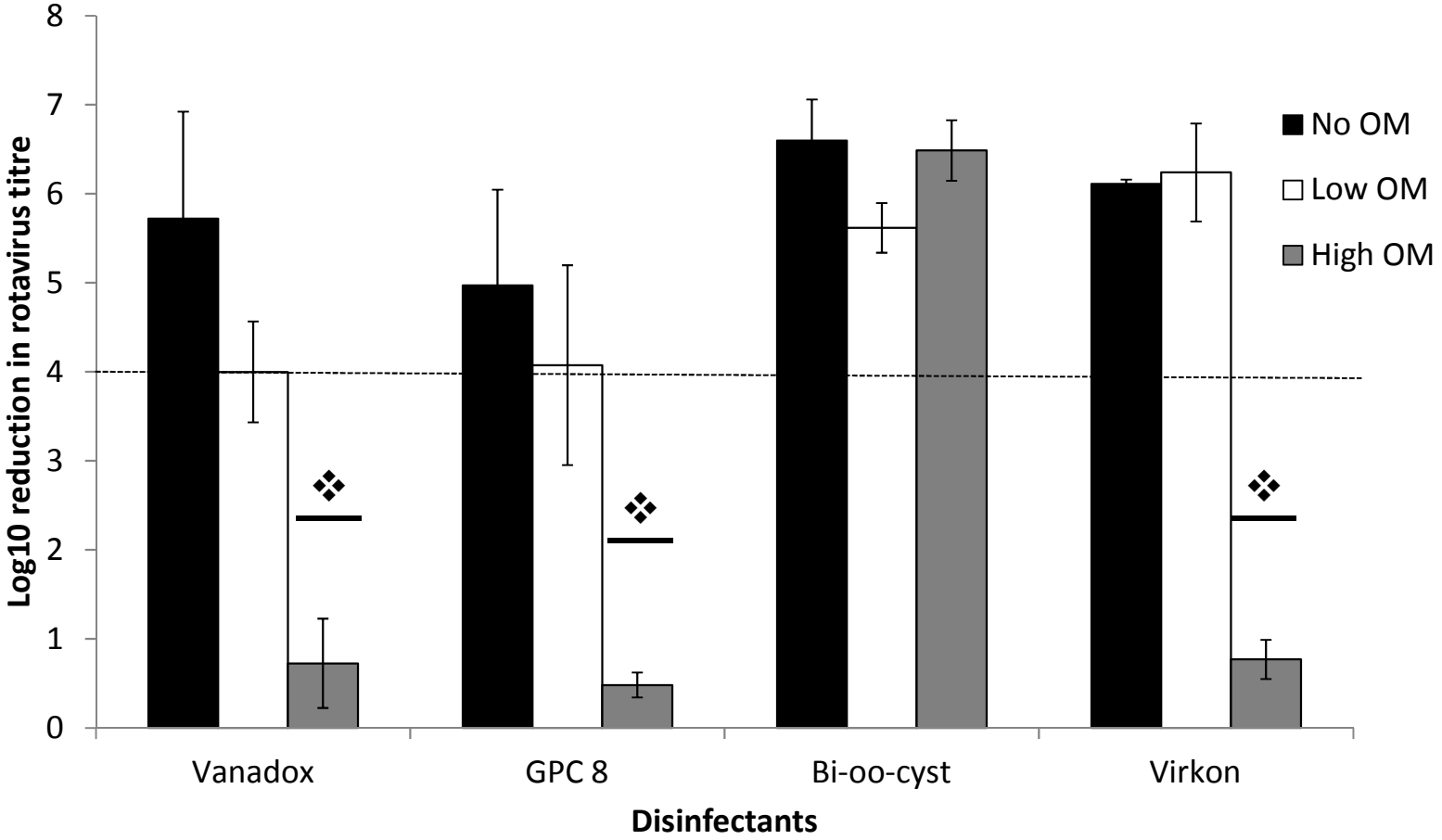

10
9
8
7
6
5
4
3
2
1
0
Log10 reduction in MS2 titre
Vanodox
Fam 30
GPC8
Bi-OO-cyst
Biophen Plus
Virkon S
Disinfectants

Figure 2


8
7
6
5
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3
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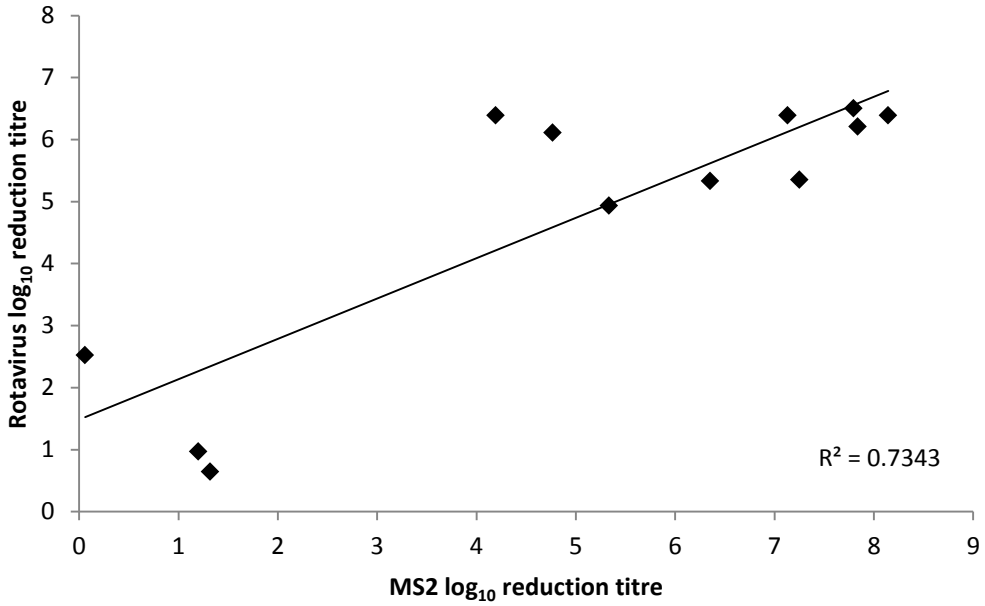
0
Log10 reduction in rotavirus titre
No OM
Low OM
High OM
❖
❖
❖
Vanadox
GPC 8
Bi-oo-cyst
Virkon
Disinfectants

Figure 3


Rotavirus log10 reduction titre
MS2 log10 reduction titre
R² = 0.7343
0
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9