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Short communication

Virucidal effect of chosen disinfectants against African swine fever virus (ASFV) – preliminary studies

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Abstract

Four commercial disinfectants were chosen for being generally accepted as effective against ASFV. Only two of them, based on sodium hypochlorite and potassium peroxymonosulfate, confirmed their effectiveness in selected concentrations. Taken together, our data supports the effectivenes of chemical disinfectants containing sodium hypochlorite (1%, 0.5% in low level soiling) and potassium peroxymonosulfate (1% in high level soiling). Furthermore, these results highlight the importance of pre-cleaning steps to remove soiling before proper disinfection which improves the effectiveness of tested disinfectants.

Key words: African swine fever (ASF), African swine fever virus (ASFV), disinfection, biosecurity, virucidal effects

Introduction

In an era of the lack of effective methods of control African swine fever virus (ASFV) (i.e. vaccine or treatment), prevention of ASF is based on the culling of infected animals and every animal that may have had contact with them (Jia et al. 2017, Quintas et al. 2017, Sánchez-Cordón et al. 2018). This method is unfavorable economically, threatens pig populations and is also unethical (EFSA Panel on Animal Health and Welfare 2015; Vergne et al. 2014). An alternative method of combating ASF is preventing virus-spread through biosecurity. Disinfection and the proper use of disinfectants is a basic, and the most important aspect of biosecurity. It is based on reducing the percentage of pathogenic micro-organisms [Food and Agriculture Organization of the United Nations (FAO) Animal Production and Health Manual 2009]. The ideal disinfectant should be characterized by: fast action, durability, non-toxicity, resistance to influence from the environment and, more importantly, it should have the widest possible spectrum of biocidal activity, including bacteria, viruses and fungi. The incorrect definition of activity parameters (i.e. concentration, contact time and range) may lead to the improper use of disinfectant products, whereby no effectiveness is achieved (Trzcińska et al. 2017, Juszkiewicz et al. 2019). There are several substances or chemical compounds generally accepted as inactivating enveloped viruses, including the ASFV. The only studies on the use of disinfectants

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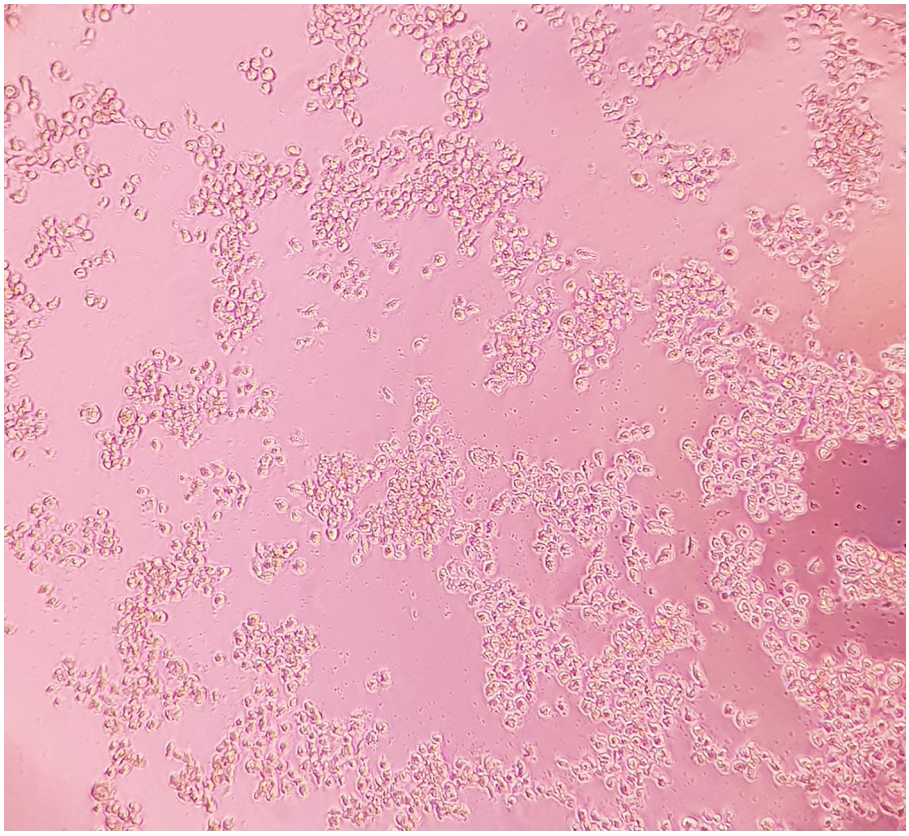

Fig. 1. Cytopathic effect observed in Vero cells infected with ASFV, after 3 days of incubation. x100.

against the ASFV concerned tests on various surfaces, as revealed (Krug et al. 2011, Krug et al. 2012, Krug et al. 2018). To the best of our knowledge, this is the first report on *in vitro* testing of disinfectants against the ASFV. The aim of this study was to find the most effective disinfectant, together with optimizing its concentrations as well as soiling level, to confirm the virucidal effect.

Materials and Methods

Four commercially available disinfectants were obtained. These were selected to be representative of the main groups of chemical compounds containing, as active substances, respectively: sodium hypochlorite, oxidant compounds, glutaraldehyde and quaternary ammonium compounds. Virus BA71V (EURL, Spain) was propagated on VERO cells (ATCC CCL-81) on which the cytopathic effect (CPE) could be seen (Fig. 1).

All of the disinfectants were diluted in hard water, containing Mg^{+}, Ca^{2+} cations and Cl, HCO_3^{-} anions (pH 7), to obtain three dilutions of each disinfectant (Table 1). A stock of the virus was mixed 1:1 with interfering substances: BSA - bovine albumin 3.0g/l (low level soiling) and BSA + YE - bovine albumin 10g/l plus yeast extract 10g/l (high level soiling), then added to the selected dilution of disinfectant, 2:8 respectively. Subsequently, mixtures were kept at 10ºC for 30 min and then titrated on 96-well plates. Afterwards, both the control virus and experimental virus suspensions were titrated in a VERO cell culture. The cytopathic effect was evaluated after 3 days of incubation (37ºC, 5% CO_2). $TCID_{50}$/ml (expressed as a negative logarithm) was estimated, using the Spearman-Karber method. Control of the virus titre was done in parallel with the tested dilutions, but the disinfectant was replaced with hard water. If the difference between $TCID_{50}$/ml of the tested disinfectant and the virus control was ≥ 4 log the disinfectant was considered as virucidal, against ASFV.

Results and Discussion

All results were compared to the control of the titre containing BSA and BSA + YE, which were respectively, 5.75log and 5.5log. After 3 days of incubation, two of the four tested disinfectants, based on glutaraldehyde and quaternary ammonium compounds (QUADs), showed a cytotoxicity, which did not allow for proper interpretation of the effectiveness of the virucidal effect of these disinfectants. Only the lowest concentration

Table 1. The effectiveness of selected commercial disinfectants, after three days of incubation.

Disinfectant	Main active substance(s)	Tested concentration	Difference* (log) $TCID_{50}$/ml		Virucidal effect	
			BSA	BSA YE	BSA	BSA+ YE
1	sodium hypochlorite	1%	4.25	4	YES	YES
		0.5%	4.25	2.25	YES	NO
		0.1%	2.25	0.75	NO	NO
2	potassium peroxymonosulfate	2%	-	-	CTX	CTX
		1%	-	4	CTX	YES
		0.1%	3.25	1	NO	NO
3	glutaraldehyde	2%	-	-	CTX	CTX
		1%	-	-	CTX	CTX
		0.1%	-	-	CTX	CTX
4	quaternary ammonium compounds	5%	-	-	CTX	CTX
		2%	-	-	CTX	CTX
		0.2%	1.25	1	NO	NO

* the difference between the control titre and the result of the disinfectant titre; BSA, 3.0 g/l bovine albumin (low level soiling); BSA+YE, 10 g/l bovine albumin plus 10 g/l yeast extract (high level soiling); CTX, the cytotoxic effect, involving cell destruction

of the second of these disinfectants (QUADs) showed a decrease in the viral titre; however, it was too weak to be considered virucidal. Of interest, glutaraldehyde (0.1%) revealed CPE in subsequent, decimal dilutions, after the cytotoxic effect, in the first wells. This confirms that no disinfectant which showed the cytotoxic effect can be considered as virucidal. The most effective against ASFV turned out to be a commercial disinfectant based on sodium hypochlorite. It was effective in a 1% concentration with BSA and BSA + YE, and a 0.5% concentration with BSA. The second, in terms of efficiency, was a disinfectant based on potassium peroxymonosulfate. This proved effective in a 1% concentration with high level soiling, while in low level soiling it was cytotoxic. A 0.1% concentration was not effective at all.

Two of the chosen commercial disinfectants turned out to be efficient against ASFV. One, based on sodium hypochlorite, the second, based on potassium peroxymonosulfate. The other two commercial disinfectants proved to be either cytotoxic or ineffective against ASFV, at selected concentrations. Research indicates that assessment of the effectiveness of the disinfectant cannot be made in the case of its cytotoxicity; the disinfectant can be cytotoxic, but not virucidal. In conclusion, choosing a tested disinfectant, the proper concentration, the contact time and taking into account of soiling level, should guarantee its effectiveness.

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