Heat-alkalinity-time pasteurization can efficiently inactivate PEDV in porcine plasma

Emergence of porcine epidemic diarrhea virus (PEDV) in North America and Asia resulted in massive losses to the pork industry. The virus is spread by the fecal-oral route and infection can cause acute diarrhea, dehydration and vomiting in pigs and may result in high mortality in neonatal and suckling piglets. The virus is not infectious to humans. Dissemination between farms occurs easily and rapidly through infected live pigs and contaminated transport vehicles, but feedborne transmission has been reported as well. Adequate biosafety measures should thus also be in place in the feed production and distribution chain. Both all-vegetal diets and diets with porcine by-products have been implicated in feedborne transmission of PEDV. Still, being a porcine by-product, spray dried porcine plasma (SDPP) is speculated to be an ingredient of concern. The present study examined the extent of PEDV inactivation by heat-alkalinity-time (HAT) pasteurization of plasma as an additional safety step in the production of SDPP.

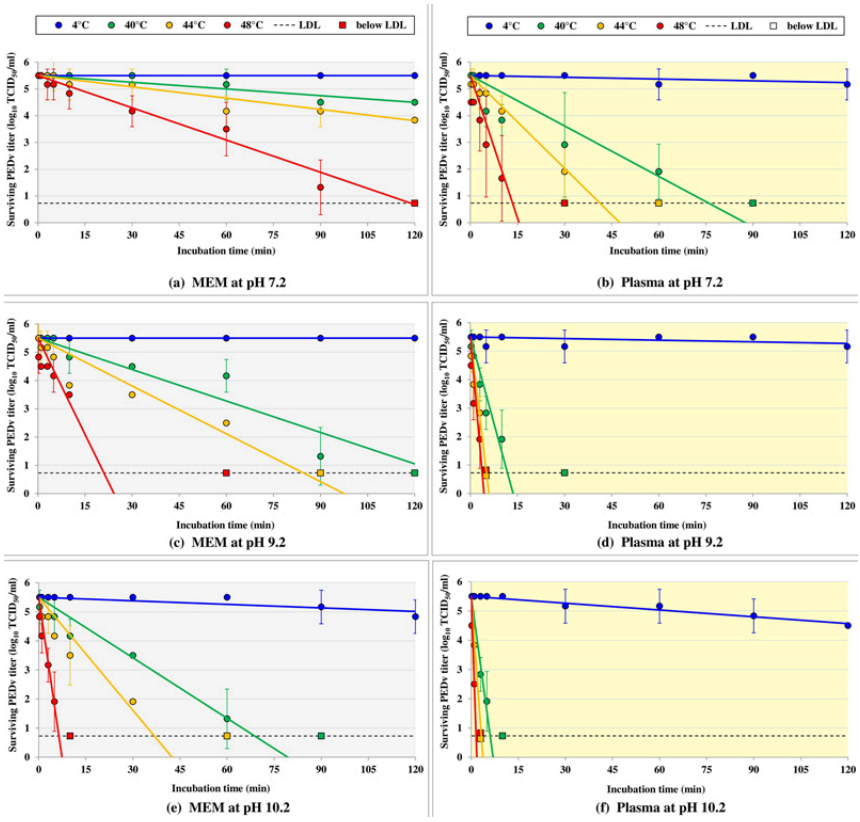

Fig. 1. Survival curves for PEDV during heat and pH treatment in the absence or presence of porcine plasma. MEM, minimum essential medium; data points are means ± SD with n = 3. (After Quist-Rybachuk et al., 2015)

Infectivity titer but not presence of genetic material determines an infectious risk because PCR does not make distinction between infectious and inactivated virus. Therefore, infectivity titer was determined by end point dilution assay. Serially diluted aliquots of test sample where added to replicate cell cultures and presence of viral replication was scored after an incubation period. The quantity of infective virus was expressed as the median tissue culture infective dose or $TCID_{50}$ per volume, in which 1 $TCID_{50}$/ml contains about 0.69 infectious particles per ml. Neutral (pH 7.2) or alkalinized (pH 9.2 or 10.2) cell culture medium (MEM) and plasma samples were mixed with PEDV CV777 strain to a final titer of $10^{5.5}$ $TCID_{50}$/ml. Aliquots (n=3) were incubated for up to 120 min at 4, 40, 44 or 48°C, and the residual virus infectivity was determined. The decimal reduction time (D-value) or time required to inactivate 90% of the initial virus load was calculated for the respective treatment conditions. D-values are presented as mean and [UCL95], where UCL95 represents the 95% upper confidence level.

Irrespective of presence of plasma, PEDV was not sensitive to pH 7.2 to 10.2 at 4°C. At neutral pH and in absence of plasma, PEDV was only moderately sensitive to heat treatment at 48°C ($D_{48°C, pH7.2}$ = 24.9 [45.5 min]). Sensitivity of PEDV to heat treatment at 48°C increased significantly by alkalinisation of the medium to pH 10.2 ($D_{48°C, pH10.2}$ = 81 [114] s). In plasma, $D_{48°C}$ was further reduced to 2.8 [6.7] min at pH 7.2 and 20 [35] s at pH 10.2. Figure 1 presents the survival curves of PEDV when subjected to heat and pH treatment in presence or absence of porcine plasma. The time needed to inactivate 10^8 $TCID_{50}$ PEDV/ml by the tested treatments is depicted in Figure 2. The calculated inactivation of PEDV reached by HAT pasteurization at 48°C and pH 10.2 for 10 min was $10^{17.4}$ and $10^{5.3}$ $TCID_{50}$/ml in plasma and MEM, respectively. Inactivation of PEDV by heating at alkaline conditions was thus greatly facilitated in porcine plasma as inoculated medium.

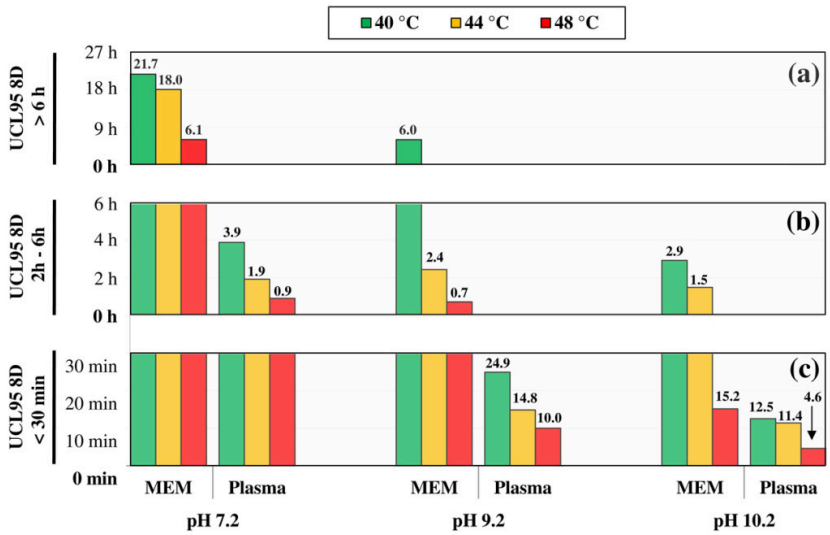

Fig. 2. Upper 95% confidence level of the time needed to inactivate 8 log10 TCID50 PEDV per ml of matrix (UCL95 8D) by heat and pH treatment in the absence or presence of 90% porcine plasma. MEM, minimum essential medium.(After Quist-Rybachuk et al., 2015)

Treatment of liquid plasma at these HAT-conditions would doubtlessly result in PEDV sterility of the highest possibly contaminated abattoir collected porcine plasma. HAT pasteurization prior to spray drying of raw plasma might not be a necessary safety step in the production of SDPP, but it eliminates any remaining doubts on PEDV safety of SDPP. In absence of HAT-pasteurization, spray-drying of plasma is reported to inactivate $10^{4.2}$ $TCID_{50}$ PEDV/ml. Moreover, PEDV is an enveloped virus that is sensitive to desiccation during storage in dry conditions. Likewise our results in liquid plasma, also inactivation by desiccation has been demonstrated to occur faster in SDPP compared to e.g. soybean meal.

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Publication

Sensitivity of porcine epidemic diarrhea virus (PEDV) to pH and heat treatment in the presence or absence of porcine plasma.
Quist-Rybachuk GV, Nauwynck HJ, Kalmar ID
Vet Microbiol. 2015 Dec 31