



Short communication

Sensitivity of porcine epidemic diarrhea virus (PEDV) to pH and heat treatment in the presence or absence of porcine plasma

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ABSTRACT

Emergence of porcine epidemic diarrhea virus (PEDV) resulted in massive neonatal mortality in the North-American and Asian pork industry. Measures to prevent its geographical spread are of utmost importance to safeguard susceptible porcine populations. The major infection route is direct or indirect faecal-oral contact. Adequate biosafety measures should be in place at all levels of the swine production chain, including feed and feed ingredients. Present study aimed to investigate the sensitivity of PEDV to thermal inactivation at neutral and alkaline pH in presence or absence of porcine plasma. Cell culture medium and porcine plasma at different pH (7.2, 9.2, 10.2) and temperature conditions (4 °C, 40 °C, 44 °C, 48 °C) were inoculated to a final titer of 5.5 log₁₀ TCID₅₀ PEDV/ml, incubated for up to 120 min and the residual infectivity was determined by endpoint dilution assay. Irrespective of presence of plasma, PEDV was not sensitive to pH 7.2–10.2 at 4 °C. At moderate temperatures (≥40 °C), both alkaline pH and presence of plasma potentiated thermal inactivation. Inactivation of 8 log₁₀ TCID₅₀/ml plasma within 30 min (8D value < 30 min) by moderate pH and temperature would denote potential industrial processing conditions that ensure safety towards PEDV while limiting denaturation of bioactive components. Virus-spiked plasma required heat treatment of 40 °C and alkalization to pH 9.2 to achieve 8 log₁₀ reduction within such time. At pH 10.2 and 48 °C, the 8D value was 4.6 min in plasma and 15.2 min in MEM. Here we propose heat-alkalinity-time (HAT) pasteurization as a highly efficient method to inactivate PEDV during industrial processing of porcine plasma.

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1. Introduction

Porcine Epidemic Diarrhea virus (PEDV) is an enveloped enteric RNA-virus belonging to the *Alphacoronavirus* genus. Infection in pigs is associated with acute diarrhea, dehydration and vomiting. In May 2013, introduction of allegedly more virulent Asian variant strains in USA's naïve pig herds resulted in high morbidity, caused up to 100% mortality in neonatal piglets, and was rapidly spread throughout the country and to other parts of the Americas (Stevenson et al., 2013). The virus is known to be transmitted via the direct or indirect faecal-oral route and is easily and rapidly spread among pig farms. Equipment, clothing, boots and, in particular, vehicles used to transport live pigs are the likely fomites. Although PEDV is sensitive to desiccation, transmission via contaminated feed has been reported as well (Dee et al., 2014, 2015). Moreover, feed was pointed out as the common risk factor through which PEDV may have been introduced into the Canadian

province of Ontario in 2014. Therefore, the implementation of biosafety measures should be in place at all levels of the pig production chain, including the feed and its ingredients. Of notice, both diets with and without animal by-products have been implicated in feed-borne transmission of PEDV (Dee et al., 2014; Pasick et al., 2014).

The time required for complete inactivation of PEDV by desiccation in dry substrates is, besides temperature and water activity, strongly dependent on the inoculated matrix. The same inoculum of PEDV retained residual infectivity in soybean meal for up to 180 days of outdoor storage, whereas in spray-dried porcine plasma (SDPP), all infectivity was lost within 7 days (Dee et al., 2015). Still, being a porcine by-product, SDPP is thought to be an ingredient of concern with respect to feed-borne transmission of PEDV. Indeed, especially in epidemic regions, raw blood may be contaminated at the abattoir and processing conditions should guarantee complete inactivation of an eventual PEDV-load in raw material. The present study aimed to examine the impact of temperature treatments of up to 48 °C and alkaline pH treatments of up to pH 10.2 on the infectivity of PEDV in the presence or absence of porcine plasma.

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2. Materials and methods

2.1. Plasma

The plasma used was a 1% Na-citrate plasma from porcine blood commercially collected at European slaughterhouses. Protein and dry matter content were 8.5% and 11.5%, respectively, and was provided by Veos N.V. (Belgium, Zwevezele). All plasma samples were negative for PEDV RNA and anti-PEDV antibodies (Diagnostic Service, www.vetvirology.ugent.be). Within 8 h of production, refrigerated plasma was sterile filtered through a 450 nm cellulose acetate- and 220 nm PES-membrane bottle top filter (VWR, Radnor, PA, USA) to minimize bacterial contamination of the cell culture, brought to the desired pH and stored at -20°C until use. Immediately prior to the assay, the heat-labile components, such as complement, were heat inactivated at 56°C for 30 min to avoid gelation during incubation in the presence of calcium-containing media.

2.2. Impact of pH, temperature and presence of porcine plasma on infectivity of PEDV

CV777 strain of PEDV (NCBI GenBank accession no. AF353511), originally isolated in this laboratory in 1977 and adapted to cell culture (Pensaert and De Bouck, 1978), was used for all experiments. In three independent replicate trials ($n=3$), $10\ \mu\text{L}$ of virus stock was mixed with $90\ \mu\text{L}$ of cell culture medium (MEM, Gibco Life Technologies, Paisley, UK) or plasma, each in advance equilibrated to 4, 40, 44 or 48°C after being accurately adjusted using 2.5 M H_2SO_4 or 2.5 M NaOH to reach a final pH of 7.2, 9.2 or 10.2 upon addition of virus stock. The resulting $100\ \mu\text{L}$ suspensions of virus in culture medium or in plasma, each at various temperature and pH combinations were made in multiple replicates and maintained for eight preset incubation periods ranging in time between 0.25 and 120 min (0.25, 1, 3, 5, 10, 30, 60 and 120 min). The residual virus infectivity following these heat-alkalinity-time (HAT) treatments was determined via a standard endpoint dilution assay on fully confluent Vero-Ba cells

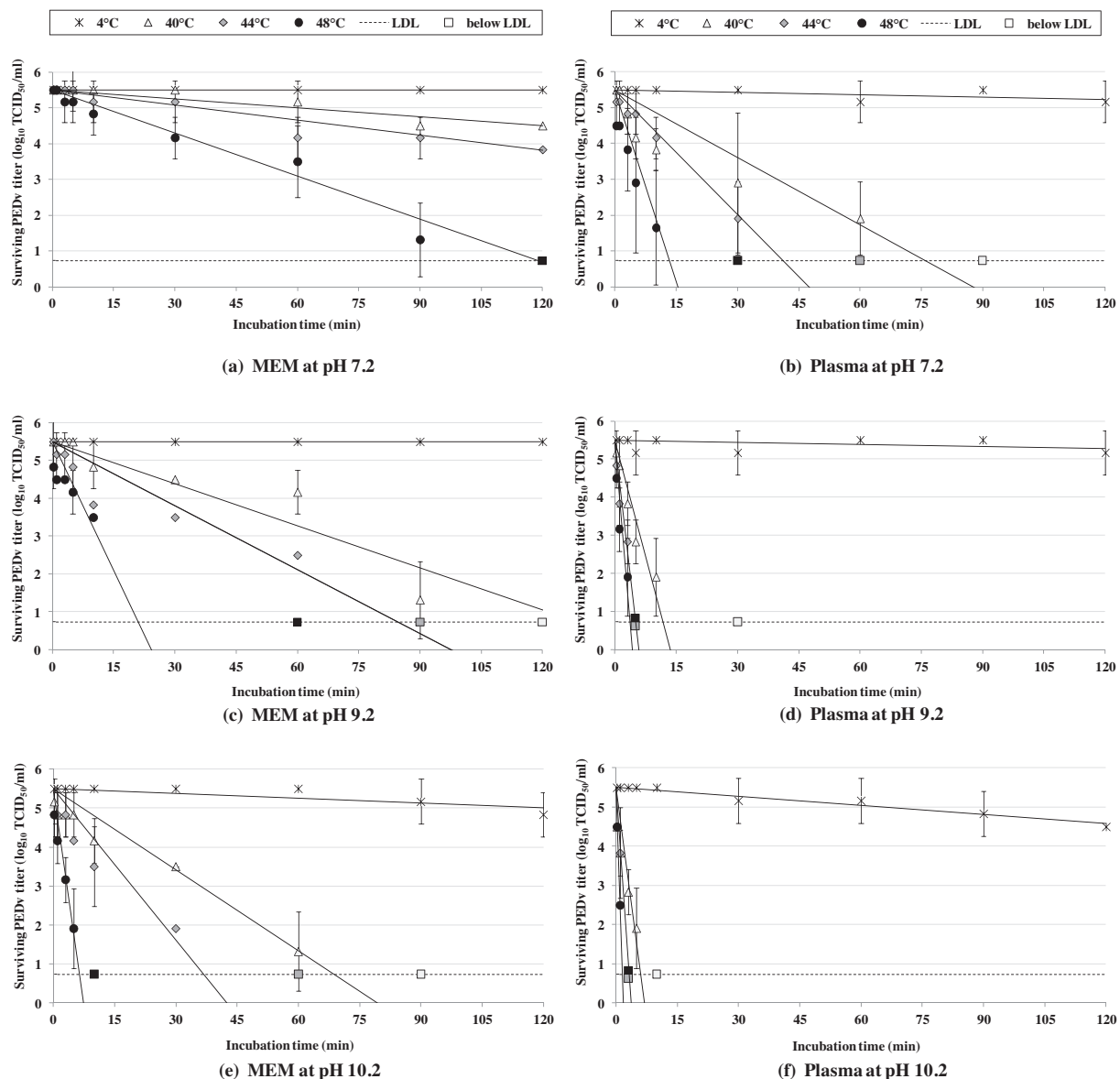


Fig. 1. Survival curves for PEDV during heat (4, 40, 44 or 48°C) and pH (7.2^{a,b}, 9.2^{c,d} or 10.2^{e,f}) treatment in the absence^{a,c,e} or presence^{b,d,f} of porcine plasma. Test samples were a 0.1 ml of a 9:1 (v:v) mixture of matrix and virus stock ($6.5 \log_{10} \text{TCID}_{50} \text{ PEDV}/\text{ml}$). MEM, minimum essential medium; plasma, porcine plasma with 8.5% crude protein and 11.5% dry matter content; LDL, lower detection limit. Data points are means $\pm$ SD with $n=3$.

as described below. Treatments were terminated by briefly cooling each sample in ice water and neutralizing the pH to 7.2 by addition of 10 μ L of premade diluted H₂SO₄ buffers ranging from 0 to 0.22 M H₂SO₄. Immediately thereafter, treatment samples were 10-fold serially diluted and used to inoculate a 96-well plate of fully confluent monolayer of Vero-Ba cells. Briefly, virus was allowed to adsorb for 1 h at 37 °C. Following attachment, the inoculum was removed, the cells were washed twice with phosphate buffered saline (PBS), overlaid with PEDV propagation medium, and further incubated at 37 °C in a humidified air 5% CO₂ atmosphere. Virus propagation medium was prewarmed (37 °C) MEM supplemented with 6% TPB of 29.5 g/L Bacto Tryptose Phosphate Broth in distilled water (BD Biosciences, Franklin Lakes, NJ, USA), 1% P/S and 40 μ L/100 ml of 0.5% trypsin-EDTA solution (Gibco Life Technologies). At 18–24 h post infection, appearance of CPE was examined under a phase contrast microscope and TCID₅₀ calculated.

A General Linear Model was used to construct first order kinetics survival curves (incubation time plotted against the log₁₀ titer). A fixed intersection point of 5.5 log₁₀ TCID₅₀/ml was used at incubation time 0 min and data points below the lower detection limit (LDL, 0.73 log₁₀ TCID₅₀/ml) were considered to have a residual infectivity of the LDL. Curve estimation was based upon all data points up to the shortest incubation time at which the residual infectivity was below the LDL in all three replicate experiments. The decimal reduction time (*D* value) or time required to inactivate 90% of the initial virus population (1 log TCID₅₀ reduction) at the respective conditions of pH, temperature and matrix, was calculated as the negative inverse of the slope of the semi-logarithmic survival curve. The *z*_{2T} and *z*_{2P} values were calculated as the negative inverse of the slopes of the 50% thermal death time curves (temperature plotted against log₂ *D*) and pH death time curves (pH plotted against log₂ *D*) and represent the change in temperature or pH, respectively, required to reduce the *D* value by 50%.

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the significance level (*p* value) of treatment effects. If significant (*p* < 0.050), the Tukeys' post hoc analysis was used to determine the significance of differences between treatments. Data are presented as the means $\pm$ standard deviations (SD) ($\mu \pm \sigma$) of three independent experiments. Where appropriate, the 95% confidence levels (UCL95) were calculated as: $\mu \pm t_{\alpha/2, n-1} \times \sigma / \sqrt{n}$, with *n* = 3 and *t* _{$\alpha/2, n-1$} = 4.303

3. Results

3.1. Sensitivity of PEDV to wet heat and the effects of alkalinity and presence of plasma

At pH 7.2, a 1:9 (v:v) dilution of 6.5 log₁₀ TCID₅₀ PEDV/ml virus stock in MEM resulted in the expected final titer of 5.5 log₁₀ TCID₅₀/ml as assessed by the endpoint dilution assay (Fig. 1). The titer was

not affected when maintained at 4 °C for at least 2 h. At neutral pH and cooled temperature (4 °C), presence of porcine plasma (90% plasma: 10% virus stock) did not affect PEDV titer. Similarly, at 4 °C and irrespective of the presence of plasma, PEDV was not sensitive to pH treatment up to pH 10.2.

In the absence of plasma, inactivation of 5.5 log₁₀ TCID₅₀ PEDV/ml test sample within a two hour treatment period required at least 48 °C at neutral pH or 40 °C at pH 9.2. Mean *D* values for these treatments were below 30 min (Table 1). With *D* values longer than 1 h, PEDV showed low sensitivity to inactivation by temperatures up to 44 °C in MEM at neutral pH. An increase in alkalinity from pH 7.2–9.2 significantly reduced the *D* values within the temperature range of 40–48 °C. A further increase in alkalinity did not significantly affect respective *D* values (*p* > 0.050). Provided a minimum temperature of 40 °C, presence of 90% plasma significantly reduced the *D* values for all tested combinations of pH and heat treatment. Specifically, in all three independent assays performed in plasma, heating to at least 40 °C resulted in complete virus inactivation within 90 min, 30 min and 10 min at pH 7.2, 9.2 and 10.2, respectively. At 40 °C and 44 °C, an increase in alkalinity of plasma from pH 7.2–9.2 significantly lowered mean *D* values, a further increase having no significant additional effect. Alkalinisation of plasma to pH 10.2 was required to significantly reduce the *D*_{48 °C} value. Similar to inactivation in MEM, an increase in temperature from 40–48 °C also significantly reduced the *D* values for inactivation in plasma. Minimal mean *D* values were 1.3 $\pm$ 0.2 min in MEM and 0.3 $\pm$ <0.1 min in plasma treated at 48 °C and pH 10.2 (Fig. 1, Table 1).

3.2. Calculated UCL95 8 *D* value

The upper 95% confidence level of the time required to inactivate 8 log₁₀ TCID₅₀ PEDV/ml matrix (UCL95 8*D* value) by heat and pH treatment in MEM and in plasma is presented in Fig. 2. The sequentially expanded Y-axis depicted in the upper (a), middle (b) and lower (c) panels show 8-log inactivation times of more than 6 h, less than 6 h and less than 30 min, respectively. At neutral pH conditions, the UCL95 8*D* value for heat treatment of up to 48 °C was over 30 min for both MEM and plasma. The maximum UCL95 8*D* value occurred at a 40 °C treatment of virus in neutral MEM and was 21.7 h. In MEM, the UCL95 8*D* value was below 30 min only at temperature and pH conditions of 48 °C and pH 10.2. Provided the presence of plasma, a combined temperature and pH treatment of at least 40 °C and pH 9.2 was sufficient to achieve an UCL95 8*D* value of less than 30 min. Although the *D*_{48 °C} value for plasma at neutral pH was merely 2.8 $\pm$ 1.6 min, the corresponding UCL95 8*D* value was over 30 min. At pH 10.2, the UCL95 8*D*_{48 °C} values were 4.6 min in plasma and 15.2 min in MEM (Fig. 2).

3.3. Interactions between matrix, pH and temperature on *z*_{2T} and *z*_{2P}

The 50% thermal death time curves and 50% pH death time curves are plotted in Fig. 3. Within the tested pH and temperature

Table 1
Decimal reduction time (*D* value, min) of PEDV at specific temperature, pH and matrix conditions (mean $\pm$ SD with *n* = 3).

	MEM ^a			Plasma ^a			p _{pH} ^{a,b,c}	
	pH 7.2	pH 9.2	pH 10.2	pH 7.2	pH 9.2	pH 10.2	MEM	Plasma
D _{40 °C}	119.9^{ax} $\pm$ 17.2	27.0^{bx} $\pm$ 7.3	14.4^{bx} $\pm$ 3.0	15.9^{ax} $\pm$ 5.3	2.5^{bx} $\pm$ 0.3	1.3^{bx} $\pm$ 0.1	0.001	0.002
D _{44 °C}	71.2^{ay} $\pm$ 25.8	17.7^{bx} $\pm$ 0.2	7.7^{by} $\pm$ 1.4	8.6^{axy} $\pm$ 2.3	1.1^{by} $\pm$ 0.3	0.7^{by} $\pm$ 0.3	0.004	0.001
D _{48 °C}	24.9^{ay} $\pm$ 8.3	4.4^{by} $\pm$ 0.4	1.3^{bz} $\pm$ 0.2	2.8^{ay} $\pm$ 1.6	0.8^{ay} $\pm$ 0.2	0.3^{by} $\pm$ <0.1	0.002	0.034
<i>p</i> _{temp} ^{x,y,z}	0.002	0.008	0.001	0.011	0.001	0.003	0.001	0.001

MEM, minimum essential medium; plasma, porcine plasma with 8.5% crude protein and 11.5% dry matter content.

Means are highlighted in bold.

^a all main effects and interactions of the full model (*D* value $\sim$ pH $\times$ temperature $\times$ matrix) are significant at *p* < 0.0001.

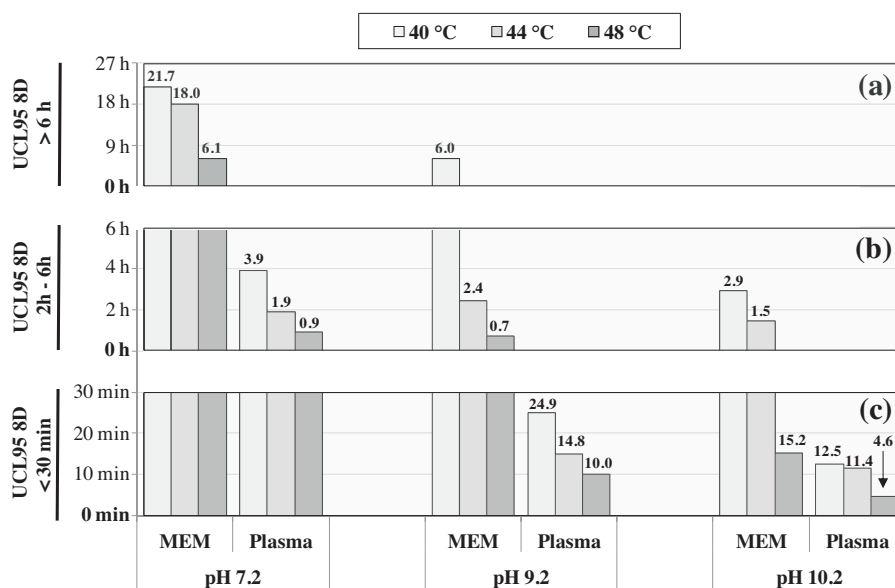


Fig. 2. Upper 95% confidence level of the time needed to inactivate 8 log₁₀ TCID₅₀ PEDV per ml of matrix (UCL95 8D) by heat and pH treatment in the absence or presence of 90% porcine plasma. MEM, minimum essential medium.

ranges, the z_{2T} and z_{2P} values were not significantly affected by the matrix's pH and temperature, respectively. Independent to the presence of plasma, an increase in matrix' alkalinity by 0.9 pH units resulted in a reduction of $D_{40-48^\circ\text{C}}$ values by 50% ($z_{2P,40-48^\circ\text{C}}$

$48^\circ\text{C} = 0.9 \pm 0.2$). The increase in temperature required to reduce the $D_{\text{pH}7.2-10.2}$ values by 50% was significantly lower in MEM ($z_{2T,\text{pH}7.2-10.2} = 3.0 \pm 0.6^\circ\text{C}$) than in plasma ($z_{2T,\text{pH}7.2-10.2} = 4.1 \pm 1.1^\circ\text{C}$) (Table 2). Specifically, at pH 10.2, z_{2T} was $2.4 \pm 0.3^\circ\text{C}$ in the

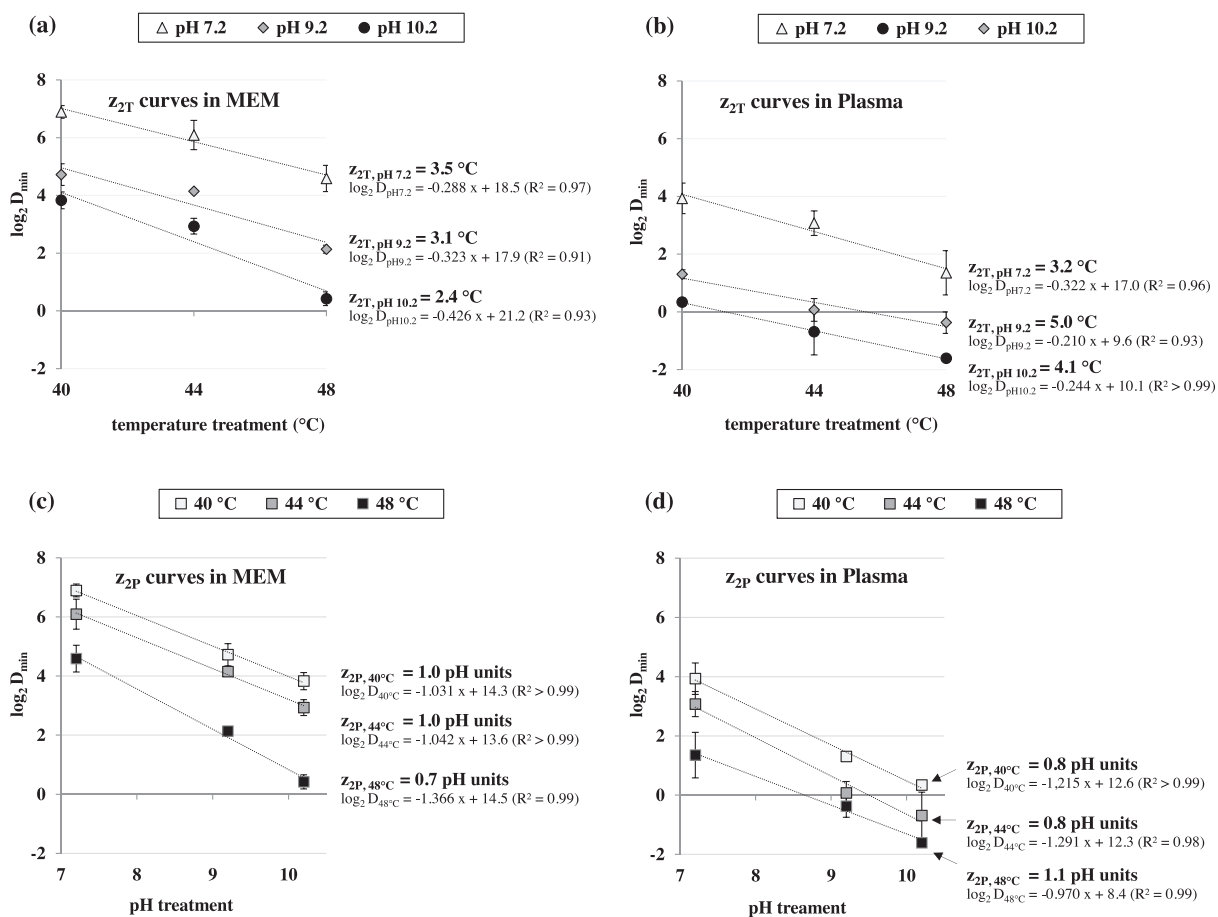


Fig. 3. Fifty percent thermal death time curves^{a,b} (z_{2T} curves) and pH death time curves^{c,d} (z_{2P} curves) for inactivation of PEDV in MEM (minimum essential medium) and in porcine plasma. Data points are means $\pm$ SD with $n = 3$.

Table 2

The z_{2T} (°C) and z_{2P} (pH units) values for inactivation of PEDV within pH range of 7.2–10.2 and temperature range of 40 °C–48 °C, as well as the effect of the matrix in which inactivation occurs (mean $\pm$ SD and 95% confidence interval with $n = 3$).

Model	MEM		Plasma		P_{matrix}	P_{pH}	P_{temp}	$P_{\text{interaction}}$
$z_{2T, \text{pH} 7.2-10.2} \sim \text{pH} \times \text{Matrix}$	3.0 $\pm$ 0.6	[2.0–4.0]	4.1 $\pm$ 1.1	[2.5–5.9]	0.006	0.133	NA	0.033
$z_{2P, 40-48^\circ\text{C}} \sim \text{Temp} \times \text{Matrix}$	0.9 $\pm$ 0.2	[0.7–1.1]	0.9 $\pm$ 0.2	[0.6–1.2]	0.899	NA	0.988	0.036

MEM, minimum essential medium; plasma, porcine plasma with 8.5% crude protein and 11.5% dry matter content.

Means are highlighted in bold.

absence of plasma and 4.1 ± 0.3 °C in its presence ($p = 0.003$; Table 3).

4. Discussion

Adequate processing of raw products that are potentially microbiologically contaminated is of paramount importance to ensure their safe use downstream. Industrially applicable processing conditions are primarily limited by their technical feasibility and their effect on desired functional properties of the end product. Use of high-temperature treatments for processing of plasma, for instance, results in heat-induced denaturation of proteins which causes gel formation. Even if gelling of plasma proteins upon high-temperature treatment would not impair further processing, the denaturation of bioactive proteins would obliterate beneficial effects of plasma-based feed ingredients on gut health. Plasma protein degradation is initiated between 43 and 45 °C (Vazquez and Larson, 2013). Vermeer and Norde (2000) report thermostability of IgG isotype 2, the main Ig in serum (Turcu et al., 2010), to at least 41 °C and a denaturation temperature of the Fab region at 61 °C at heating rate 0.5 °C/min. Endothermic processes indicative of unfolding of the native conformation occurred at slow rate at 55 °C. At 70 °C, IgG₂ was completely denatured within 20 min equilibration time (Vermeer and Norde, 2000). Here, we examined the effect of alkalinisation on heat inactivation of PEDV in an attempt to determine technically applicable processing conditions for porcine plasma that could ensure PEDV sterility whilst limiting denaturation of bioactive plasma proteins.

Whether incubated in the presence or absence of porcine plasma, PEDV was not sensitive to alkalinisation up to pH 10.2 provided no additional heat treatment. Hofmann and Wyler (1989) reported PEDV infectivity being stable over a pH range from 5 to 9. Upon a 6 h treatment period at 4 °C and pH 10, these authors observed a reduction in infectivity of about 3 log₁₀ TCID₅₀ PEDV/ml undiluted virus stock. As these authors adjusted pH by adding 2 M NaOH and 2 M HCL in the presence of the virus, which in our

observation may result in transient pH spikes, moderate sensitivity of PEDV to pH 10 may have been an overestimate. In the present assays, virus inactivation caused by pH-spikes was precluded by alkalinisation of test media prior to addition of the virus and neutralisation of test samples with maximal 0.22 M H₂SO₄. Similar to current results for treatment at 40 °C and pH 7.2 ($D = 2.0$ h, UCL95 = 3.2 h), Hofmann and Wyler (1989) reported PEDV infectivity being relatively stable at 37 °C between pH 6 and 8. Treatment for 6 h at 37 °C and pH ≤ 4 or pH ≥ 9 completely inactivated the 7.86 log₁₀ TCID₅₀/ml virus stock (Hofmann and Wyler, 1989). These results are in line with the $D_{40^\circ\text{C}, \text{pH} 9.2}$ value (27 min, UCL95 = 58.4 min) calculated here, which would have inactivated the inoculum in 4 to 7.6 h. At neutral pH, Hofmann and Wyler (1989) described a $D_{50^\circ\text{C}}$ value for PEDV of 38.2 min in undiluted virus stock. Again, this is in line with current $D_{48^\circ\text{C}, \text{pH} 7.2}$ value of 24.9 ± 8.3 min (UCL95 45.5 min).

Present data demonstrate that presence of porcine plasma potentiates heat and pH inactivation of PEDV. This effect is temperature dependent and is not lost upon prior heat inactivation of plasma for 30 min at 56 °C in neutral to alkaline (pH 10.2) environment. The underlying basis of potentiation of chemical (alkalinization) and physical (temperature) inactivation of PEDV by porcine plasma and characterization of non-specific antiviral plasma components is currently under investigation.

Incorporation of pH range as an essential condition at which heat treatment occurs broadens the concept of pasteurisation, which was initially conceived to reduce beer spoilage by bacteria and is now widely adopted as an adequate processing step to obtain desired food safety standards. HAT pasteurisation is unequivocally defined and can be accurately controlled, measured and monitored. Its efficiency to eliminate microbiological risks can easily be evaluated and extrapolation of *in vitro* results to industrial conditions is straightforward. Therefore, HAT pasteurisation can be considered an ideal good manufacturing practice (GMP) to ensure product safety towards infectious risks.

In summary, PEDV is a viral pathogen with high impact and extremely low minimal infectious dose. Maximal potential contamination of raw plasma is unknown and may depend on collection method, e.g. open versus closed blood draining systems. The calculated $H_{48^\circ\text{C}} A_{\text{pH} 10.2} T_{10\text{min}}$ inactivation of PEDV in plasma and MEM are 17.4 and 5.3 log₁₀ TCID₅₀/ml, respectively. Such levels of inactivation of infectivity will doubtlessly result in PEDV sterility of the highest possibly contaminated porcine plasma. Nevertheless, processing of fresh plasma for use as a feed ingredient usually involves spray-drying at 80 °C and is stored as a powder with low water activity for at least 2 weeks prior to distribution. Spray drying of raw plasma at 80 °C outlet temperature has been reported to inactivate at least 4.2 log₁₀ TCID₅₀ PEDV/ml plasma (Gerber et al., 2014). Similar results were obtained by spray drying at 70 °C outlet temperature (Pujols and Segalés, 2014). As mentioned before, desiccation upon post-processing contamination occurs considerably faster in SDPP compared to e.g. soybean meal (Dee et al., 2015). Of notice, infectivity titre, but not presence of genetic material, determines the infectious risk of pre and post processed materials. PCR results do not distinguish between

Table 3

The increase in heat (z_{2T} , °C) and pH (z_{2P} , pH units) required to reduce the D value (time, min) by 50%. (mean $\pm$ SD and 95% confidence interval with $n = 3$).

	MEM		Plasma		$P_{\text{matrix}}^{a,b}$
$z_{2T, \text{pH} 7.2}$ (°C)	3.5 $\pm$ 0.6	[2.1–5.0]	3.2 $\pm$ 0.7	[1.4–5.1]	0.614
$z_{2T, \text{pH} 9.2}$ (°C)	3.1 $\pm$ 0.4	[2.1–4.2]	5.0 $\pm$ 1.3	[1.8–8.2]	0.075
$z_{2T, \text{pH} 10.2}$ (°C)	2.4 $\pm$ 0.3	[1.6–3.2]	4.1 $\pm$ 0.3	[3.3–4.9]	0.003
$p_{\text{pH}} (z_{2T})$	0.054		0.120		0.033
$z_{2P, 40^\circ\text{C}}$ (pH units)	1.0 $\pm$ 0.1	[0.8–1.2]	0.8 $\pm$ 0.1	[0.5–1.2]	0.230
$z_{2P, 44^\circ\text{C}}$ (pH units)	1.0 $\pm$ 0.2	[0.5–1.4]	0.8 $\pm$ 0.2	[0.3–1.3]	0.315
$z_{2P, 48^\circ\text{C}}$ (pH units)	0.7 $\pm$ 0.1	[0.9–1.1]	1.1 $\pm$ 0.3	[0.4–1.7]	0.090
$P_{\text{temp}} (z_{2P})$	0.074		0.283		0.036

MEM, minimum essential medium; plasma, porcine plasma with 8.5% crude protein and 11.5% dry matter content.

Means are highlighted in bold.

infectious virus, virus fragments and inactivated virus. Presence of PEDV RNA in pig feed with 5% SDPP containing PEDV RNA ($5.1 \pm 0.1 \log_{10}$ RNA copies/g SDPP, $3.3 \pm 0.3 \log_{10}$ RNA copies/g feed) indeed failed to infect susceptible 3-week old piglets when fed *ad libitum* for four weeks (Opriessnig et al., 2014). The particular SDPP was spray-dried at $79 \pm 1.9^\circ\text{C}$ and stored at 18°C for one month prior to the start of the trial. In addition, presence of PEDV in pooled abattoir blood is expected to coincide with presence of anti-PEDV Ig, as shown by Opriessnig et al. (2014). Serum anti-PEDV Ig in turn, has been demonstrated to neutralize infectivity of PEDV (Hofmann and Wyler, 1989), which may passively further increase PEDV safety of blood products. All together, additional HAT pasteurisation might not be necessary to achieve PEDV sterility in SDPP. Most importantly, applied processing conditions should have demonstrated ability to completely inactivate infectivity in potentially contaminated raw ingredients. Adequate processing of raw ingredients in itself, however, is not sufficient to exclude feed-borne transmission of PEDV. Post-processing contamination of ingredients and complete feed should be prevented as well.

Conflict of interest

The authors declare they have no conflict of interest.

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