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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 $\log_{10}$ $TCID_{50}$ 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 $\log_{10}$ $TCID_{50}$/ml plasma within 30 min (8*D* 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 alkalinization to pH 9.2 to achieve 8 $\log_{10}$ reduction within such time. At pH 10.2 and 48 °C, the 8*D* 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.

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 cellolose 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 μl of virus stock was mixed with 90 μl 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 μL 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

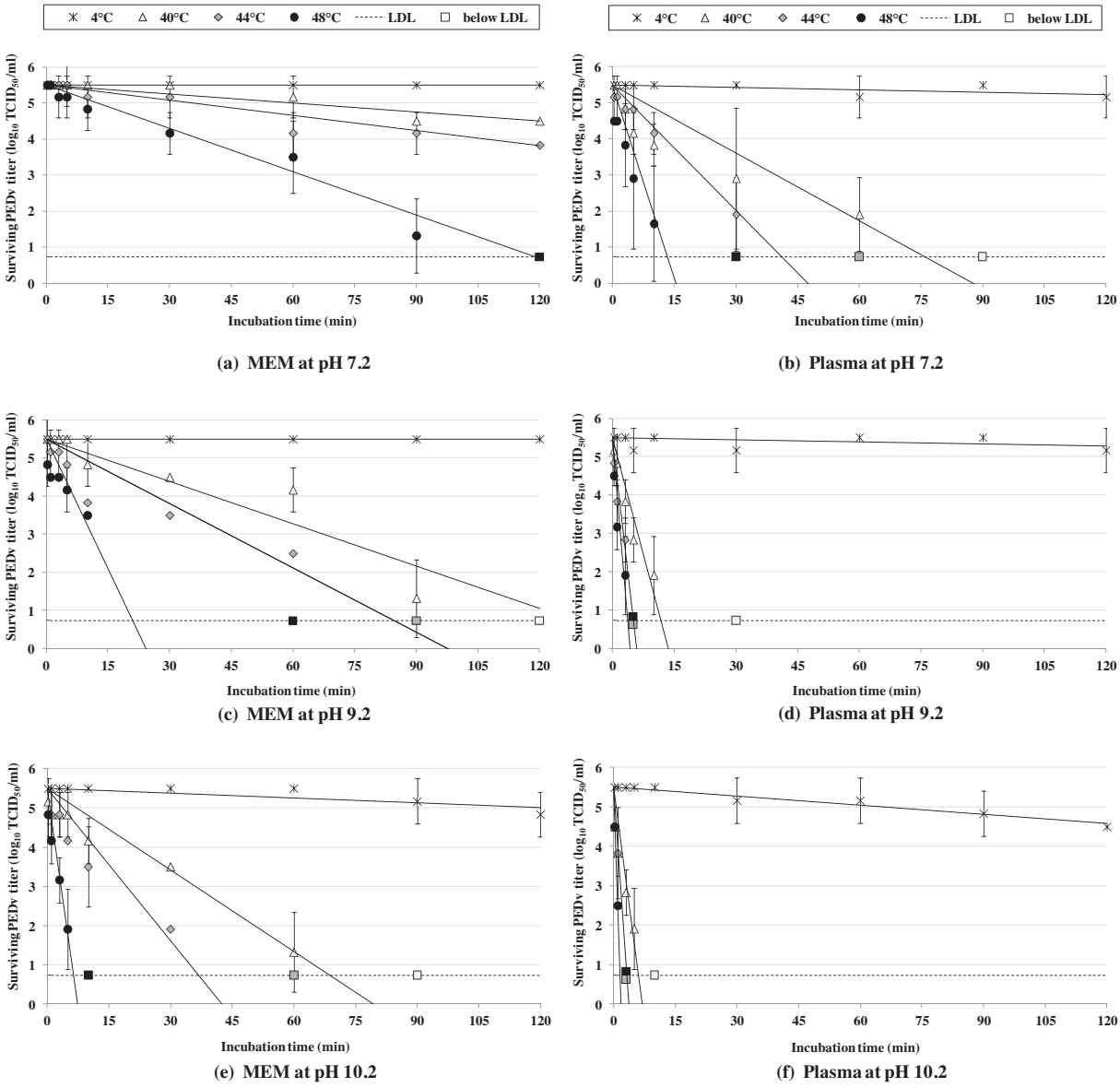

Fig. 1. Survival curves for PEDV during heat (4, 40, 44 or 48 °C) and pH (7.2[a,b], 9.2[c,d] or 10.2[e,f]) treatment in the absence[a,c,e] or presence[b,d,f] of porcine plasma. Test samples were a 0.1 ml of a 9:1 (v:v) mixture of matrix and virus stock (6.5 $\log_{10}$ $TCID_{50}$ PEDV/ml). MEM, minimum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