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NOTE

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Potential of electrolyzed water for disinfection of foot-and-mouth disease virus

Vuong N. BUI[1,2)], Khong V. NGUYEN[2)], Nga T. PHAM[2)], Anh N. BUI[2)], Tung D. DAO[1,2)], Thanh T. NGUYEN[2)], Hoa T. NGUYEN[2)], Dai Q. TRINH[1,2)], Kenjiro INUI[3)], Hiroshi UCHIUMI[4)], Haruko OGAWA[1)]* and Kunitoshi IMAI[1)]

[1)]Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada, Obihiro, Hokkaido 080-8555, Japan
[2)]Virology Department, National Institute of Veterinary Research, 86 Truong Chinh, Dong Da, Hanoi, Vietnam
[3)]Food and Agriculture Organization, 3 Nguyen Gia Thieu, Hoan Kiem, Hanoi, Vietnam
[4)]ACT Co., Ltd., 21-5, Minami-4, Nishi-21, Obihiro, Hokkaido 080-2471, Japan

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ABSTRACT. Acidic electrolyzed water (EW) (pH 2.6–5.8) and alkaline EW (pH 11.2–12.1) were examined as potential disinfectants against foot-and-mouth disease virus (FMDV). Using acidic EW with pH 2.6 and alkaline EW with pH >11.7, the viral titer decreased *in vitro* by > 4.0 log values, 2 min after the virus was mixed with EW at a 1:10 dilution. The strong virucidal effect of acidic EW (pH 2.6), but not that of alkaline EW (>11.7), seemed to depend on the chlorine level in the solution. Genetic analysis revealed that viral RNA was substantially reduced, especially by alkaline EW.

KEY WORDS: disinfectant, electrolyzed water, foot-and-mouth disease virus, virucidal

Foot-and-mouth disease (FMD) is a highly transmissible and economically important viral disease of domestic and wild cloven-hoofed animals. This disease results in devastating consequences worldwide. There had been no outbreaks for 92 years in Japan until small-scale FMD outbreaks occurred in 2000, and a large-scale outbreak happened in 2010 [2, 3, 8, 10]. It is widely recognized that prevention and eradication of FMD is a critically important issue at the global level. In most countries where the FMD has been controlled, "stamping out" is used as an essential strategy to eradicate the re-introduction of the disease. In addition, vaccination has been employed to prevent FMD virus (FMDV) infection in many other countries where it is endemic. Since the FMDV is highly contagious, rapid and effective viral disinfection is critical in the field during FMD outbreaks. Disinfectants should function effectively to prevent FMDV from gaining access to farms and spreading to other farms. Thus, washing the facilities and disinfecting trucks and hands to prevent the spread of FMDV is important for its environmental control [2, 3].

FMDV is easily destroyed by a high or low pH, but the disinfectants used might be caustic or corrosive in the concentrated form [18]. The United States Department of Agriculture (USDA) [15] has listed some disinfectants that are available for field use during an FMD outbreak. These include sodium hypochlorite, acetic acid, potassium peroxymonosulfate, sodium chloride, sodium carbonate and sodium hydroxide. However, most of these disinfectants are caustic at effective concentrations and thus could damage clothing, shoes and rubber goods; additionally, they are corrosive to steel surfaces. Many veterinarians and supportive staff workers have been injured by disinfectants, such as slaked lime during FMD outbreaks in Japan [9]. The USDA also cautioned that lye and lime are caustic and corrosive to agriculture facilities, irritant to humans, and are hazardous to the environment. This highlights the fact that there is an urgent need to find alternative disinfectants that are effective and safe [16]. Recently, Krug *et al.* [6] reported the utility of a neutralized citric acid solution as a disinfectant for FMDV.

Electrolyzed water (EW) is generated by the electrolysis of water containing sodium chloride or potassium chloride in an electrolysis chamber wherein an ion-permeable exchange diaphragm separates the anode and cathode. At the anode, acidic EW with a low pH, high oxidation-reduction potential (ORP), high concentrations of dissolved oxygen and free available chlorine (FAC) is obtained; whereas, from the cathode, alkaline EW with a high pH, high concentration of dissolved hydrogen, and low ORP is obtained. It is known that EW, especially when acidic, shows antimicrobial activity against a broad spectrum of bacteria; thus, it is widely used in the agriculture and food industry [5, 14]. Several studies have demonstrated that EW has antiviral effects against blood-borne pathogenic viruses and influenza A virus [7, 12, 13]. However, there have been no reports on the antiviral effect of EW against FMDV. Therefore, this study aimed to evaluate the virucidal effects of EW against FMDV.

EW samples, provided by ACT Co., Ltd. (Obihiro, Hokkaido, Japan) were used in this study. The acidic EW samples included

*Correspondence to: Ogawa, H., Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada, Obihiro, Hokkaido 080-8555, Japan. e-mail: hogawa@obihiro.ac.jp

Table 1. Feature of the electrolyzed water (EW) samples and tap water used in this study

Sample name		pH	FAC (ppm)	ORP (mV)
Tap water		7.5−7.7	NT	274−489
Acidic EW	Acid 2.6	2.6	48.0	1,109–1,112
	Acid 5.8	5.8	30.0−39.0	873−886
Alkaline EW	Alk 11.2	11.2	4.0	330
	Alk 11.7	11.7	1.0	46
	Alk 12.1	12.1	1.0	–48

The pH, free available chlorine concentration (FAC), and oxidation-reduction potential (ORP) were measured using a Waterproof pH meter PH-6011 (Custom Corporation, Tokyo, Japan), a Handy Water Meter AQ-202 (Sibata Scientific Technology Ltd., Saitama, Japan) and a Waterproof ORP meter ORP-6041 (Custom Corporation), respectively. NT: not tested.

those at pH 2.6 and 5.8 were named Acid 2.6 EW and Acid 5.8 EW, respectively. For alkaline EW samples, those at pH 11.2, 11.7, and 12.1 were named Alk 11.2 EW, Alk 11.7 EW, and Alk 12.1 EW, respectively. The experiments were performed at least twice for each sample. The pH, FAC, and ORP of the EW samples are presented in Table 1. The EW samples, aliquoted in small and dark bottles, were shipped to the laboratory at the National Institute of Veterinary Research (NIVR), Vietnam, and stored at room temperature until they were used for experiments. For each experiment performed, new sample bottles were opened and used. Tap water was used as a control for each experiment.

FMDV type O was kindly provided by the NIVR in Vietnam. The FMDV was propagated in BHK-21 cells cultured in a medium containing 10% fetal bovine serum, and the culture supernatant was used as the viral solution. The viral solution was mixed with each EW sample or with tap water as a control, in 1:9 or 1:99 ratio to produce a viral dilution of 1:10 or 1:100, respectively. The mixtures were maintained at room temperature for 2 or 10 min. Subsequently, the treated viral solutions were serially diluted to 1:10 and used to inoculate BHK-21 cells, grown as a monolayer in 96-well plate, to measure virus titer. The 50% tissue culture infective dose ($TCID_{50}$) was calculated by the Behrens-Kärber method based on the cytopathic effect observed on day 3–4 post-inoculation.

To determine the direct effect of EW on the viral genome, RNA was extracted from virus treated with EW using a RNeasy Mini Kit (Qiagen, Hilden, Germany). The extracted RNA was subjected to real-time reverse transcription (RT)-PCR using an QIAGEN OneStep RT-PCR Kit (Qiagen), using primers and probes detecting the 2B region of the FMDV genome, as described by Oem *et al.* [11].

Virus titer of FMDV treated with Acid 2.6 EW at a 1:10 or 1:100 dilution for 2 min or 10 min decreased by > 4.0 log values to almost undetectable levels, compared to that of the control (Fig. 1A−1D). The virus titer of FMDV treated with Acid 5.8 EW at a 1:10 dilution for 2 min decreased by 1.5−2.0 log values, but that of FMDV treated for 10 min decreased by 2.0−4.0 log values (Fig. 1A and 1C). At a 1:100 dilution, the virus titer of FMDV in Acid 5.8 EW decreased by > 4.0 log values after 2 min (Fig. 1B and 1D). These results indicate that the Acid 2.6 EW conditions exhibit strong virucidal effects against FMDV when the virus was treated with a 9-fold or larger ratio of EW to virus. Acid 5.8 EW also showed virucidal effects, but required a 99-fold ratio of EW to virus volume.

The FAC concentration and ORP of Acid 2.6 EW were 48.0 ppm and approximately 1,100 mV, respectively, which were similar to those of acidic EW that showed bactericidal and/or virucidal effects in previous studies [1, 13, 17]. Acid 5.8 EW had a lower level of FAC (≤ 39.0 ppm) and ORP (< 900 mV) compared to that of Acid 2.6 EW, which could have diminished its antiviral activity. The FAC concentration of the two acidic EW solutions decreased with time, even if the sample bottle was closed tightly (data not shown). This raises the concern that the virucidal effect of Acid 2.6 EW against FMDV might be lost over time, if this effect relies on the FAC of the solution. In a future study, the effectiveness between acidic EW and sodium hypochlorite should be compared. In our previous study on the virucidal effect of EW against H5N1 highly pathogenic avian influenza viruses, we found that the virucidal effect of acidic EW, with a pH of 6.4–7.4, depended on the FAC concentration, but not the virucidal effect of acidic EW with a pH of 2.5 or lower [13]. Since the characteristics of FMDV and influenza virus are quite different, the virucidal effects of acidic EW against these viruses might involve different mechanisms. Important factors involved in the virucidal effect of acidic EW against FMDV should be studied in future.

The titer of FMDV treated with Alk 11.2 EW was relatively unchanged even after treatment at a 1:100 dilution for 10 min (Fig. 1A and 1B). In contrast, the titer of FMDV treated with Alk 12.1 EW decreased by > 4.0 log values with treatment at 1:10 and 1:100 dilutions for 2 min (Fig. 1C and 1D). Thus, Alk 12.1 EW showed a strong virucidal effect against FMDV, which was comparable to that of the Acid 2.6 EW. The reduction in virus titer by Alk 11.7 EW was slightly less than that observed with Alk 12.1 EW after a 2-min treatment, but was comparable after a 10-min treatment (Fig. 1C and 1D). The FAC concentration of these two alkaline EWs was 1.0 ppm, which was even lower than that of Alk 11.2 EW. These results suggest that the virucidal effects of Alk EW (pH > 11.7) are independent of free chlorine in the solutions. This indicates that the virucidal effect of alkaline EW can be retained for a long period without requiring a sealing condition. This strongly highlights the beneficial features of alkaline EW as a disinfectant. Alk 12.1 EW had the highest pH and the lowest ORP among the EW samples tested in this study. The role of high pH

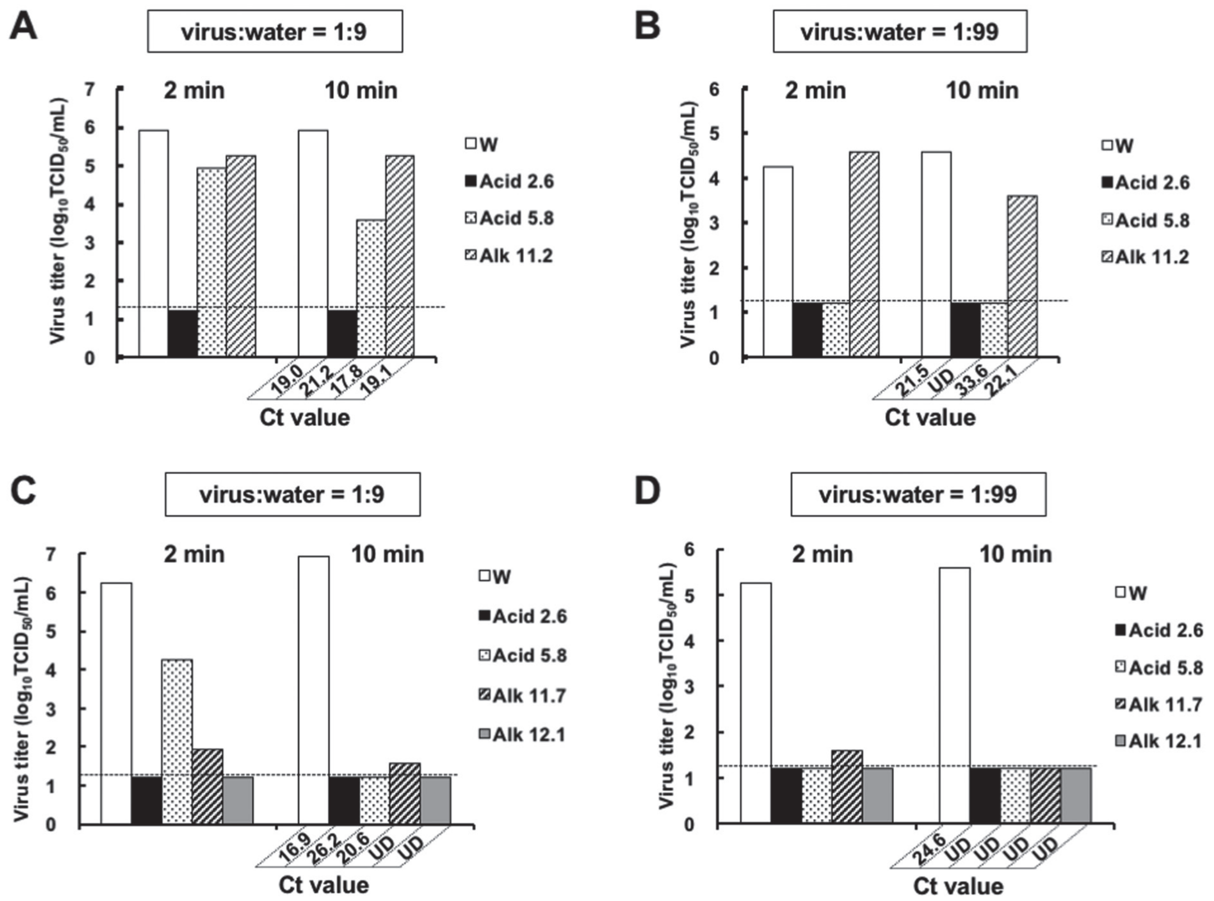

Fig. 1. Virucidal effects of acidic and alkaline electrolyzed water (EW) samples on FMDV. FMDV titers were measured using BHK-21 cells at 2 min or 10 min after viruses were mixed with EW samples or tap water (W). The results obtained with Lot 1 (A, B) and Lot 2 (C, D) of the EW samples are shown. Lot 1 samples included Acid 2.6 (pH 2.6), Acid 5.8 (pH 5.8), and Alk 11.2 (pH 11.2) EW solutions. Lot 2 included Acid 2.6, Acid 5.8, Alk 11.7 (pH 11.7), and Alk 12.1 (pH 12.1) EW solutions. Virus and water samples were mixed at 1:9 (A, C) or 1:99 (B, D) ratio. The values below the graphs, for the 10-min data, represent Ct values obtained by real-time RT-PCR for the 2B region of the FMDV genome. A Ct value of 35 was regarded as the detection limit. UD represents undetermined results (with Ct values higher than the detection limit). Representative data of 2–3 experiments are shown.

and low ORP on the virucidal effect of alkaline EW should be further evaluated.

Based on real-time RT-PCR for FMDV RNA, the Ct value for FMDV treated with Acid 2.6 EW at a 1:10 dilution for 10 min was higher than that of the control (Fig. 1A and 1C). For the 1:100 dilution, a Ct value increase of > 10 was observed for virus treated with Acid 2.6 EW (Fig. 1B and 1D); this could be interpreted as a reduction in the viral gene copy number (of > 3.0-log values) based on the nature of this analysis [4]. Similar results were obtained with Acid 5.6 EW (Fig. 1A–1D). Treatment of FMDV with Alk 11.2 EW did not affect the Ct values (Fig. 1A and 1B). In contrast, after treatment with Alk 11.7 EW and Alk 12.1 EW, the Ct values were above the detection limit (> 35.0) (Fig. 1C and 1D). These results suggest that under acidic and alkaline (pH > 11.7) EW conditions, the integrity of FMDV genomic RNA was compromised, and the effect of alkaline EW solutions seemed to be more prominent than that of the acidic EW solutions.

Numerous studies have demonstrated the safety and non-toxicity of EW, suggesting its biocompatibility. Some studies also showed lower levels of metal corrosion by acidic EW than that of a sodium hypochlorite solution. Notably EW is regarded as a non-environmental hazard, as it reverts to water or diluted salt water [5, 14]. These advantageous features of EW support its usefulness as a disinfectant. In addition, the reported advantages of EW as a disinfectant include low running cost and ease of on-site production. EW generators are commercially available and can be used to generate fresh EW on-site simply by using tap water and sodium chloride or potassium chloride [5, 14]. Therefore, EW may be used during FMD outbreak in the form of spraying, soaking, and washing solutions to disinfect the virus in the farms and surrounding areas. Previous studies showed that EW can inactivate other viruses and some bacteria. Therefore, EW may be applicable for sanitary purposes to prevent not only FMD, but also other diseases. Most previous studies focused on the antimicrobial effects of acidic EW, but not of alkaline EW. Alkaline EW showed potential as a washing solution, but had limited antimicrobial activity [5]. The present study provides a foundation for utilizing alkaline EW as an effective disinfectant.

In conclusion, this study was the first to report the virucidal effects of acidic (pH 2.6) and alkaline (pH > 11.7) EW solutions against FMDV. The prominent effect of alkaline EW on viral genome integrity and its virucidal mechanism, which is thought not to rely on FAC, emphasizes its potential utility as a disinfectant for field application.

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