Antiviral agents and disinfectants for foot-and-mouth disease (Review)

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Abstract. Fluorouracil, 5-azacytidine, 6-azauridine, ribavirin, favipiravir (T-705) and its derivative (T-1105) exhibit anti-foot-and-mouth disease virus (FMDV) effects. In particular, T-1105 exhibits promising results when administered to guinea pigs orally, and pigs in their feed. FMDV is excreted in the early stages of infection in aerosols and oral or nasal droplets from animals. T-1105 along with the FMDV vaccine can be used to combat foot-and-mouth disease (FMD) epidemics. Several studies have shown that sodium hypochlorous solutions are widely used to inactivate viruses, including FMDV. However, these solutions must be stored under cool and dark conditions to maintain their virucidal effects. Interestingly, a study indicated that the virucidal activity of a calcium bicarbonate solution with a mesoscopic structure (CAC-717) did not decrease after storage at room temperature for at least four years outside direct sunlight. Numerous lessons acquired from the 2010 FMD outbreak in Japan are relevant for the control of COVID-19. However, the widespread use of chlorite can cause environmental issues. Chlorite can be combined with nitrogen to produce chloramine or N-nitrosodimethylamine, which plays a role in carcinogenesis. Therefore, risk assessments should be conducted in aquatic environments. Moreover, there is a need to develop nonchlorine disinfectants that can be used during epidemics, including FMD. The approach of 'One Health' should be shared between the public health and veterinary fields to improve the management of viral outbreaks, including those due to FMD.

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Contents

1. Introduction

Foot-and-mouth disease (FMD) has induced disastrous effects on world livestock production and the general economy for >100 years. It is one of the most feared diseases in cattle and pigs. It infects primarily cloven-hoofed wild animals and domestic animals. In cattle viremia, anorexia with dullness are observed following a high fever (40 to 41˚C). Subsequently, blisters are observed on the tongue, lips, cheeks, gums and dental pads. Ropy saliva, smacking of the lips, lameness, and loss of movement were the conditions observed in cattle (1). The FMD virus (FMVD) belongs to the family *Picornaviridae*, genus *Aphthovirus*. FMDV is a non-enveloped virus with a single stranded RNA genome. There are seven serotypes of FMDV. These are subject to a high number of mutations, continuously generating new FMDV variants (1). All these serotypes cause similar clinical symptoms with a duration of 2-14 days after being infected.

The largest FMD outbreak in Japanese history occurred in 2010. From April to July of 2010, a total of 221,608 cattle, goats and pigs were collected from 292 farms in the Miyazaki Prefecture (Table I). The virus was identified as a serotype O prototype (Mya-98 lineage). The first case of FMD was reported on April 20, 2010. On May 19, widespread vaccination was recommended, which began on May 25. Following vaccination, a decrease in the suspected animals awaiting

culling was observed. The peak of suspected animals awaiting culling occurred on May 25 (~70,000 animals). On June 22, the last animal was sacrificed, and on July 27, all restrictions were lifted. Finally, on February 5, 2011, Japan restored its World Organisation for Animal Health (WOAH) status as an FMD-free country, where vaccination was not practiced. However, the Japanese government keeps a stock of antiviral agent T-1105 (1 ton/year) for use in any eventuality (1).

The symptoms exhibited by the animals infected with FMDV are presented in Table II. Cattle dribbled foamy saliva continuously, had fevers, and the tongues and pads were hard with blisters and erosions. Foot lesions in cattle were rarely observed (2). However, foot erosions and hemorrhages, causing lameness, were commonly observed in pigs. A total of >90% of infected pigs in farms developed lesions on their legs. In breeding farms, the sows often presented with blisters and erosions on the teats. In total, 14 goats exhibited symptoms of FMD in Miyazaki, Japan (Table I). The goats were seropositive for FMDV and only displayed small erosions in the nasal cavity.

Most outbreaks (279/292) were observed within a small area of ~20 km, from south to north, located in the central region of Miyazaki Prefecture (2). In addition, this area is a narrow plain situated between mountains to the west and the sea to the east. Several sporadic epicenters of infection were observed at distant locations.

Broad-spectrum replication inhibitors and mutagenic nucleotide analogs exert anti-FMDV effects. These include fluorouracil (3), 5-azacytidine (4), 6-azauridine (5), ribavirin (6), favipiravir (T-705) (7) and its derivative (T-1105) (8). Fluorouracil (3), 5-azacytidine (4) and 6-azauridine (5) were primarily studied in the early 2000s. Recently, ribavirin, favipiravir (T-705) (7), and its derivative (T-1105) (8) have been widely studied. Among them, T-1105 (1,9) has exhibited the greatest promise *in vivo*. T-1105 is a favipiravir derivative. Despite its higher dose (200-400 mg/kg/day), it was demonstrated to exert significant suppression of symptoms and clinical signs, such as fever, in pigs and guinea pigs (8). In addition, newly developed antiviral chemical compounds, such as brequinar (BQR) (10), mizoribine (11), merimepodib (12) and vesatolimod (13), have been discussed as potential agents against FMD. The present review describes several developments in antiviral agents and disinfectants following the large 2010 outbreak of endemic FMD in Japan.

In the last 13 years, the Japanese Ministry of Agriculture, Forestry, and Fisheries have invested a substantial sum of money to develop viral replication inhibitors against FMDV. The National Institute of Animal Health in Japan is responsible to update the scientific information of anti-FMDV chemical compounds especially T-1105 and T-705 (1,7-9). The aim of the present review was to increase awareness of the antiviral chemical compounds and disinfectants developed by the Japanese Government Institution and Universities from 2010.

Table I. Number of FMD cases in Miyazaki during the 2010 epidemic.

No. of outbreaks	No. of infected/suspected animals	
292	Cattle	37,412
	Beef cattle	36,284
	Dairy cattle	1,128
	Water buffalo	42
	Pig	174,132
	Goat	14
	Sheep	8
	Total	211,608

Table II. Clinical signs observed in the FMD cases in Miyazaki during the 2010 epidemic.

Clinical signs	Cattle	Pig
Fever	O	O
Anorexia	O	O
Excessive salivation	O	Δ
Vesicular condition (blisters, ruptured and erosion) of the feet, buccal mucosa and, in females, the mammary glands	O	O
Lameness	Δ	O

O, commonly observed; Δ, rarely observed.

2. Antiviral chemical compounds

In this section, favipiravir, that was recommended by the Japanese Government for treatment against FMDV, is mainly described and discussed. In addition, ribavirin, BQR, mizorbine, VX-497 and GS-9620, potential candidate chemical compounds which may be used in the future, are also described.

Favipiravir. Favipiravir (T-705, 6-fluoro-3-hydroxypyrazine-2-carboxamide) was developed for use against influenza viruses (14). This agent hinders ribonucleic acid (RNA)-dependent RNA polymerase within the virus (15). In addition, this agent prevents the replication of arenaviruses, bunyaviruses, flaviviruses, alphaviruses and noroviruses (9,16), and is a candidate for the Ebola virus and SARS-CoV-2 (17,18) (Fig. 1).

In a previously created and described guinea pig model, the animals were administered T-1105 (400 mg/kg/day) orally, twice daily for five days. Following the first administration of T-1105, FMDV [O1 Manisa:100 guinea pig (GP) ID_{50}] was inoculated into the intraplantar area 1 h later. A total of 14 T-1105-treated animals were completely protected from footpad lesions. A total of four days post infection (dpi), viral RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in serum, organs and oral swabs of half of the animals (19). In total, 10 animals received a single dose (2 ml) of the commercial double-oil emulsion-inactivated O1 ELISA vaccine. Three weeks later, the immunized animals were inoculated with FMDV. All vaccinated animals were protected from footpad lesions. At 4 dpi, only one out of the

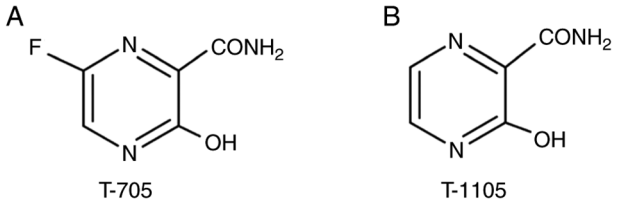

Figure 1. Structural formula of (A) T-705 and (B) T-1105.

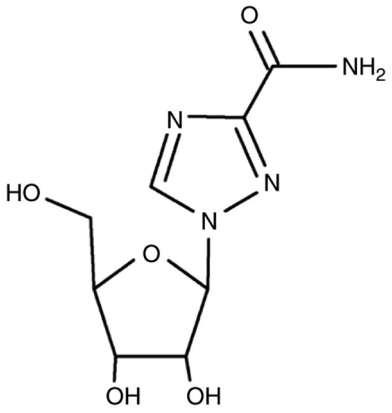

Figure 2. Structural formula of ribavirin.

ten immunized and inoculated animals exhibited FMDV in the sera, without clinical symptoms The mean viral RNA levels of the animals treated and vaccinated were significantly lower than those of the controls not treated with T-1105 and non-vaccinated ($P<0.01$). These results demonstrated the suitability of T-1105 to combat FMD outbreaks (19).

In an *in vivo* study of pigs, animals were injected with 10^6 TCID50 of FMDV O/JPN/2000 on the right side of the footpad. The T-1105-treated group was administered 200 mg/kg T-1105 orally mixed with food for 1 h before viral injection. Following the first administration, the same doses were administered twice a day for six days. The treated and non-T-1105-treated groups consisted of six and two pigs, respectively (1). For virological testing, plasma and nasal swab samples were collected for virus titration [plaque-forming units (PFU)] and quantitative PCR (qPCR). For immunological testing, liquid-phase blocking ELISA and virus neutralization tests were performed (1).

Typical clinical symptoms of FMD, such as fever, anoxia, feet blisters and lameness (Table II), were observed in control pigs (without T-1105). No clinical FMD symptoms were observed in T-1105-treated pigs throughout the experiments. In real-time qPCR, the Cq value of the treated animals reached >40 at 6 dpi in plasma, while the Cq value of the untreated animals was 20-30 at 1-3 dpi. Non-treated animals exhibited FMDV in plasma at least three days earlier than the treated animals (1).

The excretion of FMDV from the nasal cavity was assessed using a viral plaque assay (reported in PFU) and qPCR. The virus was not detected in the nasal swabs nor in the viral plaque assay in animals treated with T-1105. Aberrant Cq values were not observed in five out of the six T-1105-treated animals. Notably, one out of the six T-1105-treated animals had a Cq value of 36 at 1 dpi. However, these swabs did not isolate FMDV using a viral plaque assay. Cq values of the untreated control animals were 29-38 at 2-3 dpi on nasal swabs. Untreated control animals exhibited FMDV in nasal swab samples at 10^2-10^4 PFU/ml at 2-4 dpi (1).

ELISA antibody testing revealed that untreated controls had increased antibody levels from 4 days post infection (dpi) to >1:360 at 5-6 dpi. Serum neutralizing antibody levels reached 1:128 at 6 dpi. Conversly, the animals treated with T-1105 exhibited ELISA antibody levels of <1:45 and neutralizing antibody levels of <1:32 (1).

In a previous *in vitro* study, T-1105 was more effective than T-705 in treating FMDV (20). T-1105 exerted an antiviral effect, inhibiting viral RNA-dependent RNA polymerase (20). T-705 inhibited influenza virus RNA polymerase, and T-1105 is a derivative of T-705 (20). FMDV is excreted in the early phase of infection in aerosols and in oral or nasal droplets from animals. This occurs before the animals produce antibodies to protect themselves against infection. Combined with the FMDV vaccine, T-1105 and chemical disinfectants could provide more efficient protection against FMD outbreaks. More detailed studies are necessary to determine the most effective time point of administration and dose of T-1105 in pigs.

Recently, Nishi *et al* conducted a detailed study using T-1105, 6 h after oral infection with FMDV in pigs (21). In the aforementioned study, $10^{5.5}$ $TCID_{50}$ O/HKN/1/2015 FMDV was inoculated orally into pigs. As stated in a previously mentioned study (1), the pigs were treated orally with 200 mg/kg T-1105, which mixed with feed twice daily for 6 days (1), starting at 1 h before virus inoculation. In another group of pigs, $10^{5.5}$ $TCID_{50}$ O/HKN/1/2015 FMDV was inoculated orally, and 200 mg/kg T-1105 was administered orally to pigs twice daily for 6 days, starting from 6 h after infection. As previously mentioned, all untreated and infected control pigs exhibited clinical signs of FMD, FMDV-specific genes, and serum antibodies (1). Through the use of immunohistochemistry, the FMDV antigen was observed in the pig tissues of all untreated and infected controls. No clinical signs, FMDV-specific genes, serum antibodies, or tissue antigens with FMDV were observed in pigs treated with T-1105 twice daily for 6 days, starting at 6 h after or 1 h before infection. The results of FMDV-infected high-risk groups in the field, with T-1105 treatment or without T-1105 treatment require further investigation.

In the next section, the antiviral effects of ribavirin, BQR, mizoribine, merimepodib and vesatolimod are described as possible candidates during an FMDV pandemic. Currently, a limited number of studies have reported antiviral chemical compounds for FMD (6,22).

Ribavirin. Ribavirin is an antiviral agent (synthetic purine nucleoside analog) that acts against various RNA and DNA viruses (6,22) (Fig. 2). Different serotypes of FMDV ribavirin have been demonstrated, only *in vitro*, to have inhibitory effects (22). Ribavirin is a promising antiviral compound. Similar to favipiravir, ribavirin interferes with viral polymerase (22) and RNA capping (23). Suckling mice have been used to investigate FMDV in lethal animal models. The FMDV A strain (A/IND/40/2000) was used in C57BL/6 suckling mice (22). This viral strain was revealed to be highly

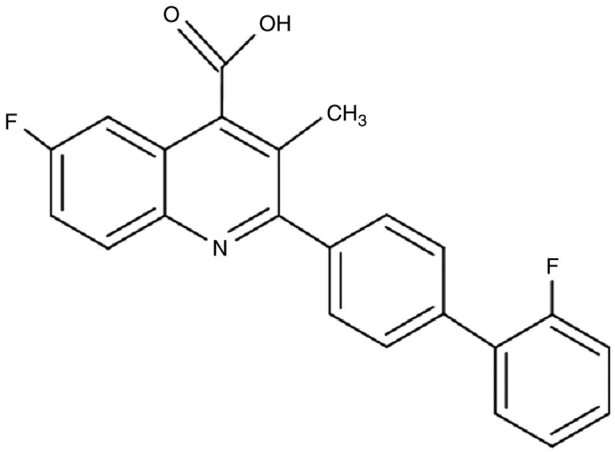

Figure 3. Structural formula of brequinar.

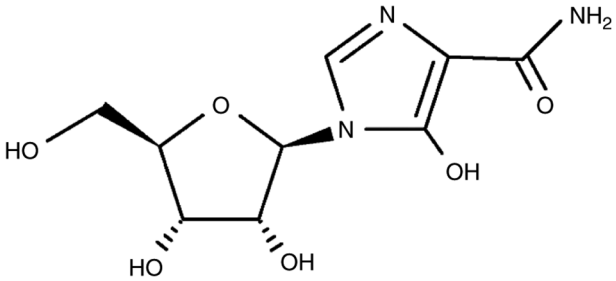

Figure 4. Structural formula of mizoribine.

virulent in suckling mice with acute flaccid paralysis and was lethal within 72 h after infection (24).

The antiviral efficiency of ribavirin against FMDV A was also determined. A single dose of ribavirin (50 mg/kg) was administered 12 and 6 h before and after treatment, respectively. A drug toxicity study was observed using 5- to 6-day-old C57BL/6 mice (18). Ribavirin (up to 80 mg/kg) was administered to mice once a day for five days. In another study, the drug was administered intraperitoneally to old ICR-suckling mice (25). Both studies reported that no mice has succumbed 15 days after administration. Therefore, ribavirin can be used in the early phase of infection or before infection with vaccine treatment. However, further testing is required in pigs.

BQR. A mechanism of action of BQR is the inhibition of dihydroorotate dehydrogenase (DHODH) (Fig. 3). The rate-limiting enzyme in the *de novo* synthesis of pyrimidines is DHODH (10). Thus, BQR, as a DHODH inhibitor, was capable of depleting the cellular pyrimidine pool. The pyrimidine pool is essential for RNA and DNA production. BQR has exerted virucidal effects against numerous types of viruses, including dengue virus (26), Ebola virus (27), and hepatitis E virus (28). Nearly all of these studies were conducted *in vitro*.

BQR against FMDV has been studied *in vitro* and *in vivo* (29). Mice were infected with 100 LD_{50} FMDV. All mice in the non-BQR group were treated 60 h after infection. This study included 3- to 4-day-old mice. The FMDV dose LD_{50} (O/MYA98/BY/2010) was determined using the Reed-Muench method with 10-fold serial dilutions. BQR (50 μg) was injected intraperitoneally into suckling mice, 2 h before virus challenge. The suckling mice were inoculated intraperitoneally with 100 μl of 100 LD_{50} FMDV, and the animals were observed for five days. After five days, 25% of the mice in the BQR-treated group had survived and histopathological changes in the hearts were observed under a microscope. Treatment with BQR significantly reduced the severity of lesions in the heart muscles of FMDV-infected mice ($P<0.05$) (29). Thus, BQR may be used as a vaccine before infection, however further studies are required in pigs and cows.

Mizoribine. Mizoribine is an imidazole nucleoside (11) (Fig. 4) that inhibits the replication of the hepatitis C virus at a 50% inhibition concentration (IC_{50}) of approximately 100 μM (30). Mizoribine was originally developed as an immunosuppressant for transplantation immunity without obvious side effects (30) and as an inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH). This inhibition is useful in renal transplantation, autoimmune diseases, and steroid-resistant nephrotic syndrome (31). Mizoribine was produced by culturing *Eupenicillium brefeldianum* M-2166 molds in culture medium (32).

Mizoribine was demonstrated to inhibit SARS-CoV (31). Additionally, mizoribine inhibited FMDV replication in suckling mice. The suckling mice were inoculated with a mizoribine solution (50 μg/0.1 ml) by subcutaneous injection into the neck. After 2 h, FMDV was inoculated intradermally (11) at 100 LD_{50} (serotype O O/MY98/BY/2010). The animals were observed for five days. Within 60 h after FMDV infection, all control mice [phosphate-buffered saline (PBS) solvent-treated] succumbed. In the FMDV-infected group treated with mizoribine, a 48-h delay in death was observed (11). A significant difference in the mortality curve was observed between the mizoribine-treated and control groups ($P=0.0014$) (11). The findings of the mizoribine study warrant further investigation using the natural host of FMDV. Mizoribine could be used to complement vaccine treatment in the future.

Merimepodib (VX-497). Merimepodib is an oral drug with broad antiviral activities (12) (Fig. 5). In mouse experiments, suckling mice were administered 30 μg of merimepodib intranasally. After administration, the nasally administered drug was moved to the stomach. Adult mice were administered orally with merimepodib. Similar to mizoribine, this drug is an oral inhibitor of IMPDH. Merimepodib has an immunosuppressive and anti-keratinocyte effect (33). This immunosuppressive effect of merimepodib disappears within 48 h after the end of administration (34). Numerous viruses, such as herpes simplex virus-1, parainfluenza-3 virus, bovine viral diarrhea virus, Venezuelan equine encephalitis virus, and dengue virus, have been demonstrated to be suppressed by merimepodib in an *in vitro* study (12). This drug is used regularly at concentrations ranging from 6 to 19 μM (34).

Oral administration of merimepodib in mice inhibits the response to the primary IgM antibody (33). Decker *et al* reported the effects of merimepodib on transplant immunology and treatment of graft-vs.-host disease (35). Li *et al* reported the antiviral activity of merimepodib using FMDV in mice *in vivo* via intranasal merimepodib administration (12). BALB/c mice, three days old were treated with 100 LD_{50} FMDV O/MYA98/BY/200. The mice were divided into three groups. One group received 100 μl of PBS containing 10%

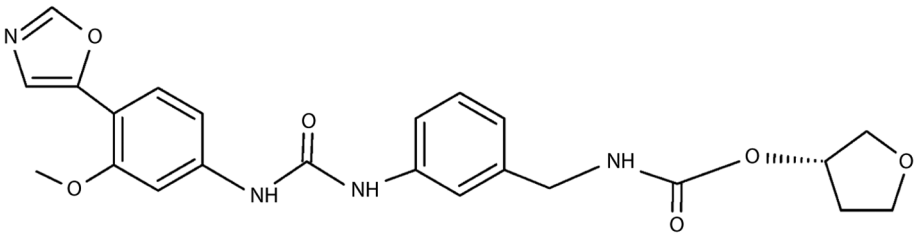

Figure 5. Structural formula of merimepodib.

dimethyl sulfoxide (DMSO) and 5% Tween-80 as a control group. The other group received 30 μg of merimepodib dissolved in 100 μl of PBS containing 10% DMSO and 5% Tween-80. After 2 h, 100 μl of diluted FMDV was subcutaneously injected into the dorsal cervical area of each mouse. The untreated and virus-infected mice began to succumb at 36 h after infection, and at 60 h, all mice had succumbed. In the merimepodib-treated and virus-infected groups, mice began to succumb 48 h after infection and all mice in this group succumbed within 108 h. The survival rate of mice administered merimepodib was significantly higher than that of mice not administered after infection with 100 LD_{50} FMDV ($P<0.0001$). Merimepodib may be used as an oral drug in the treatment of FMD (12). In future, more experiments are required to test this hypothesis in pigs.

GS-9620 (vesatolimod). Chimpanzees have been used as animal models to study the chronic hepatitis B virus isolated from humans (13). GS-9620 (Fig. 6) is a potent and selective orally active small molecule agonist of Toll-like receptor 7 (TLR7) developed in studies of hepatitis B virus (13). Orally administered GS-9620 has been used as an immunostimulant to enhance innate immunity (36). Furthermore, GS-9620 is an inducer of type 1 interferon, which induces an antiviral state in host cells via TLR7. Administration of TLR7 agonists has been attempted using various routes as adjuvants. Bhagchandani *et al* attempted oral, intravenous, topical, and intracutaneous administration (37). Lee *et al* attempted intramuscular administration of ISA 206 adjuvant (38). Moreover, the adjuvant effect of the TLR7 agonist, GS-9620, is as an antiviral agent in the early phase of FMD. Mice were injected intraperitoneally with 0.1 ml of GS-9620, ribavirin, or PBS (negative control) for 16, 24 and 72 h prior to FMDV infection. Mice were challenged with intraperitoneal injection of mouse-adapted FMDV (250 LD_{50} of O/VIT/2013). GS-9620 was injected at a dose of 5 or 10 mg/kg body weight. Ribavirin was injected at a dose of 10 mg/kg of body weight. A total of five mice were used and observed for six days after infection in each group. Both GS-9620 and ribavirin treatments increased the survival rate of drug-treated mice after FMDV infection. The survival rate of mice treated with GS-9620 (10 mg/kg) was higher than that of mice treated with ribavirin (38). Further confirmation is necessary using natural hosts for FMDV, such as pigs, cows or goats.

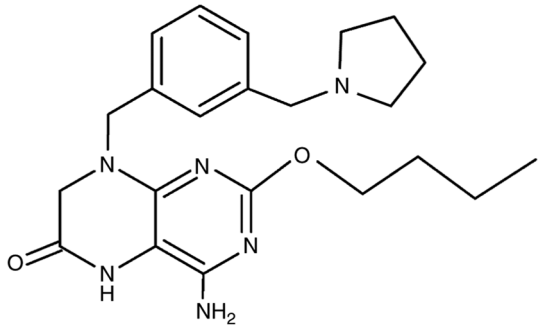

Figure 6. Structural formula of GS-9620.

3. Disinfectants for use against environmental contaminants

This section discusses recently developed disinfectants against FMDV, including CAC-717, accelerated hydrogen peroxide, and Virkon S, which can be used against environmental contaminants.

CAC-717. Sodium carbonate solution (4%) and several commercial products are widely used for disinfection of FMDV in Japan (39). In the United States, 3% sodium hypochlorite, 4-5% acetic acid, Virkon S (potassium peroxymonosulfate and sodium chlorite), 4% sodium carbonate, and 2% sodium hydroxide are recommended (40). To guarantee food safety, all surfaces must be washed or treated after sterilization before consumption. The heat-denatured egg white lysozyme is known to inactivate FMDV (39). Fukai *et al* observed that the heat-denatured lysozyme reduced the RNA content of FMDV O/Taiwan/1977 by 2.7log_{10} after treatment (39). However, the mechanisms underlying these virucidal effects are unclear. Usually, a heat-denatured lysozyme is more hydrophobic and the pH increases. This increased pH results in a virucidal effect on murine norovirus (41). Similar virucidal mechanisms may be involved in FMDV. Using FMDVsA/TAI/46-1/2015 and Asia1/Shamir (ISR/3/89), the virucidal effect of the heat-denatured lysozyme is unclear (39). Further studies are required to elucidate the virucidal effects of this enzyme.

Harada *et al* evaluated the virucidal efficiency of 13 commercially available products in Japan (42). These products included five alcohol-based disinfectants, two hand soaps, two alkaline cleaners, two quaternary ammonium compound sanitizers, and two chlorine disinfectants with short exposure times (42). Because FMDV is inactivated in acidic environments, acidic ethanol disinfectants Vir Stera and Alpet NV significantly reduced FMDV infectivity by at least 3.75log_{10} within 30 sec of exposure. Since FMDV is also inactivated under strongly alkalic conditions (pH >12), an alkaline cleaner named Degreaser FII and Start Clean significantly reduced FMDV infectivity by at least 3.5log_{10} in 30 sec of exposure.

However, strongly alkaline conditions can be harmful to human skin. A chlorine disinfectant named Zia Knock (a sodium hypochlorite product; 200 ppm) reduced the infectivity by at least 3.5$\log_{10}$ in 30 sec of exposure. The virucidal effect of chlorine is rapidly lost under dirty conditions (42). Therefore, hypochlorous acid solution should be used for virucidal effects in areas free of organic substances.

Nakashima *et al* (43) reported that the calcium bicarbonate solution (CAC-717) (43,44) did not decrease the virucidal activity against the influenza virus after incubation with bovine serum albumin (10 mg/ml). However, mixing the medium with organic materials (fetal bovine serum; protein concentration >7.0 mg/ml, final concentration) weakly reduced the effect of CAC-717 against bovine adenovirus-7 (45). As fetal bovine serum contains various inhibitors other than albumin, further studies are necessary to address these issues. Furthermore, CAC-717 works most efficiently (reduction of >3.5$\log_{10}$ $TCID_{50}$) against Asia-1, O and A type strains of FMDV compared to other reported disinfectants such as heat-denatured lysozyme (39). In heat-denatured lysozyme treatment Type O and Asia 1 FMDV were not inactivated efficiently, although Type A virus was inactivated. CAC-717 can be used internationally (Fig. 7 and Table III).

Several studies have shown that sodium hypochlorous solutions can be used to inactivate various viruses (46-49), including FMDV (40). However, these solutions must be stored in cool and dark conditions to maintain their virucidal effects (49). Conversely, Kirisawa *et al* demonstrated that the virucidal effect of CAC-717 did not decrease after storage for four years at room temperature without direct sunlight (45).

Accelarated hydrogen peroxide (AHP). The virucidal effects of AHP on wet films were assessed against FMDV and swine vesicular disease virus (SVDV) (50). The AHP solution was commercially named ACCEL TB (51). This solution was based on 0.5% accelerated H_2O_2 at a pH of 3.0. In the United States, this solution has been patented (patent no. 6.346.279) as a new generation disinfectant. To observe the effectiveness of this disinfectant, SVDV was spread on a film by spraying a 0.2-ml suspension inside the bottle, as previously described (51). The test plates inside the bottles were dried for 20 min at 20±1˚C (relative humidity, 42%). The test substances were added to 2 ml of the solution at 20˚C for the desired contact time. After scraping the test surfaces of the films, the samples were tested for infectious viruses, as previously reported (51).

AHP was effective against FMDV at a 1/40 dilution, resulting in a >5$\log_{10}$ reduction in the virus titer (50). The virucidal effect was tested immediately after reconstitution with AHP (1/20 dilution in distilled water). Subsequently, the diluted AHP was tested every two weeks at a contact time of 10 min. The results revealed that the disinfectant maintained its effectiveness for at least six weeks when kept in a sealed bottle at room temperature. A toxicity test in animals demonstrated that AHP is non-toxic, non-irritating to the skin and eyes, and immunologically non-sensitizing (50). Therefore, AHP could be useful in the field of FMDV outbreaks.

Virkon S. Virkon S (potassium peroxymonosulfate and sodium chlorite) was selected as a disinfectant for porous concrete surfaces, because the United States Environmental Protection Agency registered this chemical as broad-spectrum disinfectant and virucide that is effective against FMDV (52,53). Little has been published on the virucidal effects on agricultural porous surfaces. Wood is also a common construction material used on farms. Modeling virus decontamination in agricultural facilities is important for FMDV control (54). Virkon S has been reported to deactivate FMDV (50).

Table III. Virucidal effects of CAC-717 on the three FMDV serotypes.

	Solution		
FMDV serotype	CAC-717 60 min	Tap water 60 min	Maintenance medium 60 min
Type A	≤0.5[a]	5.00±0.13	5.38±0.13
Type O	≤0.5[b]	4.25±0.00	4.00±0.00
Type Asia 1	≤0.5[a]	4.00±0.00	4.63±0.13

Viral titer is shown as $\log_{10}$ $TCID_{50}$/25 μl (mean ± SE). [a]A reduction in virus titer of >4$\log_{10}$ compared to that of the maintenance medium-treated virus. [b]A reduction in virus titer of >3$\log_{10}$ against that of maintenance medium-treated virus. More details can be found in Ref (45). FMDV, foot-and-mouth disease virus.

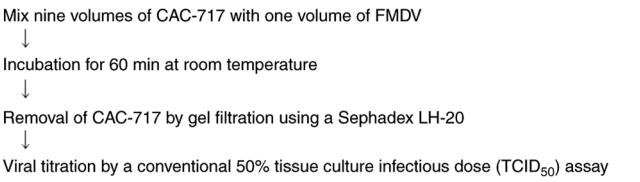

Figure 7. Disinfection procedure using CAC-717 for FMDV. FMDV, foot-and-mouth disease virus.

The FMDV strains A/24/Cruzeiro/BRA/55 and 01 Campos were identified in a porcine kidney cell line. To assess virus survival and recovery, 10 μl of virus inoculum was deposited on stainless steel and concrete surfaces and dried under desiccation for ~1 h. Carrier disinfection tests were also performed (52). A total of 50 μl (stainless steel) or 100 μl (concrete) of Virkon S were spread on the dried virus for 5 or 10 min (+3 sec). The samples were then eluted with 10 ml of medium (DMEM + 2% FBS). Quantitative disinfection experiments were performed using FMDV samples on stainless steel and concrete to determine the virucidal effects of Virkon S, and the results revealed adequate virucidal effects (52).

4. Foot-and-mouth disease and environmental contamination

CAC-717, AHP, and Virkon S are novel disinfectants that can be used to treat FMDV. Sodium hypochlorite (NaOCl) is widely used for disinfection in public places. The United States Environmental Protection Agency has listed it as one of the most used disinfectants to combat COVID-19 (55). During the COVID-19 outbreak, disinfectants containing NaOCl were widely used. However, NaOCl can negatively affect

the environment, sewage water, and farm irrigation (56). A recent report showed that NaOCl waste threatens fish, seafood and food safety (57). Therefore, there are concerns about using NaOCl-based solutions for future FMDV outbreaks. Zhang *et al* reported that China has been using chlorine disinfectants extensively indoors and outdoors. China was reported to have consumed 2,000 tons of chlorine to combat COVID-19 in Wuhan. This chemical has been identified in sewage and drinking water (57). The widespread use of chlorine during outbreaks can negatively impact the environment (57) because chlorine can react with nitrogen to produce chloramine or N-nitrosodimethylamine (58). Furthermore, these chemicals are notorious for their role in carcinogenesis (59). Therefore, risk assessments are necessary in aquatic environments prior to use. In addition, non-chlorine disinfectants that can be used during outbreaks, including FMD, must be developed.

5. Discussion

Government reports have shown that FMDV was probably introduced to Japan from other Asian countries by people or goods (19). In Miyazaki, the main route for developing the FMD cluster is the movement of people or transportation. A total of 403 disinfection posts for vehicles were established in the Miyazaki Prefecture (2). These findings were summarized in several reports (Interim Report, October 2010; Supplement Report, March 2013, Epidemiological Investigation of FMD; OIE Symposium in Tokyo, November 13, 2014) (19).

The diagnosis and containment were performed in the event of an outbreak. All laboratory tests for FMD diagnosis in Japan were conducted at the National Institute of Animal Health in Kodaira (Tokyo, Japan) (Fig. 8). To contain the spread of FMD, stamping out, disinfection, movement restrictions (a radius of 10 km around an infected farm), and tests of surrounding farms were performed. Furthermore, when the presence of FMD was confirmed, all infected and suspected animals were culled. Common burial sites were used due to the rapid increase in infected farms. This made it difficult to identify the appropriate sites on all farms. Clinical and serological surveillance was conducted on all susceptible animals within the movement restriction zone. All cases and related materials were discarded. A liquid phase blocking ELISA was performed on sera of animals within a radius of 3 km of infected and related farms (Fig. 8).

To prepare for future FMD outbreaks, the Japanese government has secured vaccines (Fig. 9) and T-1105. Furthermore, national and local authorities perform simulation exercises annually. The government also regularly updates national FMD control guidelines and annually reviews the national vaccine stock for emergency use (O1, Asia1 and A; Fig. 9).

6. Conclusion

Various disinfectants, such as 3% sodium hypochlorite, 4-5% acetic acid, Virkon S, 4% sodium carbonate, and 2% sodium hydroxide have been used to deal with FMD. Furthermore, broad-spectrum replication inhibitors and mutagenic nucleotide analogs (T-1105) are stored on Kyushu Island if necessary for future outbreaks. Recently, there has been a trend to identify antiviral agents to complement vaccination. Therefore,

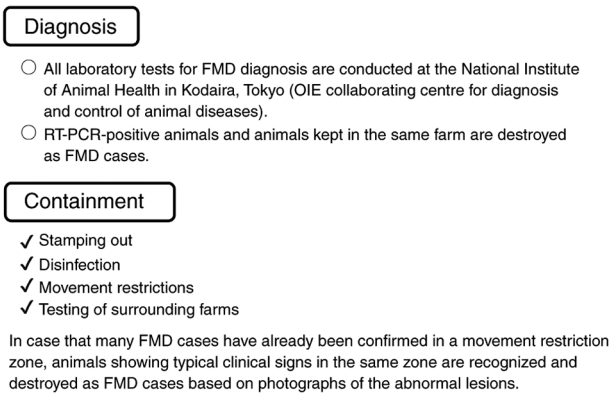

Figure 8. FMD control activities at the onset of an outbreak. FMD, foot-and-mouth disease; OIE, Office International des Epizooties; RT-PCR, reverse transcription-polymerase chain reaction.

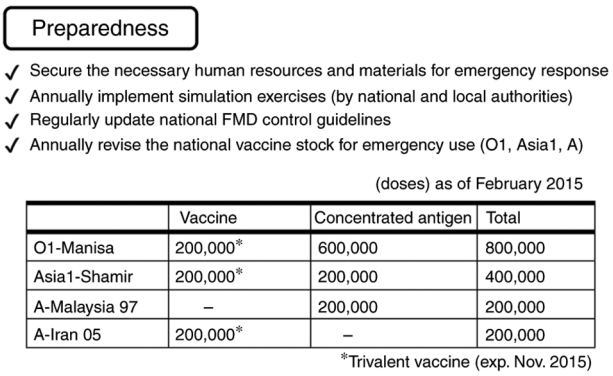

Figure 9. FMD control activities to prepare for an outbreak. FMD, foot-and-mouth disease.

T-1105 may be an important factor in enhancing the effects of FMD vaccination. A multilevel approach for disinfection and viral replication inhibitors is necessary to prepare for an FMD outbreak.

In addition, a 4% sodium carbonate solution and several commercial products are commonly used to observe the virucidal effect on FMDV. Occasionally, the effects of these disinfectants are reduced when they are mixed with organic materials. The effectiveness of surveillance and containment systems is a common concern for diseases transmitted by aerosols, such as FMD and COVID-19.

An epidemiological investigation of the 2010 FMD outbreak in Japan concluded that FMD was introduced by goods or people from countries affected by FMD in Asia. Local spread was presumed in areas highly affected by aerosols. To avoid the introduction and recurrence of FMD, various FMD control activities have been implemented. To address the aerosol transmission of FMDV, it is necessary to assess the environmental risks of using chlorite. Prompt diagnosis and containment are critical during the early stages of large-scale endemic disease outbreaks. Environmental epidemiological information is valuable in this age of global pandemics. The 'One Health' approach (60) should be shared between public health services, and the veterinary fields, with the aim that viral outbreaks, including those due to FMD, can be better managed

using social distancing, antiviral chemical compounds, environmental viral decontamination and quarantine.

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Authors' contributions

TO, AS and RK contributed to the conceptualization of the study. TO contributed to the writing of the original draft of the manuscript. TO, AS, KS, MH, KF and RK wrote, reviewed and edited the original draft of the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

KF was employed by the Mineral Activation Technical Research Center (Tamana, Japan). The remaining co-authors declare that they have no competing interests.

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