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Evaluation of the disinfectant concentration used on livestock facilities in Korea during dual outbreak of foot and mouth disease and high pathogenic avian influenza

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ABSTRACT

Background: A nationwide outbreak of foot-and-mouth disease (FMD) in South Korea caused massive economic losses in 2010. Since then, the Animal and Plant Quarantine Agency (QIA) has enhanced disinfection systems regarding livestock to prevent horizontal transmission of FMD and Avian influenza (AI). Although the amount of disinfectant used continues to increase, cases of FMD and AI have been occurring annually in Korea, except 2012 and 2013.
Objectives: This study measured the concentration of the disinfectant to determine why it failed to remove the horizontal transmission despite increased disinfectant use.
Methods: Surveys were conducted from February to May 2017, collecting 348 samples from disinfection systems. The samples were analyzed using the Standards of Animal Health Products analysis methods from QIA.
Results: Twenty-three facilities used inappropriate or non-approved disinfectants. Nearly all sampled livestock farms and facilities—93.9%—did not properly adjust the disinfectant concentration. The percentage using low concentrations, or where no effective substance was detected, was 46.9%. Furthermore, 13 samples from the official disinfection station did not use effective disinfectant, and—among 72 samples from the disinfection station—88.89% were considered inappropriate concentration, according to the foot-and-mouth disease virus guidelines; considering the AIV guideline, 73.61% were inappropriate concentrations. Inappropriate concentration samples on automatic (90.00%) and semi-automatic (90.90%) disinfection systems showed no significant difference from manual methods (88.24%). Despite this study being conducted during the crisis level, most disinfectants were used inappropriately.
Conclusions: This may partially explain why horizontal transmission of FMD and AI cannot be effectively prevented despite extensive disinfectant use.

Keywords: Disinfectants; concentration; South Korea; horizontal transmission

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Conflict of Interest
The authors have no conflicts of interest to declare.

Author Contributions
Conceptualization: Kim SJ, Chung HS, Myung D, Kim SG, Choe NH; Data curation: Kim SJ, Chung HS, Myung D, Kim SG, Choe NH; Formal analysis: Lee HS, Choi GH; Investigation: Htet SL; Jeong WS, Kim SG; Methodology: Kim SJ, Chung HS, Choe NH; Software: Myung D, Chung HS; Validation: Myung D, Chung HS; Writing - original draft: Kim SJ, Chung HS, Choe NH; Writing - review and editing: Kim SJ, Chung HS, Choe NH.

INTRODUCTION

Foot-and-mouth disease (FMD) and Avian influenza (AI) are highly contagious viral diseases affecting several species. FMD and AI have captured the attention of the international community over the years, with outbreaks in livestock having serious consequences on both livelihoods and international trade in many countries. Foot-and-mouth disease virus (FMDV) belongs to the family Picornaviridae and genus Aphthovirus, while the Avian influenza virus (AIV) is an animal disease pathogen of the genus influenza A virus of the Orthomyxoviridae. Livestock, contaminated fecal matter, contaminated equipment, wild animals, aerosol, workers, and vehicles can be transmitted FMDV and AIV [1-4]. The features of these diseases include high contagion, fast infectivity, and a sharp drop in productivity, which can cause enormous economic damage to livestock farms [5].

To control highly pathogenic livestock diseases, many countries set early-warning systems to monitor livestock and wild animals, as well as the status of pathogenic livestock disease worldwide. Moreover, many countries conduct farm surveillance, vaccinations, and official education to prevent livestock disease. Especially, vehicles are intensively monitored with movement certification because they travel through areas with a high risk of disease transmission that are associated with many outbreaks of FMDV.

The Animal and Plant Quarantine Agency (QIA) of Korea sets the Korea Animal Health Integrated System to provide the status of domestic animal diseases, information on disinfectants, location of disinfection facilities, recorded vehicle visiting times, and facility location, among others. Enforcing registration of all livestock-related facilities and vehicles makes this possible. Additionally, the official disinfection station, livestock facilities' disinfection system, and disinfection systems for livestock farms use chemical disinfectants to prevent horizontal transmission by vehicles.

Despite these efforts, FMD spread to 8 provinces, 66 cities, and 148 farms in 2010, killing approximately 3 million livestock. About 13.97 million chickens and ducks were buried due to AI between 2014 and 2015. This means that the disinfection system built to prevent horizontal transmission did not work properly.

To get effective disinfection into the field, it is necessary to understand the nature of the disinfectant and the environment in which it is used. Factors affecting the results of disinfection are temperature, disinfectant classification, pressure, organic matter, surface material type, potential hydrogen (PH), contact time, etc. [6,7]. The studies of novel chemical disinfectants and of its proper application have not been examined until recently. However, most studies focus on the development and validation of novel disinfectants, and there is no study on the environment in which disinfectants are used.

Therefore, the purpose of this study is to find problems within the domestic disinfection system by analyzing collected liquid disinfectants that were in use during FMD and AI outbreaks.

MATERIALS AND METHODS

Sample collecting

Samples were collected from the nozzles of disinfection stations located in livestock-related facilities—including farms, abattoirs, animal feed factories)—and the official disinfection station run by the Korean government. Brown media bottles (BT1060-1000, Korea Material Science Inc., Korea) were used. Liquids from disinfection station tanks were directly collected when samples could not be collected from a nozzle. In cases of official disinfection stations, samples were collected every 12 hours to find changes in concentration of disinfectant components over time. Samples were kept refrigerated until a microbiological examination is performed.

Period

Samples were collected from February to May 2017. Due to outbreaks of FMD and high pathogenic Avian influenza (HPAI), the government raised the animal disease alert to "severe" during that period.

Classification

Sample classification

Every disinfectant used in Korean farm and livestock facilities must obtain the approval of QIA by demonstrating effects of reducing pathogens by concentration. Disinfectants under testing were deemed efficacious if the virus titer was reduced by at least 10^4 (four logs) by the test. Samples were classified with AI, FMD, or both according to QIA approval. If a sample was unsuitable with the AI and FMD guidelines, they were classified as "uncertificated" or "certification canceled." Chemical ingredients of disinfectants analyzed in this study are listed in **Table 1**.

Disinfection-stations classification

Disinfection stations were classified with AI, FMD, or both. Types of disinfection stations were classified with an automatic, semi-automatic, or manual system. An automatic system routinely replenishes water and disinfectants. Semi-automatic systems only automatically replenish water, and manual systems replenish water and disinfectants by hand power. The concentrations of samples were classified into non-detective, low concentration, proper concentration, or overconcentration based on the type of disinfection station.

Analysis

Sample concentration analysis

The Korea Animal Health Products Research Institute examined 348 samples. The components and concentration analysis were conducted on 322 samples. The other samples

Table 1. The chemical ingredients of disinfectants analyzed in this study

Class	Ingredients	No. of disinfectants	Rate (%)*
Acid agents	Citric acid	180	65
	Malic acid	31	
Aldehydes	Glutaraldehydes	58	18
Oxidizing agents	Sodium hypochlorite	5	12
	Hydrogen peroxide	15	
	Sodium dichloroisocyanurate	18	
Surfactants	Quaternary ammonium chloride	15	5
Total		322	100

*Percentage of disinfectants to total disinfectants.

were labeled uncertificated, certification canceled, miswritten, or a combination of two disinfectants. Examined samples were classified into non-detective, low concentration, proper concentration, or overconcentration categories. Low concentration denotes a lower-than-recommended dilution factor. Overconcentration signifies the disinfectant was diluted more than twice the recommended amount. HPLC (1260 infinity 2 LC, Agilent technologies Inc., USA) and titrator (888 titrando, Metrohm, Swiss) are used. The HPLC method of citric and malic acid disinfectant samples employed a mobile phase of acetonitrile (ACN) and 20mM Sodium dihydrogen phosphate buffer (1:99) at 1.2 mL/min, a 4.6 × 150 mm zorbax SB-aq column with 5 µm packing, and UV detection at 210 nm. The HPLC method of glutaraldehyde disinfectant samples employed a mobile phase of 55% ACN and 2,4-dinitrophenylhydrazine (DNPH) at 1.5 mL/min, a 4.6 × 150 mm XTerra RP18 column with 5 µm packing, and UV detection at 358nm. In case of hydrogen peroxide disinfectant samples, 1 mL sample and 99 mL distilled water (DW) were mixed. 10ml of solution and 50 mL of sulfuric acid into a 100 mL conical flask, and add sufficient crushed ice to maintain the temperature below 10 deg-C during the first titration. 2–3 drops of ferroin indicator were added and samples were titrated with ceric sulfate (0.1M) to a pale blue color. 1ml of Sodium hypochlorite and sodium dichloroisocyanurate disinfectant samples and 100 mL of DW were into a 250 mL conical flask with stirring bar. 2 g of potassium iodide and 10 mL of acetic acid (6M) and 2–3 drops of starch indicator were added. Samples were titrated with standardized 0.1M sodium thiosulfate solution until the blue color just disappeared. 1ml of Quaternary ammonium chloride disinfectant samples and 75 mL of DW were into a 200 mL conical flask. Dilute hydrochloric acid were added to adjust the PH to 2.6–3.4. One drops of methyl orange indicator were added. Samples were titrated with 0.02M sodium tetraphenylborate solution until the pink color appeared.

Statistical analysis

All data was analyzed and classified using Microsoft Excel.

Ethics approval

This manuscript does not require Institutional Review Board/Institutional Animal Care and Use Committee approval because there are no human and animal participants.

RESULTS

Samples were collected from livestock farms, livestock facilities, and official disinfection stations (**Table 2**). All samples were examined except for those labeled as certification canceled, uncertificated, miswritten, and combination of two disinfectants. One parents stock farm, two broiler farms, three Korean cow farms, one dairy cow farm, 12 pig farms, and one abattoir were using certification canceled disinfectant. One broiler farm was using uncertificated disinfectant, and one abattoir was using two disinfectants together.

From all livestock farm and facilities' samples, 33 did not contain an effective disinfectant substance, 78 contained low concentration, 14 were of proper concentration, and 111 used an over-concentrated level (**Table 3**). Ninety-seven farms used an overconcentration of disinfectant, while only 13 farms used a proper concentration. Eighteen samples of livestock facilities were examined as having no effective disinfectant or low concentration, and only one used a proper concentration.

Table 2. The number of sites whose samples were collected from February to May 2017

Variables	No. of sites collecting sample	No. of sites using unexamined samples				No. of examined samples
		Certification cancelled	Uncertificated	Miss-written	Combination of two disinfectants	
Livestock farm						
Laying hens	7	0	0	0	0	7
Parents stock	7	1	0	0	0	6
Broiler	33	2	1	0	0	30
Korean native chicken	2	0	0	0	0	2
Duck	7	0	0	0	0	7
Korean cow (Han-woo)	57	3	0	0	0	54
Dairy cow	15	1	0	0	0	14
Pig	61	12	0	0	0	49
X*	48	1	2	1	0	44
Total (A) (%)	237	20 (8.44)	3 (1.26)	1 (0.42)	0	213 (89.87)
Livestock facility						
Abattoir	16	1	0	0	1	14
Chicken abattoir	6	0	0	0	0	6
Feed Manu-facture factory	4	0	0	0	0	4
Total (B) (%)	26	1 (3.85)	0	0	1 (3.85)	24 (92.31)
Official disinfection station						
C (%)	14	0	0	0	0	14 (100)
Total (A + B + C)	277	21 (7.58)	2 (0.72)	1 (0.36)	1 (0.36)	251 (90.61)

Sites are classified by facility type and the reasons for unexamined samples.
Concentration of each sample follows the Animal and Plant Quarantine Agency of Korea guidelines.
A, no effective disinfectant was detected; B, low concentration (< 1X); C, proper concentration (1X ≤ and < 2X); D, overconcentration (2X ≤).
X*, the farms raise more than two kinds of livestock.

Table 3. The concentration of effective disinfectant substance of each sample

Variables	No. of samples	Concentration			
		A	B	C	D
Livestock farm					
Laying hens	7	1	3	1	2
Parents stock farm	6	-	1	-	5
Broiler	30	4	9	4	13
Korean native chicken	2	-	2	-	-
Duck	7	2	3	1	1
Korean cow (Han-woo)	54	4	3	2	45
Dairy cow	14	3	1	1	9
Pig	49	5	19	3	22
X*	43	6	27	1	9
Livestock facility					
Abattoir	14	4	6	-	4
Chicken abattoir	6	3	2	1	-
Feed manufacture factory	4	1	2	-	1
Total (%)	236 (100)	33 (13.9)	78 (33.0)	14 (5.9)	111 (47.0)

Concentration of each sample follows the Animal and Plant Quarantine Agency of Korea guidelines.
A, no effective disinfectant was detected; B, low concentration (< 1X); C, proper concentration (1X ≤ and < 2X); D, overconcentration (2X ≤).
X*, the farms raise more than two kinds of livestock.

Samples of 14 official disinfection stations were examined (**Table 4**). Thirteen samples did not contain an effective disinfectant substance. The other 72 samples' concentrations were examined and classified according to the FMD and AIV guidelines of each disinfectant's manual. Sixty samples were labeled as over-concentrated, 8 contained proper concentration, and 4 contained low concentration according to FMD guidelines. Consulting AIV guidelines, 46 samples were considered over-concentrated, 19 contained proper concentration, and 7 contained low concentration.

Table 4. The concentration of effective disinfectant substance of official disinfection station

Disinfection station	Disinfectant substance		FMD guideline			AIV guideline		
	No detection	Detection	A	B	C	A	B	C
CN-H1	-	1	1	-	-	1	-	-
CN-B1	-	1	-	-	1	-	1	-
CN-Y1	-	1	1	-	-	1	-	-
CN-H2	-	1	-	-	1	1	-	-
CN-C1	2	8	2	3	3	4	4	-
CN-C2	5	-	-	-	-	-	-	-
CN-C3	5	-	-	-	-	-	-	-
CN-N1	-	10	-	2	8	-	7	3
CN-N2	-	10	-	3	7	-	4	6
CN-N3	-	10	-	-	10	-	-	10
CB-J1	1	-	-	-	-	-	-	-
CB-J2	-	10	-	-	10	-	3	7
CB-Y1	-	10	-	-	10	-	-	10
CB-B1	-	10	-	-	10	-	-	10
Total	13	72	4	8	60	7	19	46
%	15.29	84.71	5.56	11.11	83.33	9.72	26.39	63.89

Concentration of each sample follows the Animal and Plant Quarantine Agency of Korea guidelines.
FMD, foot-and-mouth disease; AIV, Avian influenza virus; A, no effective disinfectant was detected; B, low concentration (< 1X); C, proper concentration (1X ≤ and < 2X); D, over concentration (2X ≤).

Samples were collected 10 times at 12 h intervals from CN-N1, CN-N2, CN-N3, CB-J2, CB-Y1 and CB-B1 using same disinfectant and CN-C1 to examine changes in disinfectant concentration, according to time (**Fig. 1**). Disinfectant of CN-C1 has to maintain 400 mg/L for FMD and 666.67 mg/L for AIV, according to the disinfectant manual. Disinfectant of CN-N1, CN-N2, CN-N3, CB-J2, CB-Y1 and CB-B1 has to maintain 100 mg/L for FMD and 125 mg/L for AIV, according to the disinfectant manual. Disinfectant concentrations at CN-C1 were 0 mg/L (2 times), with low concentration (330–590 mg/L, 4 times) for the AIV guideline; for FMD guidelines, it was 0 mg/L (2 times), with low concentration (330 mg/L, 2 times) and overconcentration (890–990 mg/L, 3 times). Disinfectant concentrations at CN-N1 were over-concentrated according to both the AIV guideline (280–410 mg/L, 3 times) and FMD guideline (230–410 mg/L, 8 times). Disinfectant concentrations at CN-N2 were also over-concentrated for the AIV guideline (410–990 mg/L, 6 times) and FMD guideline (220–990 mg/L, 8 times).

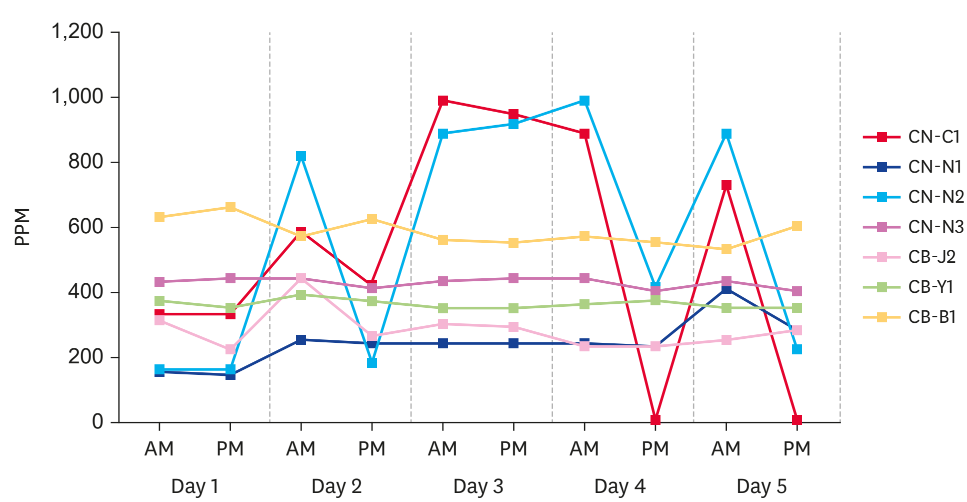

Fig. 1. Change in concentration of disinfectant used according to time in official disinfection station.

One-hundred samples were examined to determine the changes in concentration according to types of disinfection stations. Eighteen samples were not examined because of certification cancelation, being uncertificated, being miswritten, or the use of a combination of two disinfectants. 20 (24.39%), 11 (13.41%) and 51 samples (62.20%) were collected from automatic, semi-automatic and manual systems, respectively. In samples from automatic systems, 7 were labeled over-concentrated, 6 contained no effective disinfectant, and 5 contained low concentration. In samples from semi-automatic systems, 6 were labeled over-concentrated, one contained no effective disinfectant, and one contained low concentration. In case of samples from manual systems, 21 were labeled over-concentrated, 12 contained no effective disinfectant, and 12 contained low concentration. Ineffective samples comprised 55% of the automatic system testing, 47.06% of manual systems, and 36.36% of semi-automatic systems. Inappropriate samples comprised 90.91% of semi-automatic systems, 90% of automatic systems, and 88.24% of manual systems.

DISCUSSION

Since FMD spread nationwide in 2010, the Office International Des Epizooties (OIE) placed it on its List A, denoting it as a transmissible disease considered of socio-economic importance within some countries and having a significant impact on international trade for South Korea [8]. More than 5000 tons of disinfectants were used from September 2010 to May 2011 [9]. However, approximately 3.48 million animals (151,425 cattle, 3,318,299 pigs, 8,071 goats, and 2,728 deer) died and were disposed of at 4,583 burial sites [10].

More than 5000 tons of disinfectants were used in South Korea, but Sweden only used 602 tons of disinfectant for veterinary and cleaning activities in 2010 [9]. According to the Korea Animal Health Production Association, sales volume of animal disinfectants in Korea increased from 2.7 billion in 2016 to 3.3 billion in 2018. The amount of disinfectant use continues to increase compared to other countries, but cases of FMD and AI are occurring in Korea every year, except for 2012 and 2013.

Various factors that affected the action of disinfectant, to a greater or lesser degree, included organic matter, an environment's atmosphere and surface, temperature, and exposure time [11-13]. However, the most important thing is using disinfectants appropriate for valid pathogen. In this study, 23 places among livestock farms, livestock facilities, and official disinfection stations used inappropriate disinfectants (**Table 2**). Livestock farms have higher rates of inappropriate disinfectants than livestock facilities. Among them, 21 locations used certification canceled disinfectants. These results indicate that information about canceled disinfectants is lacking, and livestock farms are less informative than livestock facilities and official disinfection stations.

Livestock farms and facilities that did not properly adjust disinfectant concentrations were 93.9%, 46.9% used low concentration or no effective substance detected, and 47% were considered over-concentration (**Table 3**). Livestock facilities, such as abattoirs and feed-manufacturing factories, have high biosecurity levels, as the vehicle farm entrance directly intersects with the area where livestock gathers. However, only one of the 24 livestock facilities used a proper concentration of disinfectant. Disinfectant concentration is an important factor for antimicrobial activity. Using a lower concentration than the official recommended level may affect the disinfectant's efficacy. Based on the disinfectant concentration, study results on the titer reduction of porcine circovirus type 2 (PCV2) revealed the oxidizing agent comprised a 2.21 $\log_{10}$ reduction in titer at 1% (w/v), which is the official recommended concentration. However, it comprised a 1.15 $\log_{10}$ reduction at 0.5% and 0.16 $\log_{10}$ at 0.2%. Quaternary ammonium with aldehydes comprised a 1.74 $\log_{10}$ reduction in titer at 0.5% (w/v), which is also the official recommended concentration. However, it comprised a 0.55 $\log_{10}$ at 0.2% and 0.3 $\log_{10}$ at 0.1% [14]. Therefore 46.9% of livestock farms and facilities that used low concentration or no disinfectant were exposed to the dangers of horizontal transmission.

On the contrary, overconcentration causes serious disinfectant waste and irritability, which can cause damage to people and livestock. For example, glutaraldehyde—which was disseminated nationwide as disinfectant in 2010—is colorless and a strong skin, respiratory system, and eye stimulant [15]. A case of chemical pneumonitis caused by glutaraldehyde aspiration was reported in Korea [16].

The official disinfection station is an important factor in Korea's animal disease prevention system. It is managed and operated directly by the authorities, becoming a standard model for other disinfection facilities. Moreover, when the risk of livestock disease rises to the crisis level, all vehicles passing through livestock facilities and farms receive disinfections. Nevertheless, appropriate concentrations were not being used. In this study, 13 samples from the official disinfection station did not contain an effective disinfectant substance (**Table 4**). Among 72 samples, 88.89% were considered to contain an inappropriate concentration by FMDV guidelines, and 73.61% were considered to contain an inappropriate concentration according to the AIV guidelines.

Samples were collected from official disinfection stations at intervals of 12 hours to identify concentrations, and most were over-concentrated (**Fig. 1**). Over-concentrated levels of CN-N3, CB-Y1, and CB-B1 were being used over a five-day period. One hundred thirty-nine disinfectants can be applied to both FMD and AI, and their main substances include quaternary ammonium compounds, glutaraldehyde, formaldehyde, hydrogen peroxide, and didecyldimethylammonium chloride [17]. These substances are legally regulated against oral percutaneous inhalation toxicity as well as acute and chronic aquatic environment hazards by the Chemical Substance Management Act and are advised by the Health and Safety Corporation. Due to the characteristics of the official disinfection station, which houses laborers 24-hour a day and are closely located to natural environments, public health hazards are likely because of overconcentration.

The cause of inappropriate disinfectant concentrations was known to be a result of dilution form. However, inappropriate concentration samples on automatic (90.00%) and semi-automatic (90.90%) disinfection systems developed for the effective utilization of disinfectants also showed no significant difference from the manual methods (88.24%) (**Table 5**). Thus, the management of a disinfectant station, not its type, leads to inappropriate concentrations. The laborers currently employed to operate disinfection stations are non-experts, lacking knowledge of disinfection effects, appropriate concentration, and operation. The city government conducts education and inspection to compensate for this, but it does not appear to be enough. Expert management and inspection are required for the stable operation of disinfection stations. Another reason is the absence of indicators that can assess disinfectant concentration that are being sprayed. This requires further study.

Table 5. The concentration of samples classified with types of disinfection station

Variables	Automatic system				Semi-automatic system				Manual system			
	A	B	C	D	A	B	C	D	A	B	C	D
Laying hens	1	-	-	-	-	-	-	-	3	1	1	-
Broiler	1	-	2	3	1	1	-	1	4	2	4	1
Duck	1	-	-	-	-	-	-	-	-	-	-	1
Chicken abattoir	-	-	-	-	1	-	-	-	-	-	-	-
Korean cow (Han-woo)	-	-	-	1	-	-	-	-	5	1	-	3
Dairy cow	1	-	-	1	-	-	1	2	4	-	1	2
Pig	-	2	-	1	-	-	-	2	4	2	5	5
X*	-	-	-	-	-	-	-	-	1	-	-	-
Abattoir	1	1	-	-	-	-	-	1	-	-	1	-
Feed manufacture factory	1	2	-	1	-	1	-	-	-	-	-	-
Total (%)	6 (30)	5 (25)	2 (10)	7 (35)	2 (18.18)	2 (18.18)	1 (9.09)	6 (54.55)	12 (23.53)	12 (23.53)	6 (11.76)	21 (41.18)
Ineffective (%)	11 (55.00)				4 (36.36)				24 (47.06)			
Inappropriate (%)	18 (90.00)				10 (90.91)				45 (88.24)			

Concentration of each sample follows QIA of Korea guidelines. Automatic system replenishes water and disinfectant automatically. Semi-automatic system only automatically replenishes water. Manual system replenishes water and disinfectants by hand power. Ineffective, A+B; Inappropriate, A+B+D.
A, no effective disinfectant was detected; B, low concentration (< 1X); C, proper concentration (1X ≤ and < 2X); D, over concentration (2X ≤); X*, the farms raise more than two kinds of livestock.

The study was conducted when the crisis level of animal disease was raised to “severe” due to the outbreak of FMD and AI. To strengthen movement control during this level, the prevention headquarters installed by the Ministry of Agriculture, Food, and Rural Affairs was expanded to the pan-governmental central accident control headquarters and disinfection stations set on all major roads across the country. This was a longer-than-usual period of education and management. Therefore, the survey of disinfectants at the “severe” level could recognize the degree of effectiveness, and the usual stage of the disinfectant survey indicated whether prevention was conducted efficiently. Therefore, it is necessary to investigate the status of disinfectants when they are not in a serious level to determine if they are properly disinfected for preventing horizontal transmission. This study conducted on-site assessments of disinfection facilities that had not been previously performed in Korea, providing an explanation for the continuous occurrence of horizontal transmission despite the use of disinfectants. Furthermore, this study can act as support data for supplemental examinations following disinfection station set.

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