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A quadruple protection procedure for resuming pig production in small-scale ASFV-positive farms in China

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ABSTRACT

African swine fever (ASF) outbreak has caused serious economic losses in Asia since 2018. As ASF is a new emerging disease, many farmers hesitate to raise pigs before biosafety procedures were evaluated to be effective. To support small-scale farms in resuming pig production, a comprehensive procedure, called the quadruple protection procedure (QPP), was tested in 35 small farms which had been confirmed with African swine fever virus (ASFV). The QPP takes care of the farms' construction, environmental disinfection, regular immunization, and feed quality. Qualified daily management was supplemented as well. During a one-year survey four disinfectants and one piece of equipment were used in higher frequency. A 7- or 15-day empty period after the disinfection was suitable when it was combined with the rest of the protection measures from QPP. Totally 18,730 porkers and 3,006 sows were healthy by the end of the study with percentage of 100 and 98.8, respectively, indicating that QPP could protect pigs in small-scale farms from pathogens within China. This study developed an effective protective procedure system for small-scale farms to produce pigs under the risk of ASF outbreak.

1. Introduction

African swine fever (ASF) is a hemorrhagic disease with high infectivity and high mortality which not only causes huge economic losses, but also affects food safety and pig trade (Gallardo et al., 2015). In 2018, ASF broke out in China, subsequently sweeping many other Asian countries (Heilmann et al., 2020; Kim et al., 2020; Nga et al., 2020; Zhou et al., 2018). At the same time, the death of wild boars from ASFV infection in China were reported, which further increased the difficulty of eradicating ASFV (Li et al., 2019; Sanchez-Cordon et al., 2019). In addition, COVID-19 (Coronavirus disease 2019) has been slowing down various industries since 2019 December (Pan et al., 2020), affecting pig raising, pork production and supply transportations for those already hit hard by ASF (Gao et al., 2020; Kedkovid et al., 2020). Therefore, there is an urgent necessity to support societies by raising more pigs and producing more pork while fighting the epidemics.

Under the influence of ASF, it would be very important to support small-scale pig farms (which produce and sell pigs fewer than 10,000 per year) to successfully raise pigs, since small-scale farms not only produce a large portion of pork, but also play an important role in transmission of pathogens (Correia-Gomes et al., 2017; Riedel et al., 2012). Up to now, there is no vaccine and effective treatment available for dealing with the causative virus, ASFV (Galindo and Alonso, 2017). The only countermeasure is implementation of strict biosafety procedures in pig farms, such as on-site diagnosis, early detection, safe disposal of carcasses, avoidance of feeding with swill, disinfection of pig farm vehicles, and so on. (Danzetta et al., 2020; Lyra, 2006; Penrith et al., 2013)

ASF is a new disease in China. Many farmers hesitated to take the risk of raising pigs until biosafety measures are confirmed to be effective under the various weather and environmental conditions, which vary within thousands of kilometers from north to south of China. In this study, to establish suitable comprehensive guidelines for prevention and control of ASF in small-scale pig farms, a QPP will be tested in 35 ASFV positive farms which were selected from 9 provinces in China.

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No.	Disinfectant Or Equipment	Major Characteristic	Mostly Applied On
1	Sodium Hydroxide	Strong alkalinity	Floor, no-pig zone, waste system
2	Quicklime (calcium hydroxide)	Strong alkalinity	Floor, sole disinfection, no-pig zone
3	Flame Gun	High temperature	Floor, no-pig zone
4	Glutaraldehyde	Protein denaturation	Floor, pens, facilities, vehicles
5	Formalin	Protein denaturation	No-pig zone, fumigation
6	Glutaraldehyde Decyl Ammonium Bromide	Surface active agent	Floor, pens, spraying disinfection of indoor air, facilities, vehicles
7	Potassium Persulfate Compound Salt	Strong oxidation	Floor, spraying disinfection of indoor air
8	Chlorine Dioxide Effervescent Tablets	Strong oxidation	Floor, pens, drinking water, facilities, vehicles
9	Povidone Iodine	Strong oxidation	Floor, pens, drinking water, facilities, skin disinfection
10	Potassium Permanganate	Strong oxidation	Spraying disinfection of indoor air
11	Ozone	Strong oxidation	Fumigation of supplies
12	Sodium Trichloroisocyanurate	Strong oxidation	Floor, no-pig zone, waste system

B

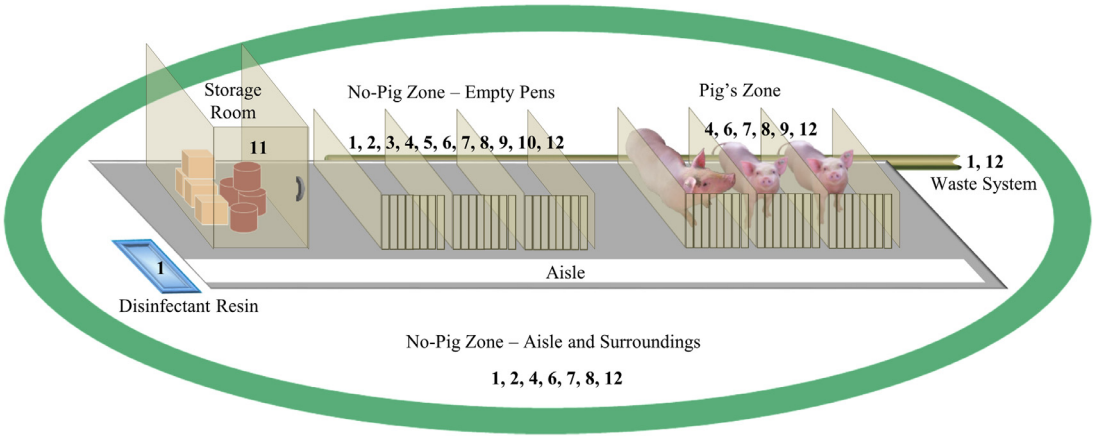


Fig. 1. The 12 D&Es and their usage in the farms. (A) The major characteristic of the D&Es and the places where they were applied at. (B) Model of a pig farm labeled with D&Es at the places where they could be used. The numbers correspond to the D&Es as listed in (A).

2. Materials and methods

2.1. Pig farms

35 pig farms that have been previously reported as ASFV-positive were selected from southern and eastern areas of China. Of these, 34 farms were left empty after the disease had occurred which will be used for testing QPP. There were 30 to 6000 pigs raised per farm. The remaining farm was set as a control to evaluate the effectiveness of the step of empty farms. The only difference between the testing farms and the control farm is that the control farm was disinfected by the owner who also raised 50 sows there before this study started, while the testing farms had no pigs. All of the farms will be treated the same way during this study using the same methods. All methods were performed in accordance with the relevant guidelines and regulations which have been approved by the ethics committee of animal experiments of Guangdong Haid Group Co., Ltd.

2.2. Disinfectants and equipment (D&E)

As shown in Fig. 1A, there were 12 D&Es which include chemicals and equipment. The chemicals were strong alkalis, surface active agents, or strong oxidants, etc., such as, 3% and 5% sodium hydroxide purchased from Cangzhou Zhongtai Chemical Products Co., Ltd., quicklime (calcium hydroxide) purchased from Gongyi Xingyuan Water Purification Material Co., Ltd., glutaraldehyde purchased from Hubei Xinjing New Materials Co., Ltd., formalin and potassium permanganate purchased from Chengdu Minsheng Disinfectant Co., Ltd., potassium persulfate compound salt, chlorine dioxide effervescent tablets, povidone iodine, glutaraldehyde decyl ammonium bromide solution, and sodium trichloroisocyanurate purchased from Guangzhou Hesheng Animal Pharmaceutical Co., Ltd. The equipment includes flame gun and ozone generator. Flame guns were purchased from Guangzhou Qinbao Animal Husbandry Equipment Co., Ltd. and ozone generators were purchased from Guangzhou Baifeng Environmental Protection Technology Co., Ltd.

2.3. The QPP

To reduce the risk of pathogen infection, QPP covers multiple aspects that may affect pig health, it includes protective wall construction, environmental disinfection, regular immunization, and standardized feed. The details will be explained shortly.

2.3.1. Construction of "protective walls"

According to publication (Fasina et al., 2012; Olesen et al., 2018), three types of "protective walls" were constructed to cut off the transmission of pathogens through multiple channels, including solid pen wall, mosquito net, and rat-proof wall. All of the pen walls including those between pens were made of cement. Mosquito nets covered the open windows to stop mosquitos and other flying insects from entering. Rat-proof walls surrounded the pens which were made with metal planks or with brick walls covered with ceramic tiles.

2.3.2. Procedure of disinfection

The places where the D&Es applied were illustrated in Fig. 1B. To prevent cross-contamination, the disinfection followed the order from the inside of pens to outside of pens, from the staff living area to the outside of the farms. The type of D&Es was selected according to the actual situation of the farms (Fig. 1A).

The whole process of disinfection can be divided into four sections. In brief, the first section was carried out in the empty farms. Disinfection

Table 1
Detection of viruses.

Viruses	Samples	Targets	Methods	Test kits produced by
ASFV	Blood or swabs of nasal, saliva, or of feeding environment	Virus gene	qPCR	Guangdong Haid Group Co., Ltd
CSFV	Blood	Erns protein	ELISA	IDEXX Laboratories, Inc.
PRRSV (highly pathogenic)	Blood	Virus gene	qPCR	IDEXX Laboratories, Inc.
PRV (wild type)	Blood	gpI antibody	ELISA	IDEXX Laboratories, Inc.
PEDV	Feces	Virus gene	qPCR	Guangdong Haid Group Co., Ltd

Table 2
The contents of major nutrients in the pig feeds.

Feed for	The Contents of Major Nutrients (percentage of weight)						
	Total Protein ≥	Crude Fiber ≤	Crude Ash ≤	Calcium	Total Phosphorus ≥	Salt	Lysine ≥
Suckling pig	19.0	5.0	8.0	0.6 to 1.3	0.35 to 1.00	0.30 to 0.80	1.45
Weaner	18.0	7.0	10.0	0.5 to 1.2	0.35 to 1.00	0.25 to 0.80	1.30
Young Pig	16.0	7.0	10.0	0.5 to 1.2	0.35 to 1.00	0.25 to 0.80	1.00
Adult Pig	15.0	8.0	10.0	0.5 to 1.2	0.35 to 1.00	0.25 to 0.80	0.90
Gilt	16.0	8.0	10.0	0.5 to 1.2	0.35 to 1.00	0.25 to 0.80	1.00
Pregnant Sow	13.0	10.0	12.0	0.5 to 1.2	0.35 to 1.00	0.25 to 0.80	0.70
Lactating Sow	14.0	10.0	12.0	0.5 to 1.2	0.35 to 1.00	0.25 to 0.80	0.70

was performed twice a day for a week at the pens (fences, exhaust fans, feed troughs, floors, etc.), staff living area (canteen, dormitory, etc.), the water dispensing system (drinking fountains, water towers, automatic material supply pipes, etc.), consumables transportation pipelines, stocking rooms, and waste system, etc. This was followed by collection of samples from feed troughs, the floor, waste system, and water dispensing system for testing of ASFV nucleic acid with the methods in Table 1. If the testing results were ASFV negative, the farms would be kept empty for at least one week. Then environmental ASFV contamination was tested again before starting to raise pigs. The virus detection information will be explained later. The pigs would be raised according to the rule of all-in-all-out.

The second section is the daily disinfection after the healthy pigs were put into the farms. As shown in Fig. 1B, low toxic chemicals, such as potassium persulfate compound salt, povidone iodine, etc. were used inside pens to spray into air, on floor and wall, or on pig skin, while strong alkali were used outside the pens.

The third section focused on the time of selling pigs. In brief, the pigs must be transferred one-way to the delivery vehicles through interchange stations, that is, once pigs are transferred out of the farm they cannot go back in. There was a “red line” surrounding the pig farms, people from each side of the “red line” were forbidden to walk to another side during transfer of pigs. All of the areas, facilities, as well as clothes that were involved must be fully disinfected during and after the selling process.

The last section is about daily management. All of the farms were kept with a comfortable feeding environment, real-time monitoring of humidity, temperature, and ventilation. The pigs entered and left each separated piggery at the same time through a one-way path. For staff, they were quarantined at the farm living quarters for 2 to 3 days if they came to the workplace first time. Then they worked at the piggeries by following the rules step by step: in brief, for entering, they stepped on the mat which was soaked with 2 to 5% sodium hydroxide solution at the entry door, then took baths and changed clothes, followed by disinfecting the hands and boots before entering the pens. To prevent cross contamination, staff normally were forbidden to walk across the different production areas. If they have to, they must change shoes and wash hands before walking across. When leaving the piggeries, they must wash hands and change and disinfect work clothes.

2.3.3. Virus detection

The ASFV nucleic acids test was performed in the feeding environment three times. The first test and second test was carried out before and after the first section of disinfection, respectively, in which the first test checked the virus contamination in the farms, and the second test confirmed that the virus has been removed by disinfection. The third time was done after the empty period to re-confirm that the ASFV had been completely cleaned for re-raising pigs. The test samples were collected by swabbing of the floor, feed trough, waste systems, and water dispensing systems. The gene of ASFV was tested by using qPCR (as shown in Table 1).

Before entering into the farms, the pigs needed to show negativity in five types of viral infections, such as ASFV, Classical Swine Fever Virus (CSFV), highly pathogenic Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), wild type Pseudorabies Virus (PRV), Porcine Epidemic Diarrhea Virus (PEDV). The test methods were listed in Table 1.

2.3.4. Comprehensive immunization program

As shown in Fig. 2A, the pigs were routinely vaccinated against infection of a total of 11 pathogenic microbes. They include 7 different vaccines for yearly vaccination of sows and extra 4 vaccines for brood sows before and after farrowing. Porkers would accept 4 different vaccines within 10 weeks after birth (Fig. 2B). The inactive PEDV vaccine was developed by Guangdong Haid Group Co., Ltd. and used in the control farm when the sows developed PEDV infection.

2.3.5. Standardized feedings

To standardize the nutrients, all of the feedings was provided by Guangdong Haid Group Co., Ltd. As summarized in Table 2, the percentage of the nutrients was adjusted according to the needs of the different types of pigs.

2.4. Data collection

A one-year follow-up survey was carried out from the beginning of February 2019 to the end of February 2020.

3. Results

3.1. Contamination of ASFV in the pig farms

As shown in Fig. 3A, the farms were located in 9 provinces of China, most of which come from Shandong, Guangdong, Jiangxi, and Jiangsu, respectively. Of these, 20 farms from 6 provinces were in the subtropical zone and 15 farms from Hebei, Henan, and Shandong, respectively, were in the temperate zone.

Before disinfection, the first test of ASFV nucleic acids by qPCR (as shown in Table 1 and Fig. 3B) indicated that all of the farms displayed

A

No.	Vaccine Against Infection With	Produced By
1	CSFV (C strain developed from tissue culture)	Boehringer Ingelheim
2	Circovirus (inactivated)	Harbin Harvac Biotechnology Co., Ltd.
3	PRRSV (attenuated)	Qilu Animal Health Products Co., Ltd.
4	Food-and-mouth disease virus (inactivated, trivalent)	Tecon Biological Co., Ltd.
5	PRV (attenuated)	Sichuan Hailinge Biological Pharmaceutical Co., Ltd.
6	Swine encephalitis virus (attenuated)	Wuhan Keqian Biology Co., Ltd.
7	Porcine transmissible gastroenteritis virus & PEDV (attenuated, bivalent)	Qilu Animal Health Products Co., Ltd.
8	Bacillus erysipelatos-suis (attenuated)	Harbin Harvac Biotechnology Co., Ltd.
9	Porcine parvovirus (inactivated)	Qilu Animal Health Products Co., Ltd.
10	Escherichia coli (inactivated, trivalent)	Qilu Animal Health Products Co., Ltd.
11	Mycoplasma hyopneumoniae (inactivated)	Qilu Animal Health Products Co., Ltd.

B

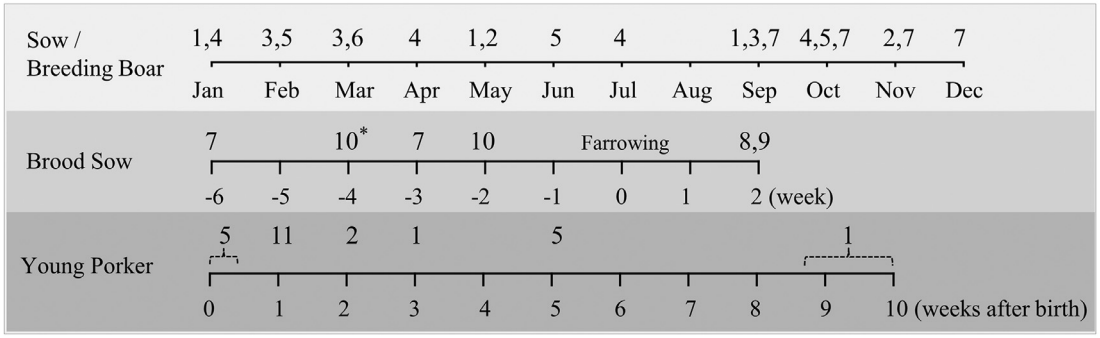
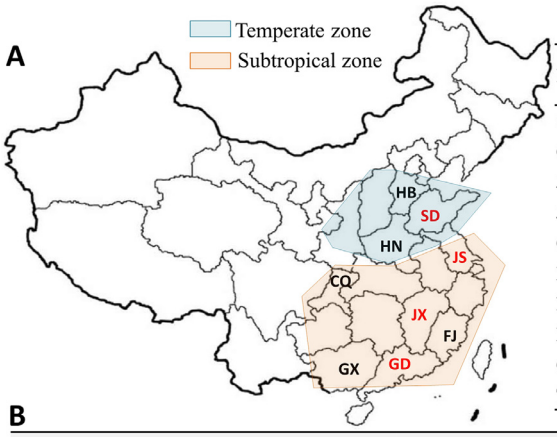


Fig. 2. Regular immunization procedure in the pig farms. (A) 11 vaccines were purchased from 6 different companies. (B) The procedure of the vaccination for the sows and porkers, respectively. The numbers above the axis correspond to the vaccines as listed in (A). * The primiparous sows were given an extra trivalent inactivated vaccine against E. coli infection at 4 weeks before birth of babies.

A



Province	Number of Farms
Shandong (SD)	13
Guangdong (GD)	7
Jiangxi (JX)	5
Jiangsu (JS)	4
Chongqing (CQ)	2
Fujian (FJ)	1
Hebei (HB)	1
Henan (HN)	1
Guangxi (GX)	1
Total	**35**

B

Testing Place	Tested Farms	Positive Farms	Positive Percentage (%)
Waste Systems	33	24	72.7
Feed Trough	28	19	67.9
Floor	34	19	55.9
Water Dispensing Systems	29	9	31.0

Fig. 3. Geographical distribution of the 35 pig farms in China and their ASFV contamination situation. (A) Farms were from 9 provinces, which were located in both the temperate zone and the subtropical zone. Four provinces that contributed more farms than the rest of the provinces are highlighted in red. (B) The contamination of ASFV was confirmed by detection of viral nucleic acid at four different places (as listed) in the farms. For every farm, at least one place tested positive. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

positive for the virus in at least one of the testing places. The contamination in waste systems and feed troughs were more serious as 72.7% and 67.9% were positive for virus nucleic acid, respectively. For the floor and water dispensing systems, 55.9% and 31% were positive, respectively, (Fig. 3B).

After disinfection, ASFV were re-tested twice in these farms, and there were no positive results reported again.

3.2. The D&Es and their combination applied in the farms

After a one-week disinfection, the effect of the disinfection procedure on empty farms was evaluated with ASFV nucleic acid testing (with the methods in Table 1). All of the samples collected from different places of the farms displayed negative results, which indicated that this group of the D&Es was suitable for disinfection of small-scale farms in China.

Table 3
The usage of the D&Es in the pig farms.

Index	Disinfection With	Concentration For No-Pig Zone	Concentration For Pig's Zone	Number of Farms Using	Usage Ratio (%)
1	***Sodium Hydroxide*** [a]	2–5%		***35***	***100***
2	***Quicklime (calcium hydroxide)***	10%–20%		***29***	***82.8***
3	***Flame Gun***	NA [b]		***26***	***74.3***
4	***Glutaraldehyde***	2%	0.5%	***27***	***77.1***
5	Formalin (formaldehyde)	35%–40%		4	11.4
6	Glutaraldehyde Decvl Ammonium Bromide	2%	0.5%	6	17.1
7	***Potassium Persulfate Compound Salt***	0.5‰	0.1‰	***27***	***77.1***
8	Chlorine Dioxide Effervescent Tablets	0.5‰	0.05‰	12	34.3
9	Povidone Iodine	2%–3%	1%	6	17.1
10	Potassium Permanganate	0.5%–1%		4	11.4
11	Ozone	0.35–0.45ppm		9	25.7
12	Sodium Trichloroisocyanurate	0.5‰	0.15‰	2	5.7

[a] The D&E which were set in bold and italic were used in more than 70% of the farms.
[b] Not applicable.

Table 4
The combination of the D&Es.

Combination (with indices) [a] Before and after raising	During raising – no-pig zone	During raising – pig's zone	Number of Farms Adopted
1 + 2 + 3 + 4	1 + 2 + 3 + 4	7	10
1 + 2 + 4 + 11	1 + 2 + 4 + 11	7	7
1 + 2 + 3 + 4	1 + 2 + 3 + 4	7 + 8 + 9	6
1 + 3 + 5 + 6 + 10	1 + 3 + 5 + 6 + 10	8	4
1 + 2 + 3 + 11	1 + 2 + 3 + 11	7 + 8	2
1 + 3 + 4	1 + 3 + 4	7 + 12	2
1 + 2 + 3 + 4	1 + 2 + 3 + 4	4	2
1 + 2 + 6	1 + 2 + 6	6	2
Total			35

[a] Indices correspond to those listed in Table 3.

As shown in Table 3, the most commonly used D&E was 2% to 5% sodium hydroxide; all of the farms used it for empty pens and environment disinfection. The second most commonly used was 10% to 20% quicklime (calcium hydroxide), having 29 of 35 farms using it. Flame gun, 2% glutaraldehyde, and 20% potassium persulfate compound salt were another three most commonly used D&Es compared with the rest of the D&Es. The least used D&E was sodium trichloroisocyanurate, as only 2 farms chose it. In addition, 6 D&Es which were glutaraldehyde, glutaraldehyde decvl ammonium bromide, potassium persulfate compound salt, chlorine dioxide effervescent tablets, povidone iodine, and sodium trichloroisocyanurate could be used at any place in the farms, but their concentration was 2–10 times lower for zones with pigs than no-pig zones.

During the entire pig raising period, multiple D&Es were applied in the farms. As shown in Table 4, at least three D&Es were used for disinfection of no-pig zones and at least one D&E was used in zones with pigs. A popular combination, 1 + 2 + 3 + 4, which includes sodium hydroxide, quicklime, flame gun, and glutaraldehyde, was adopted by 18 farms for before and after raising and for no-pig zones as well.

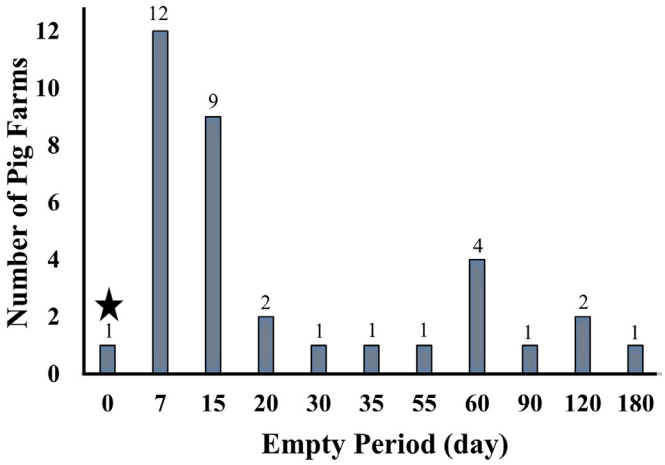


Fig. 4. The empty period after disinfection. The star indicates that there was no vacant period in the control farm.

3.3. The empty period

34 farms were left empty without any further cleaning procedure after the first section of the disinfection procedure finished. As shown in Fig. 4, 21 of 34 farms were kept empty for 7 and 15 days. The longest empty time was 180 days. The control farm didn't have empty time.

3.4. Successfully raising pigs during one year of observation

Among the 35 pig farms, 14 farms only raised porkers, 18 farms only raised sows, and the other 3 farms raised both at the same time. As summarized in Table 5, by the time of data collection, all of the 34 farms excepting the control farm had been raising pigs for more than 50 days, in which 9 farms kept their herds healthy more than 3 months (101–150 days) and 12 farms kept the herds healthy more than 5 months (151–200 days). This leads to a total of 10,280 porkers and sows successfully sold from 9 farms, 205 sows from 5 farms farrowed, and 8650 porkers and 2806 sows still raising in 20 farms.

Sows from control farms presented health problems, such as loss of appetite, diarrhea, vomiting etc. at the beginning of the feeding. PEDV was detected by qPCR in feces swabs. Then the sick sows were removed from the farms, and the rest of the sows were immediately injected with an inactive PEDV vaccine. One of tested farms reported ASFV infection again after 111 days of feeding, which resulted in termination of the feeding immediately. The survival rate of porkers at all farms was 100%, and of sows, 98.8%.

Table 5
Results of raising pigs.

Raising time (day)	Number of pig farms	Number of porkers	Number of sows
0 to 50	0	0	0
51 to100	8	1160	392
101 to 150	9	6700	136 [a]
151 to 200	12	10,800	1958 [b]
201 to 250	4	0	355
251 to 300	1	70	0
301 to 350	1	0	200
Mature to sale	9	10,080	200
Farrowing	5		205
Still in raising	20	8650	2806
Termination	1 [a]		
Survival rate		100%	98.8%
Total	35	18,730	3041

[a] 28 sows from one farm were infected with ASFV after 111 days of feeding, and the feeding was immediately terminated.

[b] 7 sows from the control farm were sick and removed at the early stage of the raising period, the rest of pigs were health by the time of collection of data.

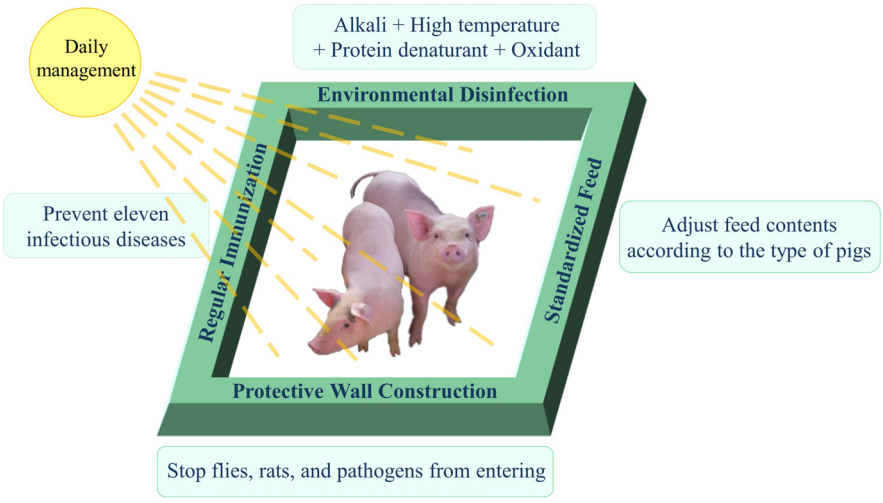


Fig. 5. Illustration of the QPP. The pigs were protected by four different procedures (labeled on the green walls). The summary of each procedure is, respectively, listed in the light blue rectangles. As a necessary supplementary procedure, daily management (the yellow circle) covers the entire raising period to timely monitor the health status of the pigs and maintain the biological safety of the environment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Usually the large-scale pig farms are equipped with high quality facilities and perform comprehensive biosafety procedures to maintain a pathogen-free environment. However, the small-scale pig farms in China scatter across the country and their constructions and facilities are varying (Costard et al., 2015; Riedel et al., 2012; Zhang et al., 2017). Thus it is hard to use one standard procedure to manage different farms. Moreover, as temperature and humidity are involved in pathogen transmission (Lowen et al., 2007; Wilkinson and Donaldson, 1977), different local weather from the north to south of China would bring more challenges into the operation of small-scale farms. As illustrated in Fig. 5, in this study, QPP supplemented with daily management almost covers every aspect in managing the farms. Of the four procedures, environmental disinfection has to be carried out in various ways to adapt to different situations.

For disinfection of pig farms, many chemicals and equipment are available. They destroy pathogens by denaturing their proteins or destroying their structures. ASFV is a double layer enveloped virus. It can tolerate pH of 3.9 to 11.5 but are sensitive to very strong acids and bases. It also can be inactivated by lipid solvents and chloroform, etc. (De Lorenzi et al., 2020) The recommended chemicals are sodium hydroxide, sodium hypochlorite, formalin, o-phenylphenol and iodine compounds (Juszkiewicz et al., 2019; Shirai et al., 2000). In this study, 2% to 5% caustic soda (sodium hydroxide) and quicklime (calcium hydroxide) were used most frequently. Quicklime not only has the function of sterilization, but also has strong hygroscopicity which can inhibit microorganism growth by decreasing the humidity of the environment (Gehring et al., 2020; Herbst, 2000).

Glutaraldehyde was widely used in this study because of its characteristics including high efficiency in inactivation of microorganisms, low toxicity to animals, low corrosivity of metal facilities, and stability (Lin et al., 2018). It is an ideal reagent for sterilization of the breeding facilities and precision instruments. Another popular disinfectant is potassium persulfate complex salt, which is a good product for waterline disinfection and fumigation. Its function results from the ability to oxidize oxygen ions with reduced valence state to oxygen. Chlorine then dissolves in water to produce hydrochloric acid and hypochloric acid, which will kill the pathogens in cooperation with potassium hydrogen sulfate (Ghanizadeh et al., 2015; Sonthipet et al., 2018). Beside of the chemicals, it is also recommended to apply flame to the surface of the installations, equipment, wall, and floor (Delhalle et al., 2008).

In addition, it is should be noted that heavy use of chemicals in farms may increase the difficulties in treatment of manure and wastewater (Vazquez et al., 2018). Therefore it is necessary to consider other prevention and control measures simultaneously.

One of the supplementary measures for disinfection is vacancy. It is generally suggested that the pig farms should be fully disinfected and laid idle for one month, after which sentinel pigs would be kept there for one to two months before officially raising pigs (Technical guidelines; Chinese Academy of Agricultural Sciences, n.d.). In this study, 7 or 15 days of vacancy time was acceptable when it was combined with other

measures from QPP. However, pigs from the control farm developed clinical symptoms of diarrhea and vomiting at the early stage and was diagnosed with porcine epidemic diarrhea virus. This result emphasized that it is important to do complete disinfection in farms without pigs, then leave the farms empty for one to two weeks before bringing healthy pigs in.

The incubation period of ASFV in pigs is 3 to 21 days (Animal and Plant Health Inspection Service, 2020). In this study, we would assume a farm was ASFV free if this farm demonstrated no clinical symptoms and etiology surveillance results were negative for 60 days after breeding pigs. If a positive case of ASF is reported again after 60 days, it would indicate that there were loopholes in either the daily management or the biosafety prevention and control system. During this study, the pigs would be qualified for selling or farrowing by testing for ASFV gene in the specimen of feces or saliva. After more than 60 days of raising, in total 10,080 porkers and 200 sows were sold with negative results in ASFV gene tests, and 205 sows farrowed without the virus gene in their specimen as well. In addition, the virus gene was also undetectable in the specimen of the rest of pigs before this article was submitted. However, there was one farm which reported ASF-like clinical symptoms after 111 days of feeding. Nasal/oral swab testing of virus nucleic acid also verified ASFV infection. Which indicated that even though the environment disinfection procedure was qualified, daily management needed to improve further (Fig. 5) to oversee the farms in real-time, in ways like more frequently training employees with standard biosafety protocol and maintaining protective walls regularly.

In addition, keeping herds healthy also depends on prevention of common diseases such as classical swine fever, pseudorabies and foot-and-mouth disease, etc. using a comprehensive vaccination program (Postma et al., 2016). At the same time, using good feed is also very important for keeping the swine immune system functioning optimally (Rakhshandeh et al., 2012). Meanwhile, the feed should meet nutritional requirements of different types of pigs.

5. Conclusion

As ASFV is still circulating in China, it is urgently necessary to set up a comprehensive procedure for protection of small-scale pig farms. The QPP in this study started with the construction of protective walls followed by complete inactivation and disinfection of empty farms until the ASFV nucleic acid tested negative. Then a reasonably long empty period was set up before bringing healthy pigs in. Thereafter it was combined with daily biosecurity management, regular vaccination, and qualified feeding. The results demonstrated that almost all of the pigs were kept healthy during the one-year study. As such, QPP provides a method to small-scale pig farms throughout the country for raising pigs under the influence of ASF.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Lang Tian: Writing - original draft, Methodology, Validation, Formal analysis, Investigation. **Yilin Luo:** Methodology, Validation, Formal analysis. **Tanqing Wen:** Methodology, Validation, Formal analysis. **Weizheng Yang:** Methodology, Validation, Formal analysis. **Yulin Zhao:** Methodology, Validation, Formal analysis. **Pan Huang:** Methodology, Validation, Formal analysis. **Hongbo He:** Conceptualization, Investigation. **Jianmin Wu:** Resources, Data curation. **Zhongsheng Li:** Resources, Data curation. **Chungen Pan:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Project administration.

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Ethical Statement and consent of owners

This study was approved by the ethics committee of animal experiments of Guangdong Haid Group Co., Ltd., and performed with the informed consent from the owners of the pig farms.

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