Successful Depopulation and Reopening of an Isolation Facility

within a Large Production Compound without Allowing ASFV Spread to the Main Breeding Herd

Onset of ASFV, Diagnosis and Disease Progression
The isolation room as described in this paper was inside the same production compound, 54 metres away from the B/G area of the main herd, separated by a road. This facility was installed with air filtration (HEPA) and was fenced to create a separation from the main herd and constructed to receive replacement pigs. The details of biosecurity had been planned and implemented for the isolation assuring a biosecure transfer of genetics after isolation monitoring procedures were performed.

Millions of years ago when life began on earth, numerous species emerged and co-evolved sharing earth's resources and habitat. *Homo sapiens*, which evolved as the most intelligent species, dominated and exploited the major share of resources pushing the other species towards extinction or (at the very least) struggling to thrive. Humans expanded their habitat, croplands and livestock into the forests, which disrupted the natural ecosystem and thus ruined the harmonious coexistence with other wild species. Meanwhile, broken barriers by close human-animal interfaces enabled the interspecies transmission of pathogens to distant and diverse species. Incidences of novel pathogen emergence by animal to human transmissions and the extermination of millions of humans from Earth have been witnessed on various occasions. Outbreaks from emerging infectious diseases have been reported to increase every decade since the 1980s and most of them have been linked to wildlife.

Pens inside the isolation room

The delivery of 176 boars from source X and 352 replacements from source C were moved into the isolation starting on December 20th, 2019 with the C source shipment, and the X shipment arrived on Dec 21st.

On Dec 23rd, a single pig from X started to show watery diarrhoea.

Watery diarrhoea on Dec 26th, 2019

On Dec 26th, most of the pigs in the isolation began showing watery diarrhoea. Rapid tests indicated positive for PEDV. Population exposure was practised with watery faeces spread to all pens to ensure simultaneous exposure/infection. A PEDV elimination programme was instituted and additional biosecurity measures were put in place to avoid leakage and/or people tracking from the isolation barn to the resident pigs in the main herd.

On Dec 25th, the first pig died, and on 27th, the second. Both pigs were sudden deaths without any clinical signs. Nasal swabs were collected from dead pigs for lab ASFV diagnosis.

On Dec 27th, all the roads in the farm and surroundings of the isolation room were covered with dry lime, to avoid potential spread of the diseases by the fomites. Dry hydrated lime is frequently used in China as a disinfectant to curb contamination because of movements of pigs, trucks and materials in the farm environment.

Oral swabs from the pigs in the pens, and 300 serum samples and 60 rectal swabs were collected for diagnosis of PEDV (faecal samples) and ASFV and PRRSV (serum samples).

On Dec 29th, a third death occurred.

By Dec 30th, all isolation pigs had shown diarrhoea signs.

Pen No.	Boars C	Boars C	18 C	17 C	16 C	15 C	14 C	13 X	12 X	11 X
Pen No.	1 C	2 C	3 C	4 C	5 C	6 C	7 X	8 X	9 X	10 X

Note: C: C source X: X source

Layout of the pens inside the isolation barn

Dead pig seen on Dec 29th

On Dec 31st, the fourth pig died without clinical signs, and by then most pigs had stopped scouring and were recovering, raising suspicion that another agent may be present.

The PEDV sequencing on Jan 6th indicated a different genetic strain compared with previous sequencing at source farm C and another farm having a pig source linkage to C, pointing to a likely contamination during transport or at the isolation facility.

On Jan 1st, 2020, the fifth pig died from apparent stress, without clear clinical signs.

On Jan 3rd, the test results (collected on December 27th and 28th, sent on 29th) came back, indicating PEDV positive, and TGE and porcine delta coronavirus negative. Testing of 300 serum samples via RT-PCR PRRSV indicated the pigs were PRRSV negative.

However, eight of 107 pooled oral fluids swabs were positive for ASFV with the IDEXX kit RT-PCR, but the positive samples are negative when tested on a local Chinese manufacturer ASFV RT-PCR test. CT values were close to the critical CT value of 37, possibly indicating the samples were negative.

On Jan 4th, it was decided to collect serum samples from all pigs in the pens that were tested ASFV positive using a different test kit by Thermo Fisher on each individual pig from the positive pool samples.

On Jan 12th, the results came back from a different laboratory indicating negative results for ASFV (Thermo Fisher RT-PCR test kits).

On Jan 5th, another pig died, and rectal body temperatures of 10 pigs in the same pen were randomly taken to see if there were any fevers, but no feverish pigs were found.

On this same day, a boar was observed with black faeces and paleness, and on Jan 7th, it died.

On Jan 8th, a gilt died from flatulence.

A boar died with black faeces

On Jan 9th, most of the pigs in the 9th pen started to show signs of inappetence, lethargy, red skin, and high temperature (41°C–41.5°C). Five whole blood samples from affected pigs were collected and sent to a private lab. These pigs from pen 9 came from X farm. The sick pigs in this pen were treated with ceftiofur and flunixin meglumine with no clinical improvement.

Pigs showing reduced feed intake, red skin and lethargy

On Jan 10th, pigs in the neighbouring pen 10 began to show fever. One pig had a body temperature measured at 41.3°C.

On Jan 11th, four pigs in pen 9 died.

On Jan 12th, six pigs in pen 9 died, and two were seen vomiting with blood. Some pigs in pen 8 started to show skin discoloration and off-feed with high body temperature.

On Jan 12th, the private lab results showed that the five whole blood samples collected from pen 9 were positive for ASFV (Thermo Fisher: CT 22; IDEXX: CT 18).

On Jan 13th, the team decided that this was an ASFV infection and an outbreak, and determined to depopulate the isolation herd. A plan was developed to prepare for depopulation. Details were discussed to ensure biosecure logistics and supplies needed to humanely euthanise the pigs, and disposal could be performed in a manner preventing contamination of the adjacent breeding herd.

On Jan 13th, the pigs from pens 7 and 8, and more pigs in 9 and 10, began to show clinical signs. Five from pen 9 died, and five from pen 8 died.

On Jan 14th, one from pen 10 died, and 10 from pen 9 died.

By this time the disease was progressing quickly and escalating after an inapparent period of transmission in the isolation.

Depopulation of the Isolation Herd
Since Jan 13th, the team worked to find a burial place to dispose these pigs, and one site was found.

On Jan 15th, all the pigs in the isolation barn were euthanised.

On Jan 16th in the evening, the pigs were moved out of the facility in a biosecure way to avoid leakage.

- Trucks entering the farm grounds were washed and disinfected before getting close to the loading gate of the isolation room;
- Interior of carriage of trucks were covered with impermeable plastic film;
- The roads inside the facility grounds were paved with dry lime;
- A burying site at a different location 4 km away from the farm was selected;
- A minimum of 2 metres of soil on top of the disposed carcasses was required.

Cleaning and Decontamination (C&D) of the Isolation Facility Post Removal of the Pigs
After the depopulation was completed on Jan 17th, biosecurity work was initiated to thoroughly clean and disinfect the facility and the environment, and final inspection of the isolation occurred on May 24th. At this time the isolation was determined to be ready to place sentinel pigs as a final step before reopening the isolation.

Trucks hauling the euthanised pigs

Site cleanup standards and priorities:

- No organic matter inside and outside the isolation barn;
- Ensured that the isolation barn was strictly separated from the main compound;
- Covered all the roads linked to the isolation facility and all the roads inside/outside the main compound with dry hydrated lime to minimise contamination;
- Ensured that the pits were soaked with caustic soda to get the pH higher than 13.5, and kept it for a minimum of two weeks before cleaning and disinfection of the pits;
- All the disposables supplies were being disposed, and equipment being dismantled and thoroughly washed and disinfected;
- Filters dismantled and replaced with new;
- Adopted a multiple-step cleaning, washing and disinfection approach; use multiple methods such as drying, flaming, pit treatments, and fumigation at different points of time to ensure effectiveness.

Emergency Response Plan and Communication
An action plan was formulated for the decontamination and repopulation of the isolation facility, targeting complete cleaning and decontamination (C&D, several rounds). Details of biosecurity were discussed for actions, and work progress were reported and communicated daily. An action plan to monitor the health status of the pigs in the main herd was formulated and implemented.

This response plan had five main pillars:

- Define who should be in the communication and the decision-making loop;
- Complete cleaning and decontamination of the isolation room and surrounding area; to eliminate/minimise residual ASFV virus in the environment;

Examples of cleaning and decontamination process

- Avoid leakage and spread of virus to the main herd beyond the separation road/fence;
- Measure the effectiveness of all procedures as best as the team could, to demonstrate measurable results and assure confidence in re-stocking the isolation facility;
- Ensure the virus would not be re-introduced to the isolation after it is thoroughly decontaminated, and after environmental testing assured the facility was ASFV negative before introducing sentinels. Monitor sentinels as the last step of assurance that the facility was no longer contaminated with ASFV.

Investigation of Source of Contamination:
The investigation pointed to these potential sources of contamination:

Potential contamination of a hauling truck from source X either because of non-effective decontamination or while routing to the destination farm. As suspicious clinical signs first appeared in pen 9, 10, 8, 7 etc., all sourced from farm X, the team had a legitimate reason to speculate that the farm X delivery truck was the source of infection.

Source X had no clinical or diagnostic indication that the pigs were infected at the source farm before shipment and remained unaffected at the time of this investigation and later, so the source farm was ruled out as a potential source of the virus.

Prior to this shipment, this truck had hauled pigs from X to another herd owned by clients on Dec 4th, then washed in an outside washing site. A second wash and disinfection were done after returning to the X washing facility (or truck wash facility) on Dec 5th. It was very cold in the north China winter, and in X truck wash facility, freezing and icing was an obstacle for thorough washing and cleaning, and the site was not equipped with a heated drying facility. On Dec 18th, prior to shipping pigs to the isolation facility, the truck carriage was covered with a tarpaulin and heated by blowing/flowing hot air under the tarpaulin at 20°C with a forced air heater. In retrospect, the team had good reason to believe that effectiveness of the truck wash procedures against ASFV was doubtful.

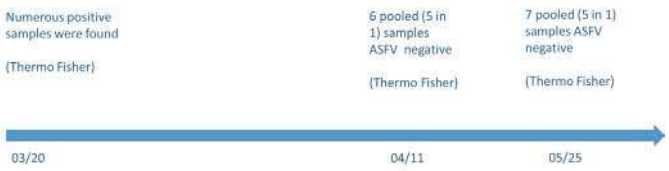
In addition, for this long-distance haul, the contamination could have occurred during the trip because it had to park in resting areas five or six times, and at toll gates where the cashiers had to check and take photos, which added biosecurity risks.

In the investigation, it was found that some potential tracking mistakes were also made during the loading process.

However, the slowness of the development of disease prompted some questions. The breeding herd feedback exposure to the isolation was done on Dec 26th soon after arrival, due to containment of PEDV added complexity as this approach might spread the virus, if there was contamination, from some single infected individuals to others. Likelihood of other contamination sources exist, such as inadequate decontamination of the barn itself, or because of contamination from people or supplies, such as contaminated cell phones owned and brought in by farm staff, or from people not properly showered in to the isolation room.

Surveillance, Sentinels and Re-introduction into the Isolation Facility
As the cleaning and decontamination were underway, on March 20th, April 11th, May 25th, sets of environmental samples from various surfaces, including corners, equipment, cooling cells, filters, and pits were collected and tested for the presence of ASFV nucleic acids. Some samples collected on March 20th were found PCR ASFV positive but subsequent samplings all indicated negative for ASFV.

Introduction of Sentinel Pigs
The isolation barn was prepared, thoroughly foamed, washed, disinfected and flamed, then closed to people traffic for three days, before receiving the sentinel pigs.

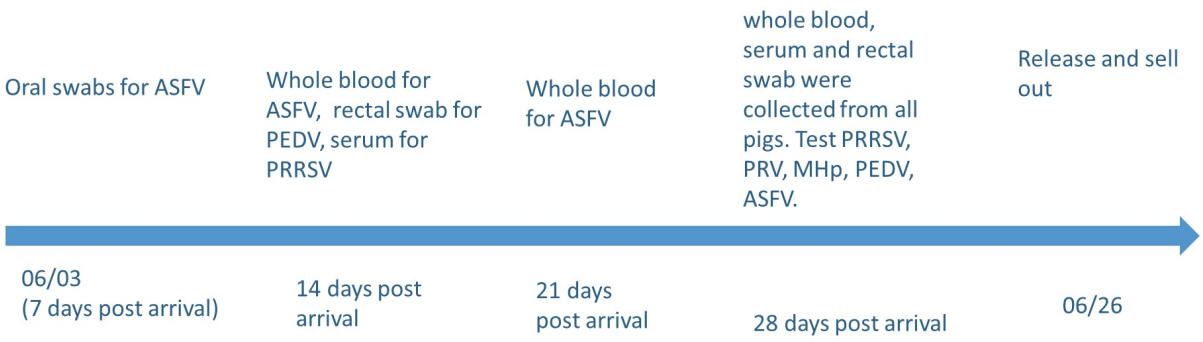


The surveillance of environmental samples for presence of ASFV nucleic acids

On May 26th, 20 sentinel pigs were introduced into the isolation barn and allowed complete access to all parts/surfaces of the facility. Oral swab samples were collected at seven days post arrival to test for ASFV, and whole blood and rectal swabs were collected at 14 days, 21 and 28 days post arrival to test for the presence of ASFV, PEDV and PRRSV. No positives were found, and sentinel pigs were released for slaughter sales on June 26th.

Repopulation
It was decided that the barn was safe to repopulate after sentinel pigs were not showing clinical signs and were tested free of diseases of concern, and thus it was prepared to re-stock. On July 2nd, one day prior to re-stock, the barn was thoroughly flamed as a final biosecurity assurance step.

On July 3rd, 500 gilts were introduced into this isolation facility, and monitored for ASFV before releasing them into



The surveillance of ASFV for sentinels

Flaming the barn

the main herd in mid-August. At the time of completion of this paper, six months after introducing the pigs into the main herd, there has been no evidence of ASFV infections in the farm.

Repopulation

Conclusions

This case has demonstrated an unusual progression of ASFV infection in a population of naive replacement stock in an isolation facility. Early detection by accurate diagnosis was a big challenge, as initially there were no clear clinical signs pointing to ASFV infection, and diagnostic kits and laboratories gave inconsistent results.

Via considerable efforts on biosecurity, the team was able to prevent the spread of the virus to the main herd, which was only 54 metres away, and was able to decontaminate then successfully reintroduce replacement pigs in the isolation barn and into the breeding herd six months after a diagnosis of ASFV was first established.

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