Detection and Control of Low Pathogenic ASFV

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It is difficult to explain ASF in recent years.

- **2018:** the disease entered into China, posing a challenge causing industrial panic
- **2019:** continuous pandemic, bio-safety, tooth extraction for self-rescue
- **2020:** soaring pig prices, fake vaccine influx, resume production to guarantee supply
- **2021:** giant expnsion, capital boost, plunge in pig prices
- **2022:** rampant variants, disillusioned vaccine dream, reverse pig prices
- **2023:** recurrent pandemic? rising pig prices? eliminating ASF?

ASF did not go far.

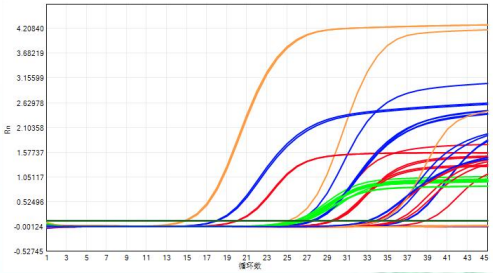
- **Genotype:** type II > type I > mixed type > recombinant type
- **Strain Diversity:** wild strain, artificial deletion strain, recombinant strain and natural variant
- **Pathogenicity:** high pathogenicity, moderate pathogenicity and low pathogenicity
- **Prevalence range:** local transmission and regional epidemic

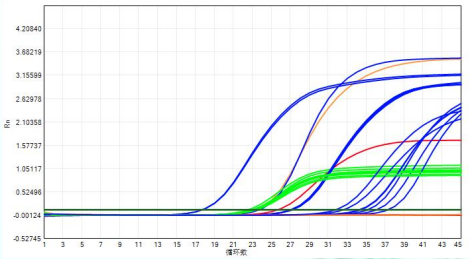
Attenuated ASFV causing a mess.

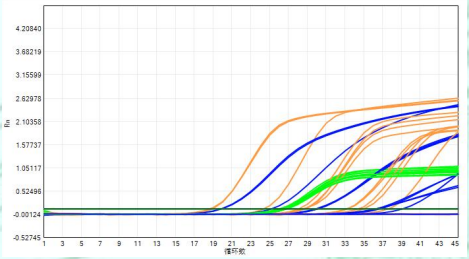
- **strain type:** type II (main), type I and recombinant type
- **deleting gene:** CD2v、MGF、I177L and combinations thereof
- **possible mark:** green and/or red fluorescent protein or none

The detection rate of type I ASFV increased significantly in 2023.

sample type	sample number	P72/MGF/CD2v conventional triplet					P72/eGFP/mCherry mark triplet					type I and type II			
		P72	internal mark	MGF	CD2v	results	P72	internal mark	MGF	CD2v	results	type II	internal mark	type I	results
		FAM	HEX	ROX	Cy5		FAM	HEX	ROX	Cy5		FAM	HEX	ROX	
swab	8-C14	33.02	26.74	34.21	33.86	wild strain?	31.71	23.29	NoCt	NoCt	wild strain	36.26	26.78	33.16	type I
		33.10	26.63	NoCt	33.81		32.54	23.40	NoCt	NoCt		36.29	26.72	33.16	
	30-45	NoCt	26.41	NoCt	NoCt	negative	37.24	23.12	NoCt	NoCt	positive	39.07	26.65	37.50	suspected
		NoCt	26.44	NoCt	NoCt		NoCt	23.12	NoCt	NoCt		NoCt	26.60	NoCt	
	8-C12	36.63	26.34	NoCt	NoCt	MGF variation	35.13	23.08	NoCt	NoCt	wild strain	NoCt	26.64	NoCt	negative
		36.56	26.29	NoCt	38.74		36.08	23.14	NoCt	NoCt		NoCt	26.71	NoCt	
	31-39	27.56	26.35	NoCt	29.56	MGF variation	27.14	23.16	NoCt	NoCt	wild strain	29.82	26.62	28.06	type I
		27.63	26.25	NoCt	29.61		27.18	23.12	NoCt	NoCt		30.04	26.55	28.53	
	8-D3	33.77	26.30	NoCt	35.70	MGF variation	34.24	23.17	NoCt	NoCt	wild strain	36.25	26.57	34.32	type I
		34.32	26.28	NoCt	36.17		34.24	23.15	NoCt	NoCt		36.65	26.51	34.92	
blood sample	3-4-5	27.44	26.31	NoCt	29.33	MGF variation	27.03	23.13	NoCt	NoCt	wild strain	30.07	26.63	28.70	type I
		27.43	26.32	NoCt	29.46		27.06	23.11	NoCt	NoCt		30.02	26.70	28.53	
	zhuangzi	18.04	25.64	14.92	20.07	wild strain	17.67	23.05	NoCt	NoCt	wild strain	19.75	26.02	18.85	type I
		17.88	25.71	14.67	19.99		17.64	23.01	NoCt	NoCt		19.85	26.04	18.56	
negative control		NoCt	25.73	NoCt	NoCt	/	NoCt	22.49	NoCt	NoCt	/	NoCt	26.11	NoCt	/
positive control		26.05	27.30	25.11	27.49	/	23.67	23.08	23.29	25.62	/	25.14	26.52	24.31	/





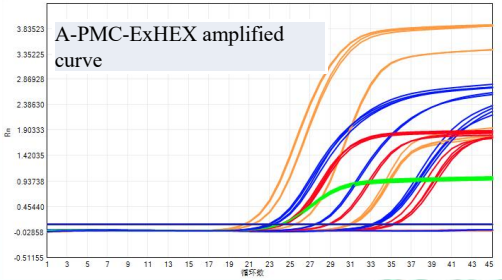


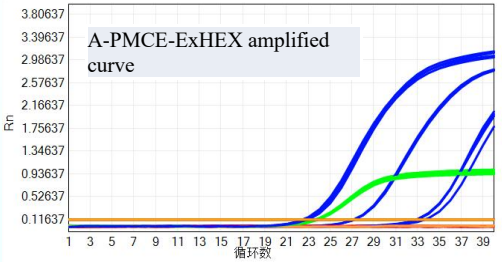
It is not necessarily judged as wild strain if eGFP or mCherry test negative.

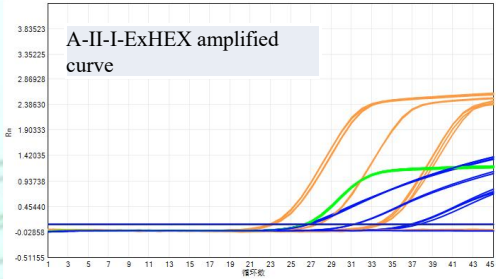
Clinical symptoms: off-feed
Time of pathogen nucleic acid testing: Mar. 9, 2023
Follow-up (Mar. 18) monitoring was positive for antibodies.

Suspected type I ASFV wild strain was detected.

sample type	sample number	experiment number	A-PMC-ExHEX					A-PMCE-ExHEX					A-II-I-ExHEX			
			FAM	HEX	ROX	CY5	results	FAM	HEX	ROX	CY5	results	FAM	HEX	ROX	results
			P72	internal mark	MGF	CD2V		P72	internal mark	eGFP	mCherry		II	internal mark	I	
cotton swab	vomiting 99186772	1	23.28	24.10	21.24	24.37	wild strain	22.82	23.65	NoCt	NoCt	wild strain	26.84	26.34	23.26	positive type I
cotton swab	vomiting 99186772	2	23.37	24.11	21.30	24.42	wild strain	22.98	23.68	NoCt	NoCt	wild strain	26.73	26.35	23.21	positive type I
cotton swab	death 99186668	3	22.80	24.12	20.39	24.07	wild strain	22.43	23.75	NoCt	NoCt	wild strain	26.43	26.28	22.81	positive type I
cotton swab	death 99186668	4	22.97	24.12	20.47	24.19	wild strain	22.51	23.71	NoCt	NoCt	wild strain	26.40	26.24	23.04	positive type I
cotton swab	1 vomiting and 2 around 99186844	5	33.30	24.20	30.75	35.07	wild strain	32.92	23.89	NoCt	NoCt	wild strain	36.81	26.39	33.36	positive type I
cotton swab	1 vomiting and 2 around 99186844	6	33.04	24.13	31.00	34.28	wild strain	33.62	23.6	NoCt	NoCt	wild strain	37.03	26.35	34.02	positive type I
cotton swab	1 vomiting and 2 around 99186603	7	27.39	24.17	25.41	28.72	wild strain	27.17	23.73	NoCt	NoCt	wild strain	31.17	26.34	27.65	positive type I
cotton swab	1 vomiting and 2 around 99186603	8	27.56	24.13	25.46	28.95	wild strain	27.11	23.73	NoCt	NoCt	wild strain	31.24	26.30	27.49	positive type I
cotton swab	1 vomiting and 2 around 99186615	9	33.48	24.14	31.11	35.31	wild strain	32.98	23.7	NoCt	NoCt	wild strain	36.77	26.29	33.34	positive type I
cotton swab	1 vomiting and 2 around 99186615	10	33.15	24.17	31.06	35.09	wild strain	32.84	23.71	NoCt	NoCt	wild strain	37.62	26.49	33.56	positive type I
negative control	/	/	NoCt	24.12	NoCt	NoCt	/	NoCt	23.64	NoCt	NoCt	/	NoCt	26.4	NoCt	/
	/		NoCt	24.14	NoCt	NoCt	/	NoCt	23.61	NoCt	NoCt	/	NoCt	26.4	NoCt	/





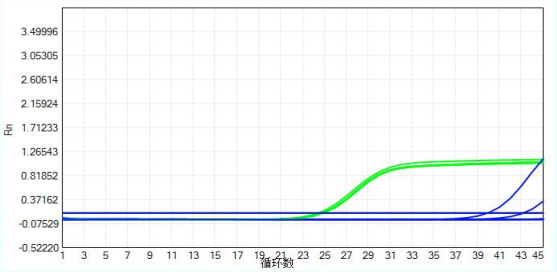


Clinical symptoms: Sudden death, vomiting

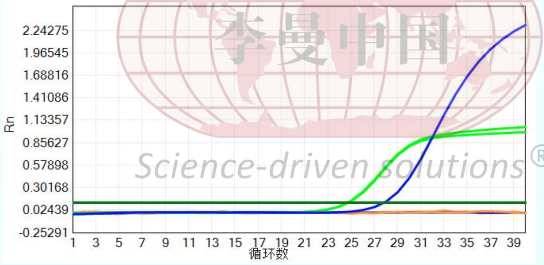
Time of pathogen nucleic acid testing: Jun. 1, 2023

Suspected I177L deletion strain was detected.

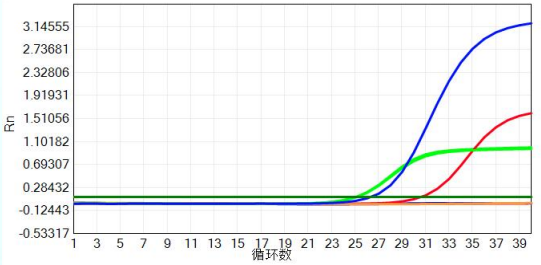
original number	A-II-I-ExHEX				A-PMC-ExHEX					A-I177L-ExHEX		
	FAM	ROX	HEX	results	FAM	ROX	Cy5	HEX	results	FAM	HEX	results
	type II	type I	internal mark		P72	MGF	CD2V	internal mark		I177L	internal mark	
14	27.78	NoCt	24.24	positive type II	26.30	NoCt	30.59	24.81	MGF variation	NoCt	24.61	I177L deletion?
negative	NoCt	NoCt	24.20	/	NoCt	NoCt	NoCt	24.76	/	NoCt	24.69	/



A-I177L amplified curve



Type A-II-I amplified curve



A-PMC amplified curve

Clinical symptoms: Sudden death, vomiting
Time of pathogen nucleic acid testing: Aug. 15, 2023

Have the biological characteristics of attenuated ASFV changed?

Its pathogenicity is reduced, but tooth extraction is less effective (not easy to perceive, not easy to detect, not easy to eliminate, not easy to trace):

- **The mode of transmission has not changed.**
- **The route of infection hasn't changed.**
- **Physical and chemical properties: Sensitivity to high temperature, drying, strong acid, strong alkali has not changed.**
- **Disinfectant sensitivity: Disinfectants applicable to ASF wild strain are still effective.**
- **Control strategy: Biosecurity measures applied to ASF wild strain remain effective.**

Is attenuated ASFV really weak?

- **weak: for healthy pigs**
- **not weak: for sick and weak pigs, sub-healthy pigs and pregnant sows**
- **It is continuously virulent and mutated, irregularly shed the virus, and activated under stress conditions (herd transfer, high temperature, coldness, castration, injection, mating, food changing and so on.)**

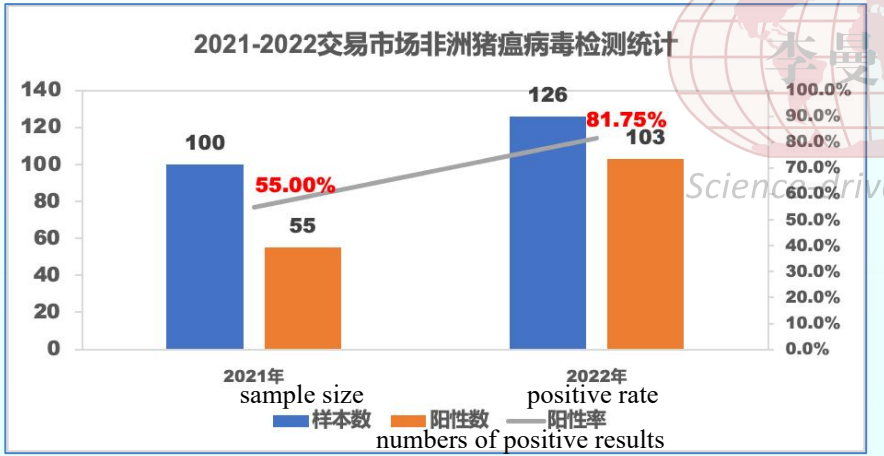
How does ASFV get into pig farms?

- **Pigs and related products:** introducing, pork import, seminal fluid...
- **Personnel:** clothes, boots, supplies...
- **Carrier:** drinking water, feed, vehicles, supplies, ingredients, aerosols...
- **Organisms:** vectors (mosquitoes, flies and ticks), wildlife (rodents and birds), pets (dogs and cats)...

Risk Points of ASFV Transmission

- The detection rate of ASFV nucleic acid in the trading market was high and showed an increasing trend, which was a high risk point.
- The positive rate of ASFV in abnormal pigs was higher, mostly above 40%.
- Hazard-free treatment, slaughtering, transportation and other links have the risk of transmission of the epidemic.

2021-2022 trading market ASFV detection statistics



Surveillance of ASF in a regional trading market in 2021-2022

2021-2022 high-risk pig herds ASFV detection statistics

Surveillance of ASF in high-risk pig herds in 2021-2022

Risk of Different Media Carries ASFV

- Detection rate of different media: personnel > materials > vehicles > environment

sample type	test number	positive number	positive rate (%)
personnel	50834	371	**0.73**
materials	27897	136	**0.49**
vehicles	76991	277	**0.36**
vehicles	114088	67	**0.06**
total	269810	851	0.32

How does ASFV get into pigs?

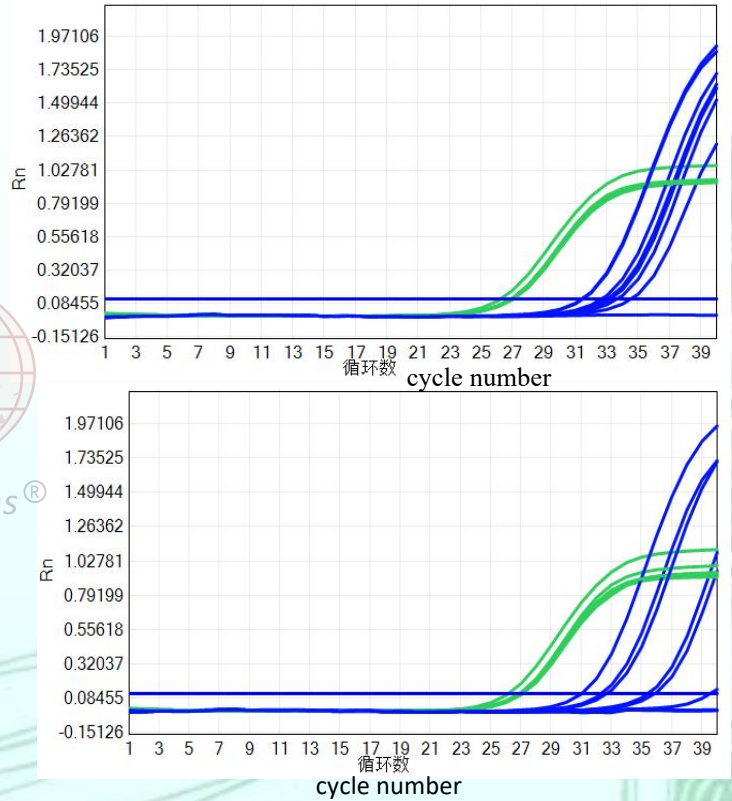
- **water and feed:** infected with ASFV through tonsil as ingesting contaminated drinking water and feed
- **wound:** enters the body through damaged skin membranes
- **injection:** injected into the bloodstream through contaminated needles and vaccines
- **mating:** enters the reproductive tract through contaminated vas deferens or semen
- **breath:** inhales virus-carrying dust, aerosols...

How to detect attenuated ASFV?

- **Screening for more sensitive detection methods:** Regular testing of viral nucleic acids and antibodies
- **Strengthen stall inspection:** artificial plus intelligence, focus on details (fluctuation of feed intake, abnormal behavior, changes in body temperature, changes in production indicators)
- **Abnormal pigs must be checked:** pigs die of diseases, culled pigs, underfed pigs in high-risk period, treatment fluid for piglet tail cut-off, placenta and umbilical cord blood
- **Introduced pig must be checked: replacement pigs, seminal fluid,** source pig farms and their surroundings
- **Key parts:** regular sampling and inspection of doorways, outlets of draught fan and isolation houses

Mixed sample detection of low copy samples is prone to missing detection.

sample	1#		average value	2#		average value
	target gene	internal mark		target gene	internal mark	
single inspection	31.47	26.93	31.47	31.03	26.87	31.03
3 mixed with 1	31.40	26.95	31.86	32.30	26.97	32.51
	32.67	26.96		32.72	27.09	
5 mixed with 1	33.23	27.04	33.44	35.87	26.99	35.64
	33.65	27.06		35.40	27.10	
10 mixed with 1	33.06	26.89	33.8	NoCt	27.04	/
	34.53	26.95		39.55	27.04	
negative control	NoCt	26.23	/	NoCt	26.16	/



- Environment, material and water samples carry less virus.
- Mixing ratio should not be higher than 3 mixing 1.

Case 1: In a pig farm with 5000 sows, farrowing units were detected with double-gene deletion strain and subsequently tested negative for antibody.

sample number	sample type	sample source	clinical symptoms	notes	joint inspection number	P72 unit weight	P72/CD2V	MGF	strain
1	tail vein	P3		5A53	J1	36.38	41.86/0	-	double-gene deletion vaccine virus
2	throat swab	P3		5A53	J2	30.52	29.6/0	-	
3	blood	P3		5A53	J3	34.97	35.99/0	-	

- **Solutions:**

1. Personnel isolation: Isolate the feeder of the line where the positive unit is located, and feeders of other lines are isolated in terms of different lines.
2. Pig handiling: The positive pigs were killed, sealed and wrapped, and then treated harmlessly.
3. Pig culling: On the morning of the second day, transferred the sows in this unit and their corresponding units to breeding units, and cull a total of 500 pigs in another unit that the feeder is responsible for.
4. Disinfection: The production line is thoroughly disinfected once, and the pig unit is culled. Continue to disinfect for 3 to 5 days until the samples are negative.
5. Sampled abnormal pigs daily (tail vein, throat swab).

- **Results:** If the test result was negative after 30 consecutive days, the isolation was lifted, and the treatment was successful.
- **Conclusion:** If positive cases are found early and properly handled, the epidemic will be quickly controlled. Production personnel should be highly responsible, daily monitoring of abnormal pigs, and timely inspecting, which is conducive to early detection of positive pigs.

Case 2: A pig farm introduced 3000 pigs in July, ending isolation in early August, the whole herd was sampled to detect nucleic acid and antibody.

Test results: The nucleic acid test was negative, with 1 antibody positive, 2 suspected.

	IDV-blocking retest					IDV-indirect retest				
sample number	OD value	S/N value	results			OD value	S/P	results	N	P
7-4	0.6289	37.23	positive (+)	1.551	0.082	0.3286	0.270	negative (-)	0.0356	1.1212
8-133	0.7708	46.89	suspected (+/-)			0.3323	0.273	negative (-)		
8-155	/					0.067	0.029	negative (-)	0.036	1.121
7-4	0.6087	35.85	positive (+)			0.3937	0.330	suspected (+/-)		
8-133	0.7529	45.67	suspected (+/-)			0.298	0.241	negative (-)		
8-155	0.6929	41.59	suspected (+/-)			0.0722	0.033	negative (-)		

- **Solutions:**

1. Antibody positive or suspected pigs were resampled and retested with other kits, and the results were negative.
2. Three pigs were dissected and their tissues (lung, lymph gland, spleen, bone marrow) were taken for nucleic acid detection, and the results were negative.
3. Two weeks later, the whole herd was sampled again for nucleic acid and antibody detection, and the results were negative.

- **Results:** If the test result is negative for 2 consecutive months, the risk is removed.
- **Conclusion:** Introduction detection is very important for the prevention and control of vaccine virus.

1. Detected the nucleic acid and antibody of the whole herd before and after introduction.
2. If antibodies are detected, they need to be resampled, retested with a different kit, collecting tissue to detect the pathogen.
3. If the proportion of positive antibody is high, it is recommended to stop introduction.

Case 3: Single gene deletion strain was detected in the mating and pregnant line of a pig farm, in which 2 antibodies were positive.

location of stall	inspection sample	antigen			antibody		
		positive sample	P72/CD2v	P72/MGF	positive sample	first inspection	re-inspection
A9-94	throat swab + anterior venous blood	throat swab	30.86/31.77	30.56/--	anterior venous blood	positive	positive
PA9-89	throat swab + anterior venous blood	throat swab	27.51/28.14	26.18/--	anterior venous blood	positive	positive
A9-92	throat swab + anterior venous blood	throat swab	34.19/35.36	35.86/--			
B3-12	throat swab + anterior venous blood	throat swab	33.04/34.16	34.81/--			

- **Solutions:**

1. Isolated interms of different lines.
2. Culled pigs in positive area of matingand pregnant house, and thoroughly deinfected.
3. Start the whole herd screening, once 2 weeks, daily sampling and inspecting abnormal pigs.

- **Results:** The infection spread to the whole herd, depopulating after 1 month.
- **Conclusion:** The first case was positive for nucleic acid and antibodies, indicating that the farm had been infected for some time (≥2 weeks), and cross-contamination and transmission may have occurred.

Key Point of Low Pathogenicity Strain Elimination

- ✓ **Key:** Early detection of "Case Zero" (first positive pig)
- ✓ **Guarantee:** Perfect monitoring system (program, technology, team)
- ✓ **Core:** Regular culling of sick and weak pigs (trigger)
- ✓ **Foundation:** Clear division of labor, well-trained, well-handled, attention to details

Key Points of ASF Prevention and Control

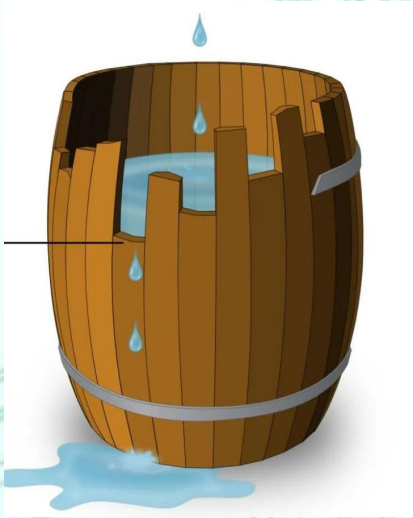
multi-pronged approach, systematic prevention and control

multi-barrier and mucous membrane maintenance

- **Stay ahead of the virus:** regular testing + abnormal pig screening, pathogens + antibodies
- **Strict control of introduction:** source, introduction monitoring, process risk control, isolation and domestication after introduction
- **Prevent disease from entering the mouth:** drinking water, feed (raw materials, high temperature granulation, cooling, transportation), air (positive/negative pressure ventilation, air filtration)
- **Strengthening body resistance to eliminate pathogenic factors:** flies do not bite seamless eggs
- **Prevention:** emergency drills, anti-cross, anti-transmission

Preventive Measures for Attenuated ASFV

- **Introduction management:** 2~3 tests, nucleic acid + antibody
- **Drinking water management:** fermented organic acid, ozone water, ultrafiltration, bleaching powder
- **Wound management:** mucosal disinfectant (oral iodine, ozone water, chlorine dioxide)
- **Pig sale management**: off-site transfer out of the pig platform, one-way flow, external pig cart control
- **"Seven degrees" health management:** temperature, humidity, density, illumination, air freshness, water cleanliness, nutrition balance
- **Preventive culling of sick and weak pigs**: barrel principle, piping principle
- **Materials and ventilation management:** reduce the frequency of materials, proper resting, conditional air filtration



Raising pigs is to raise the intestines, and the key is to protect the intestines.

- The intestine is the largest digestive organ and the largest immune organ in the body
- The intestine is a fine absorber of water and grain and a converter of matter and energy
- The large intestine and the lung are mutually related (constipation and respiratory diseases are mutually causal)
- At great risk of infection (water is a carrier of both nutrients and pathogens)

How to Maintain Intestinal Health

- **Mycotoxin control** (raw materials, towers, lines, tanks)
- **Prevent constipation** (dietary fiber, fermented feed, proper exercise, adequate water intake)
- **Scientific health care** (strengthening body resistance to eliminate pathogenic factors, anti-stress, antioxidant health care products, multi-dimensional)
- **Rational feeding** (trickle feeding, liquid feeding, dynamic feeding)
- **New feeding technologies** (in vitro pre-digestion, acidopeptidase, biological feed, intelligent feeding)

Current misunderstanding of ASF prevention

- **Pay attention to biosafety rather than health management, making nutrition and prevention are opposite**
- **Excessive disinfection, abuse of antibiotics, breaking the ecological balance inside and outside the pig**
- **Use harsh disinfectant to disinfect pigs, damaging the skin and mucous membrane, and destroying the microecological barrier**
- **Confuse the primary with secondary, failure to grasp key points (drinking water, introduction, wounds, mucous membranes)**
- **Trick thinking, gambler's psychology: bet on God medicine, God vaccine, God program**

Prevention and Control of Other Pig Diseases

- **Piglet diarrhea:** environmental control, prevention of sows constipation, milk increase, vaccine supplement
- **PRRS:** domestication of disposable activated vaccines + inactivated vaccine strengthening, herdclosure management, anti-stress
- **Foot-and-mouth disease:** vaccine content and stability, antibody assessment, maternal antibody monitoring
- **Aujeszky's disease:** vaccination and decontamination (gE-ELISA), anti-stress
- **Swine fever:** vaccination and decontamination (nucleic acid antigen + E^{rns} antibody）
- **Bacterial diseases:** conditional, environmental, clean water and feed house, careful use of drugs for health care

The Way Out for the Family Farm

- **Give full play to advantages:** low cost, high efficiency, good turn-around
- **Overcome disadvantages:** equipment, talent, capital, market
- **Joint development:** Relying on the group, integrating resources, joining cooperatives and consortia
- **Differentiated development:** integrated planting and breeding, diversified feed, ecological breeding, characteristic products, brand management

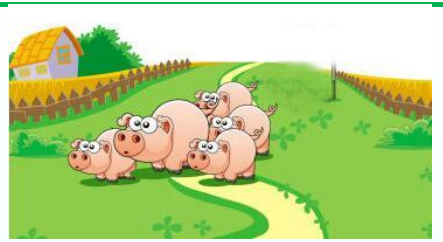
Main Factors Restricting the High Quality Development of Pig Industry

- Good pig breeds are controlled by others.
- Feed is controlled by others.
- Serious breeding pollution
- Complex and changeable diseases
- ...

What is the modern way of pig production with Chinese characteristics?

- Good pig breeds are controlled by others. vs excellent pig breeds from over 50 regions
- Feed is controlled by others. vs over 2 billion tons of agricultural, animal husbandry and fishery by-products
- Serious breeding pollution vs natural way of returning manure and urine to the field
- complex and changeable diseases vs healthy way of ecological breeding
- ...

Future Prospect of Pig Industry in China



- **Safe industries:** Chinese breeding pigs, Chinese pork
- **Delicious pork:** Healthy production, delicious meat
- **Ecological farm:** ecological breeding circle, healthy industrial chain (combination of farming and breeding, moderate scale, ecological breeding, brand pork, circular agriculture, low-carbon economy)
- **Rich and beautiful countryside:** rural revitalization, beautiful homes

"Let people can see the lucid waters and lush mountains, and can keep their homesickness."

In the future, large-scale, automated, intelligent, **ecological** and **beneficial** breeding is the only way to achieve high-quality development of animal husbandry and build a powerful country in breeding.

- efficient production
- safe products
- save resources
- environment friendly
- effective regulation

Thank you!

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Thanks to the following people:

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