Novel porcine circovirus type 5
（Porcine Circovirus type 5, PCV5）
的Discovery, identification and virus characterization research

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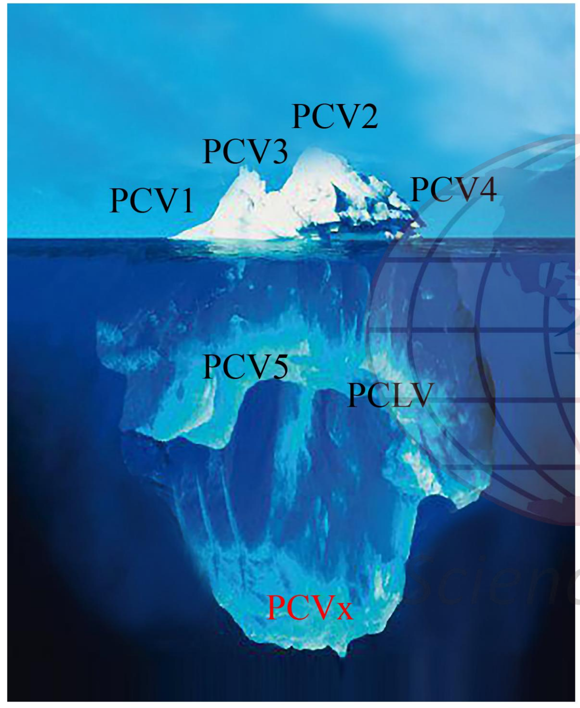


Background

Research status of porcine circovirus（PCV1–PCV4)

猪圆环病毒（Porcine cicrovirus, PCV)

It belongs to the genus Circovirus and the family Circoviridae, and four porcine circovirus genotypes have been reported so far, which are not known to be disease-knowingPCV1、PCV2、PCV3与PCV4（Meng XJ, 2013; Palinski R, 2016; Zhang HH, 2020)。

The disease caused by PCV2 infection is called Porcine circovirus-related diseases (PCVAD) mainly include multiple system failure syndrome, pig dermatitis and nephrotic syndrome and reproductive system diseases in weaned piglets.

PCV3 has been widely prevalent around the world since it was reported in 2016, and PCV3 has been associated with a range of related porcine circovirus diseases.

圆环病毒名称	基因组大小	Cap蛋白大小（aa）	Rep蛋白大小（aa）
PCLV	3921-3950	187-189	310
PCV1	1758-1760	230-233	312
PCV2	1766-1768	233-236	314
PCV3	2000	214	296-297
PCV4	1700	229	292

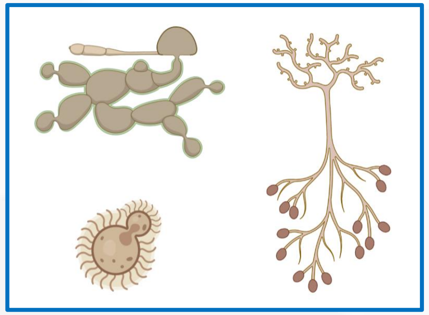
Circoviruses belong to the family Circoviridae and the genus Circovirus, but what family does circovirus belong to??

Background

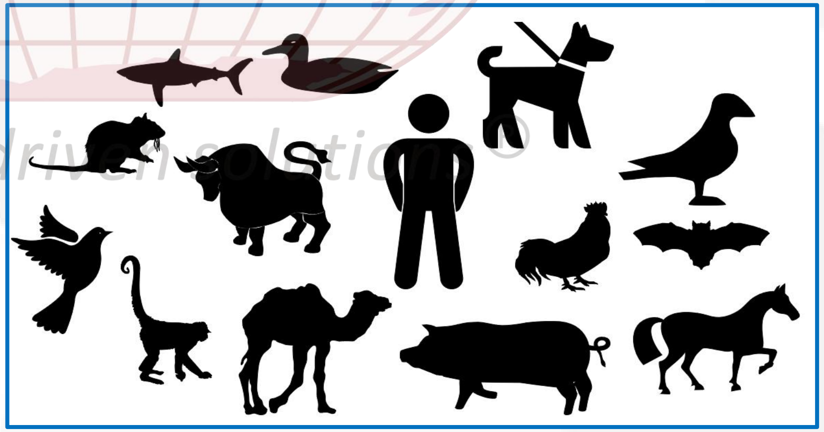
CRESS DNAVirus diversity

- Eukaryotic Circular Rep-Encoding Single-Stranded DNA (CRESS DNA) Viruses: 真核生物复制酶蛋白编码的单股环状DNA病毒
- Small genomes(1-8 kb)
- Wide range of hosts

Plants
植物



Fungi
真菌

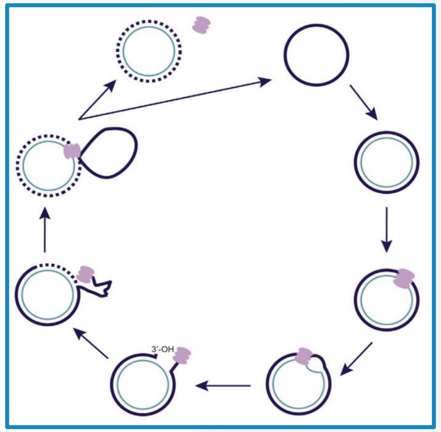


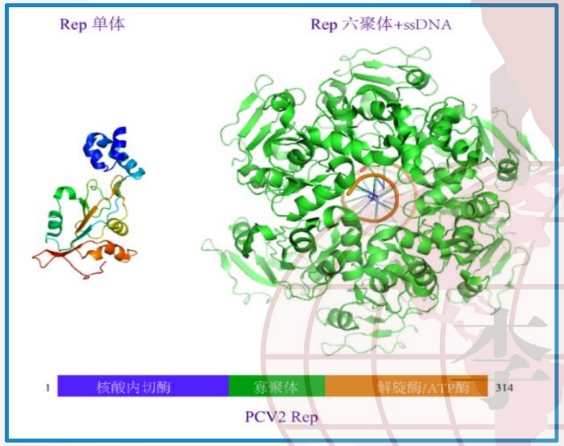
Animals and human 动物和人

Diatoms
硅藻

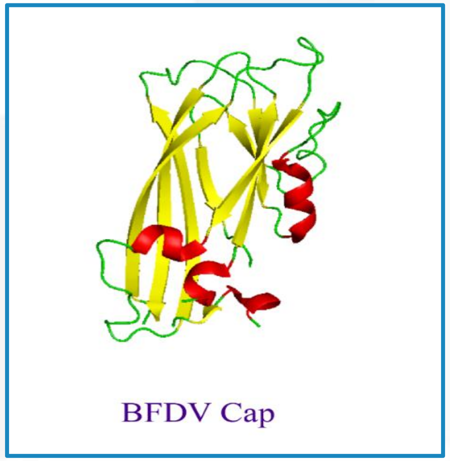
Background

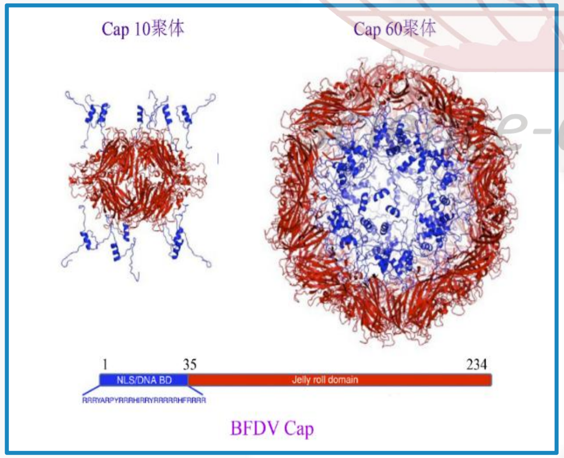
Replication and assembly of circovirus





Rep is a versatile enzyme responsible for cleaving and ligating ssDNA and capable of unwinding double-stranded DNA (dsDNA). In the nucleus, the ssDNA genome of the virus is separated from the capsid of the virus, and the ssDNA interacts with Rep to carry out rolling loop replication, and the rolling loop replication mechanism is shown in the figure on the left。





Cap is the only structural protein of circovirus, forming a viral capsid that wraps the virus's genome and has antigenic determinants on the surface of the capsid. In addition, capsid proteins play a key role in viral replication, including adsorption, cell entry, genome release, and translocation to the nucleus。

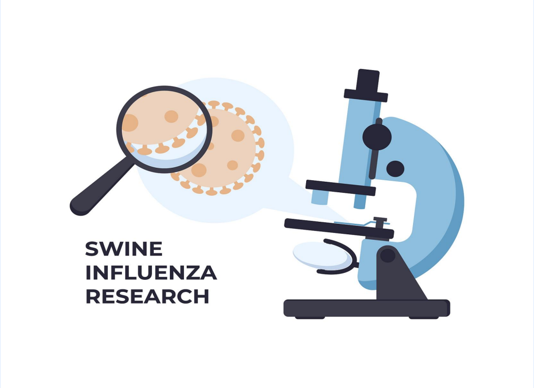
Background

Surveillance and research on emerging viruses can help us be more scientific Respond to the epidemic of emerging infectious diseases。

In view of the fact that it is difficult to accurately identify infectious diseases in some large-scale pig farms causes, and lack of targeted prevention and control measures, this paper is carried out based on this problem Study.

Purpose and significance of the study

It provides a new theoretical basis for disease prevention and control in pig farms



This study enriches the etiology and genetic evolution of circovirus

Materials and methods

1 Sample collection (clinical background check)

2 Whole gene sequencing and conserved primer design

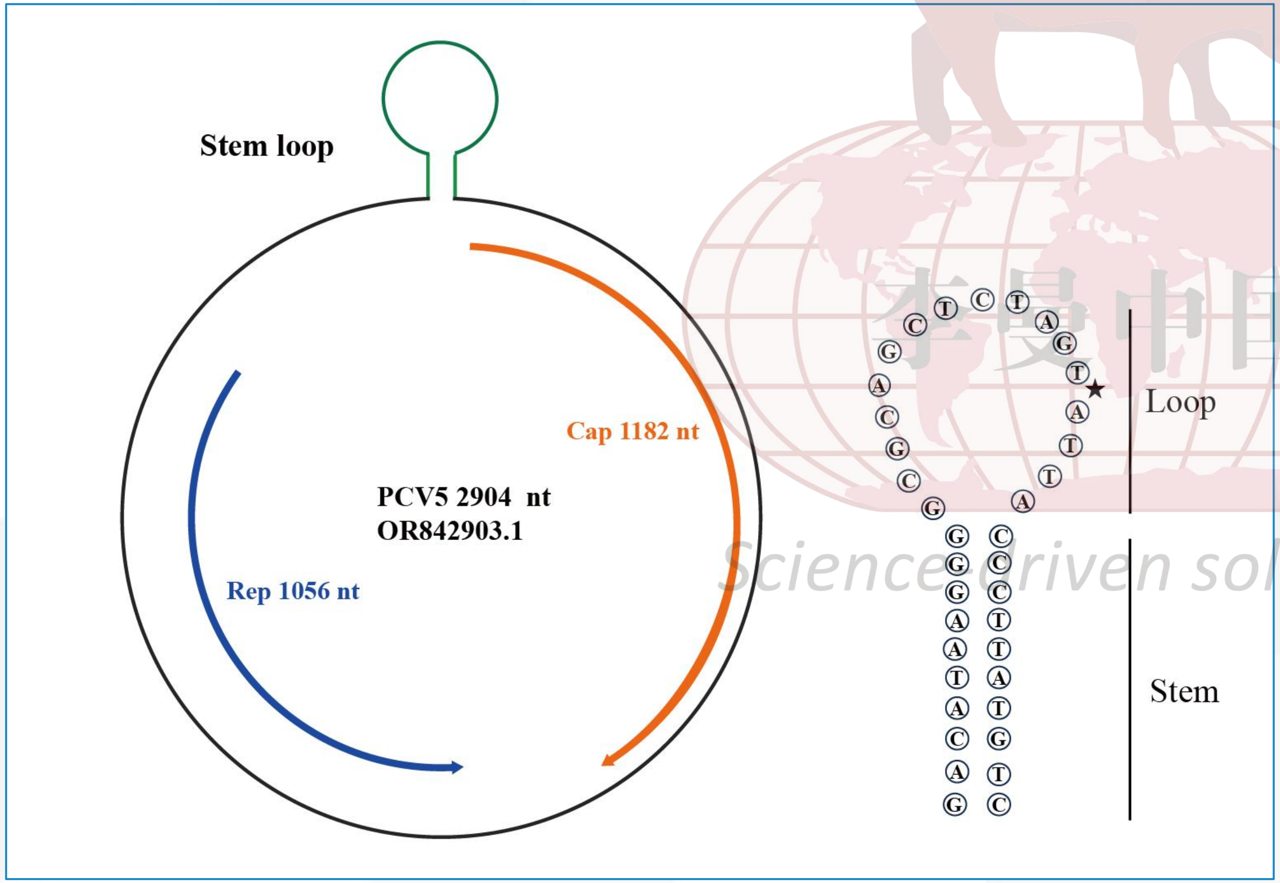
3 Virus isolation, purification, and morphological identification

4 Structure prediction (AlphaFold), protein expression and VLP assembly

5 Serological testing (ELISA) was established with qPCR

Findings

Genome organization of the PCV5



PCV5 has a single strand circle genome (2904 nt): the Cap (1182 nt), the Rep (1056 nt) and a stem ring (stem: 9 nt and loop: 17 nt).

Findings

Geographic distribution of the PCV5

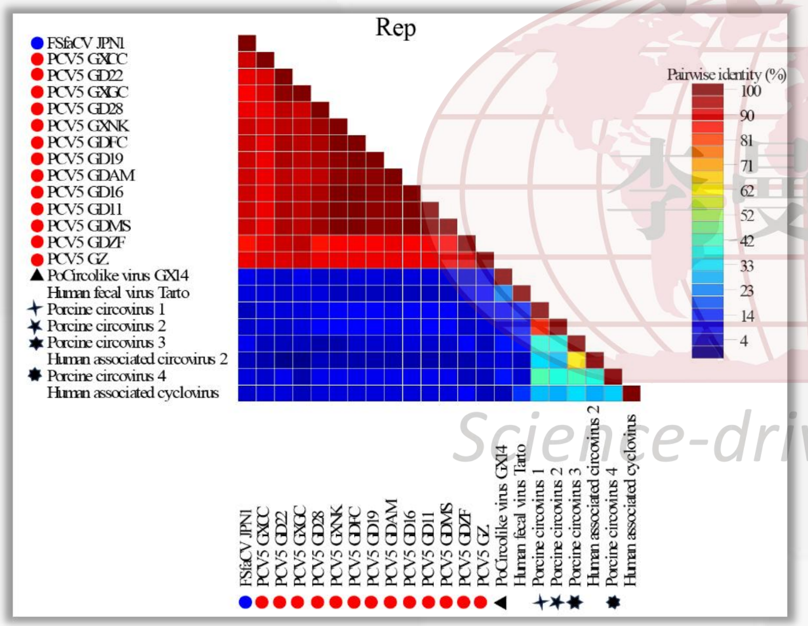
Areas where surveillance of PCV5 was carried out are shown in blue. Red circles indicate the locations of farms with confirmed PCV5 infection. A total of 17 pig farms in Guangxi, Guangdong, Hunan, Yunnan and Hainan provinces were PCV5 positive.

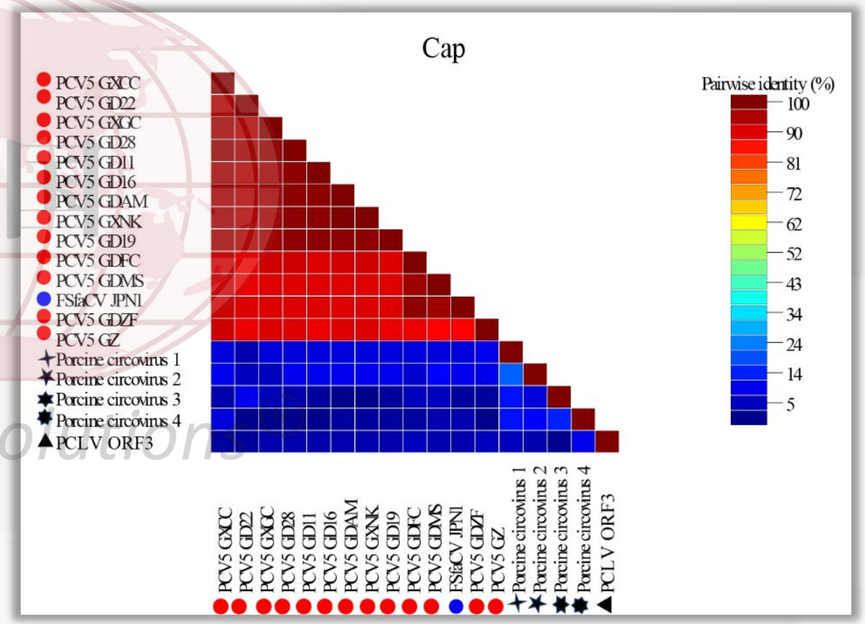
The positive detection rate for PCV5 in tested samples was 54.4% (294 out of 540), with 24.3% (17 out of 70) of pig farms in China testing positive for the virus.

Findings

Genetic relationship of PCV5 strains to PCV1, PCV2, PCV3, PCV4, PCLV and several human circoviruses

The viral Rep and Cap showed low amino acid sequence identities to the four PCV species, with **the highest identity of 26.2% to PCV2 Rep and 20.8% to PCV4 Cap.**

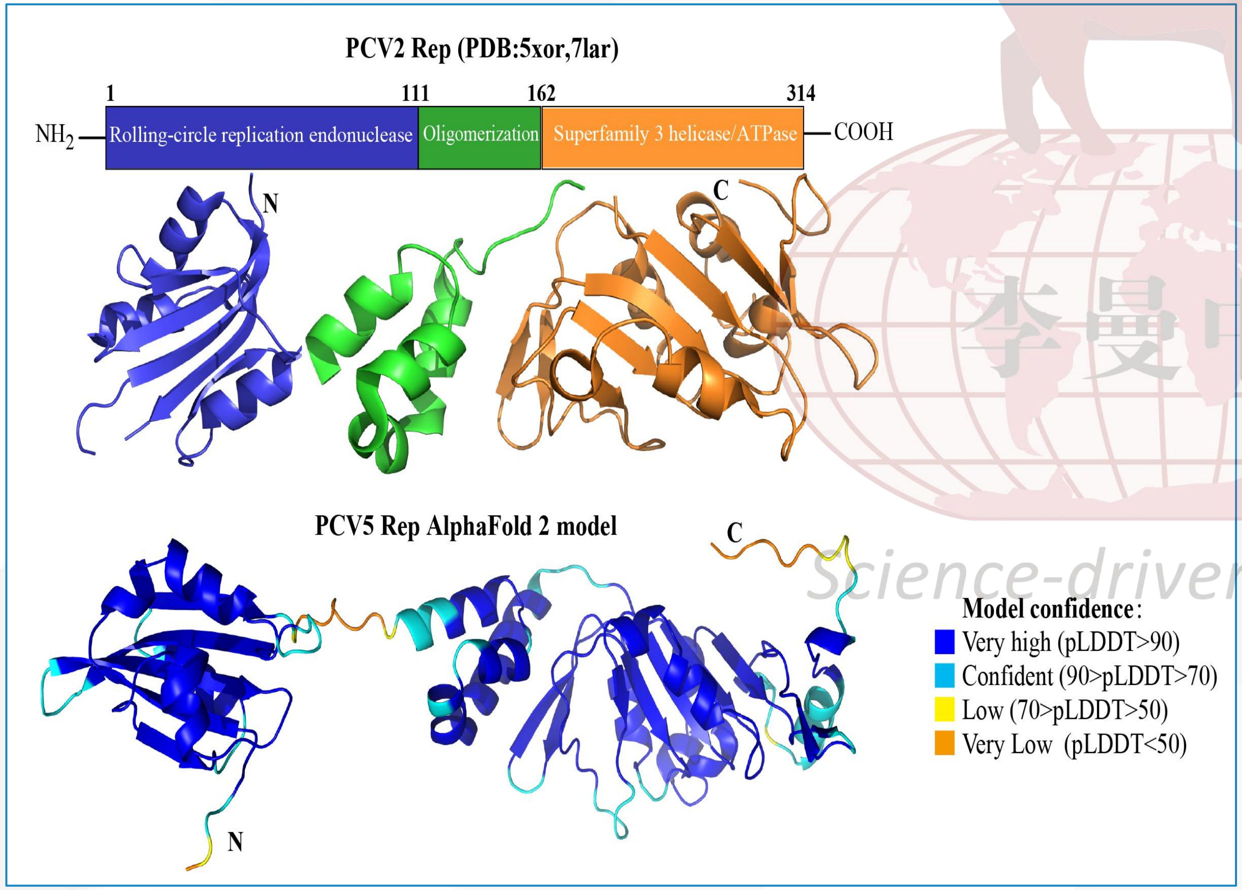


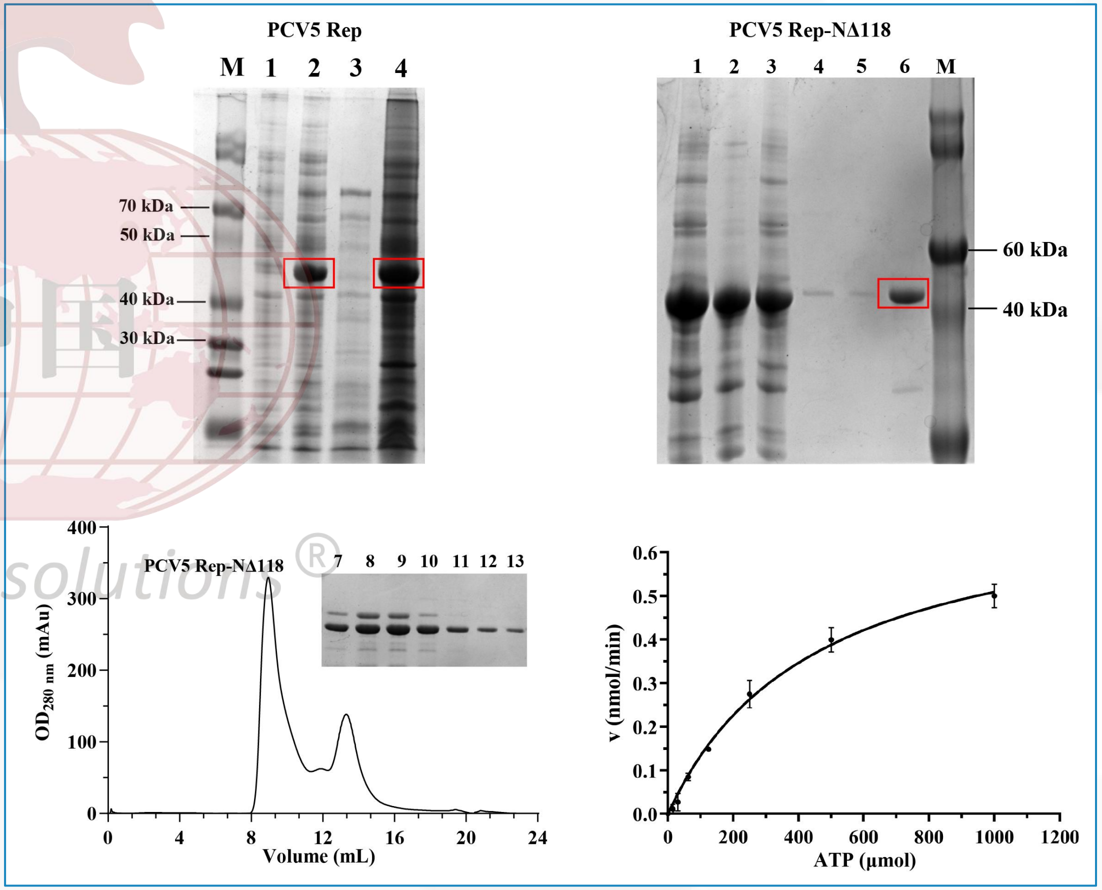


Pairwise identities of the Rep and Cap amino acid of twelve PCV5 strains to other circoviruses. The black star indicates PCV1, PCV2, PCV3, PCV4 and PCLV, the blue triangle represents the fur seal faeces associated circular DNA virus (FSfaCV, accession KF246569.1) strain.

Findings

Biochemical characteristics of PCV5 Rep



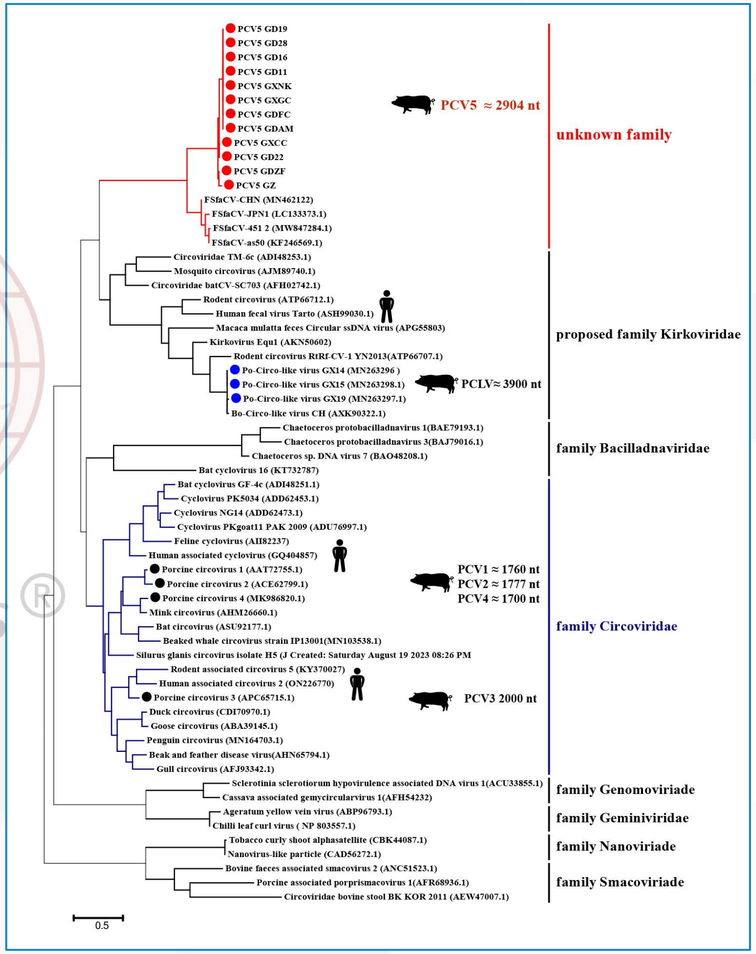


Findings

Phylogenetic analysis based on Rep

The phylogeny tree suggests that PCV5 is genetically distinct from PCV1, PCV2, PCV3, PCV4, PCLV and several human CRESS DNA viruses, indicating PCV5 does not belong to the known family.

The PCV5 strains in the present study are marked with red circles. Black circles indicate five porcine circoviruses: PCV1, PCV2, PCV3 and PCV4. Blue circles indicate PCLV.



Findings

Histological lesions associated with the presence of PCV5

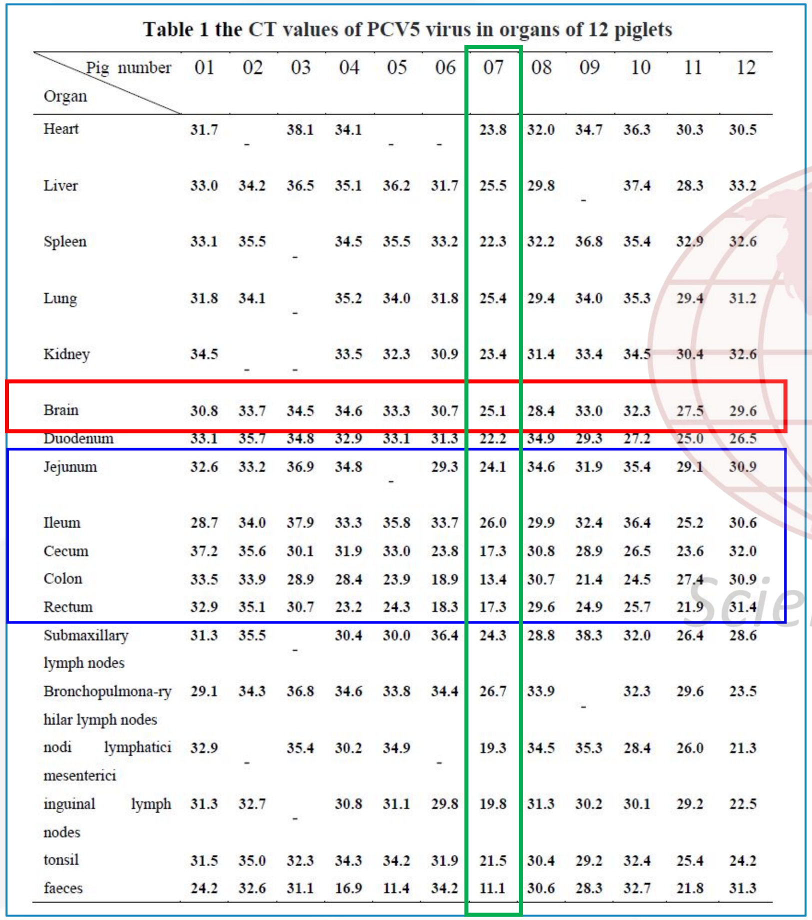


Table 1 the CT values of PCV5 virus in organs of 12 piglets

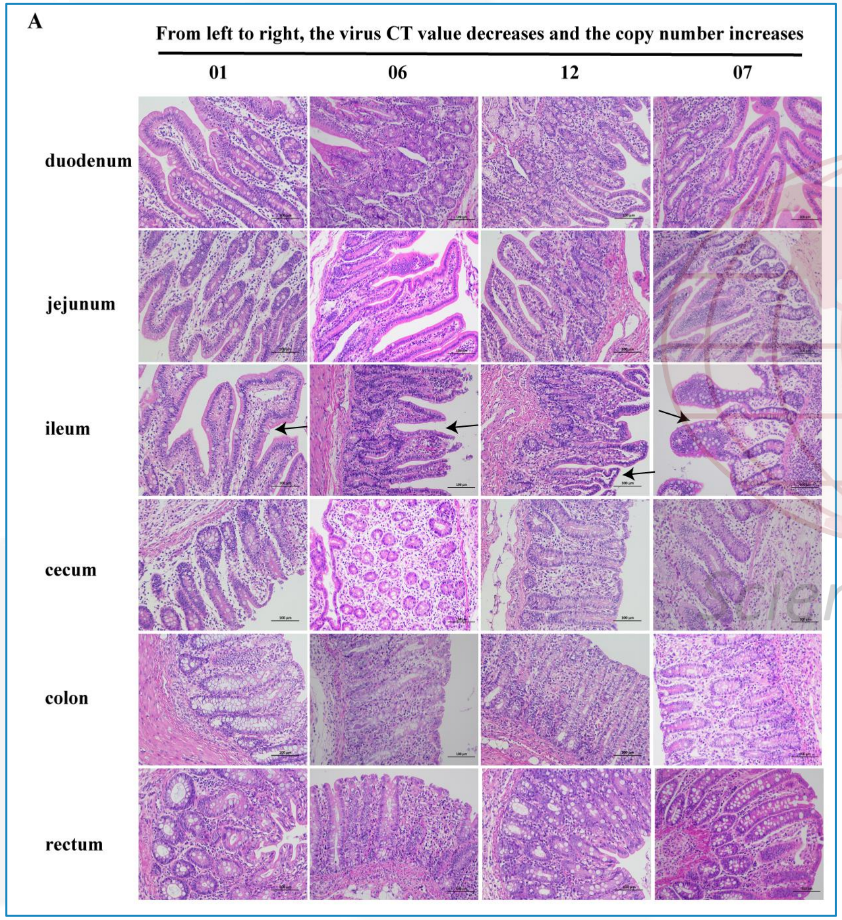
Pig number / Organ	01	02	03	04	05	06	07	08	09	10	11	12
Heart	31.7	-	38.1	34.1	-	-	23.8	32.0	34.7	36.3	30.3	30.5
Liver	33.0	34.2	36.5	35.1	36.2	31.7	25.5	29.8	-	37.4	28.3	33.2
Spleen	33.1	35.5	-	34.5	35.5	33.2	22.3	32.2	36.8	35.4	32.9	32.6
Lung	31.8	34.1	-	35.2	34.0	31.8	25.4	29.4	34.0	35.3	29.4	31.2
Kidney	34.5	-	-	33.5	32.3	30.9	23.4	31.4	33.4	34.5	30.4	32.6
Brain	30.8	33.7	34.5	34.6	33.3	30.7	25.1	28.4	33.0	32.3	27.5	29.6
Duodenum	33.1	35.7	34.8	32.9	33.1	31.3	22.2	34.9	29.3	27.2	25.0	26.5
Jejunum	32.6	33.2	36.9	34.8	-	29.3	24.1	34.6	31.9	35.4	29.1	30.9
Ileum	28.7	34.0	37.9	33.3	35.8	33.7	26.0	29.9	32.4	36.4	25.2	30.6
Cecum	37.2	35.6	30.1	31.9	33.0	23.8	17.3	30.8	28.9	26.5	23.6	32.0
Colon	33.5	33.9	28.9	28.4	23.9	18.9	13.4	30.7	21.4	24.5	27.4	30.9
Rectum	32.9	35.1	30.7	23.2	24.3	18.3	17.3	29.6	24.9	25.7	21.9	31.4
Submaxillary lymph nodes	31.3	35.5	-	30.4	30.0	36.4	24.3	28.8	38.3	32.0	26.4	28.6
Bronchopulmona-ry hilar lymph nodes	29.1	34.3	36.8	34.6	33.8	34.4	26.7	33.9	-	32.3	29.6	23.5
nodi lymphatici mesenterici	32.9	-	35.4	30.2	34.9	-	19.3	34.5	35.3	28.4	26.0	21.3
inguinal lymph nodes	31.3	32.7	-	30.8	31.1	29.8	19.8	31.3	30.2	30.1	29.2	22.5
tonsil	31.5	35.0	32.3	34.3	34.2	31.9	21.5	30.4	29.2	32.4	25.4	24.2
faeces	24.2	32.6	31.1	16.9	11.4	34.2	11.1	30.6	28.3	32.7	21.8	31.3

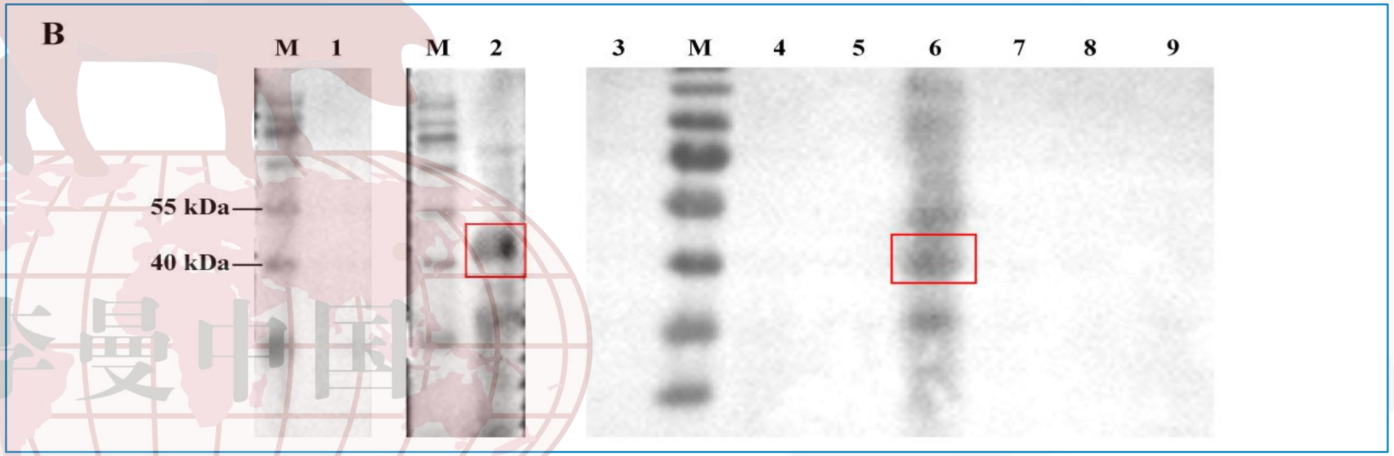
The virus CT values of 12 pigs were closely correlated with their clinical symptoms. When viral DNA levels were low, higher concentrations of PCV5 DNA is found in intestinal tissue, intestinal lymph nodes and faces.

It was particularly to find that the virus can also cross the blood-brain barrier, as high levels of viral DNA were identified in brain samples. In addition, PCV5 DNA was detected in stillbirth piglets from other farms with outbreaks of reproductive disorders (data is not shown)

Findings

Histologic lesions of tissue of swine infected with PCV5

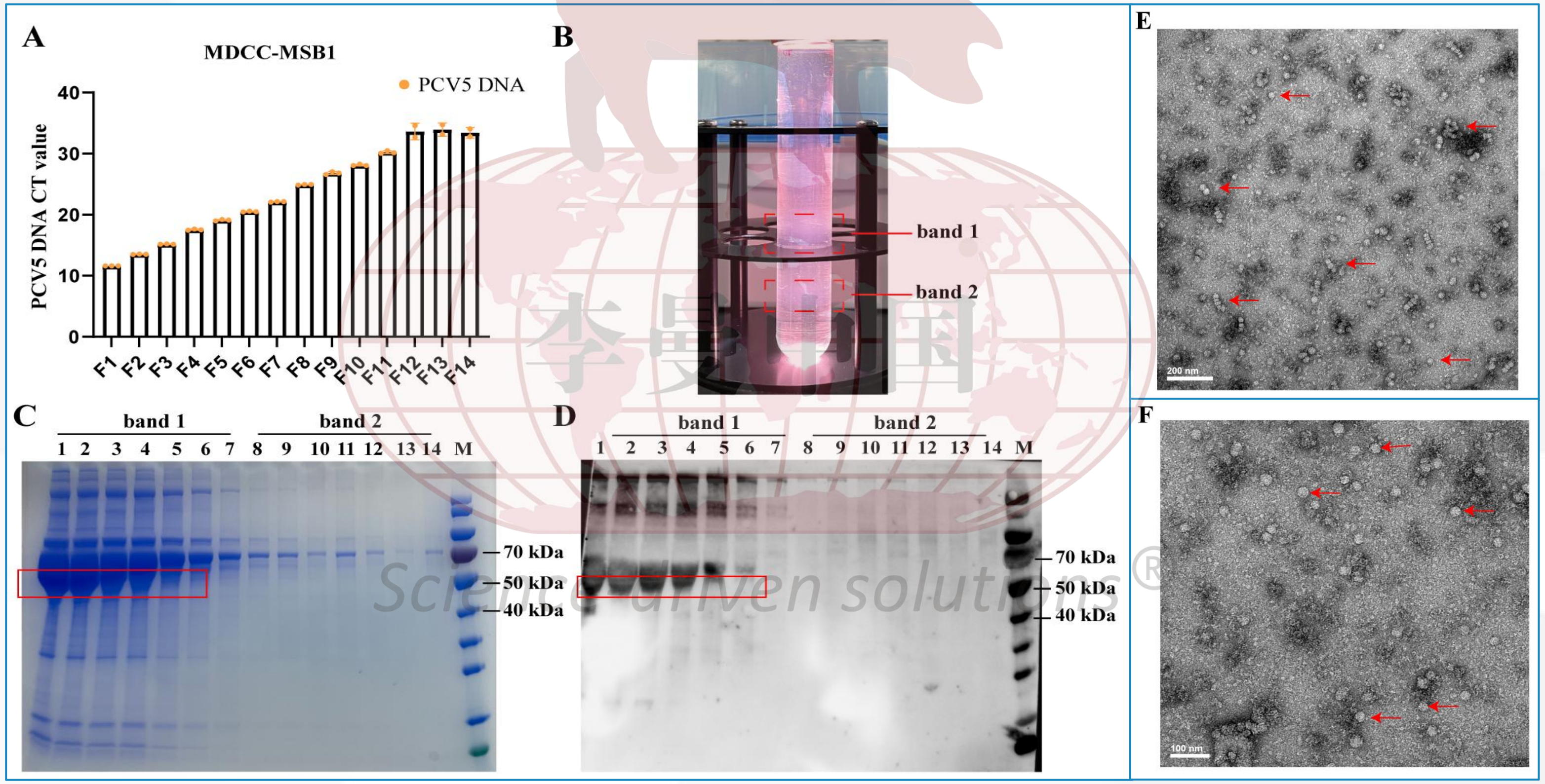




The pathological examination of the four piglets revealed that the organ lesions were associated with the copies of PCV5 DNA (Fig A), and pathological injury of ileum was serious in the four piglets. These results suggest that a close correlation between the copy number of PCV5 and the severity of infectious diseases.

Findings

Virus isolation and visualization

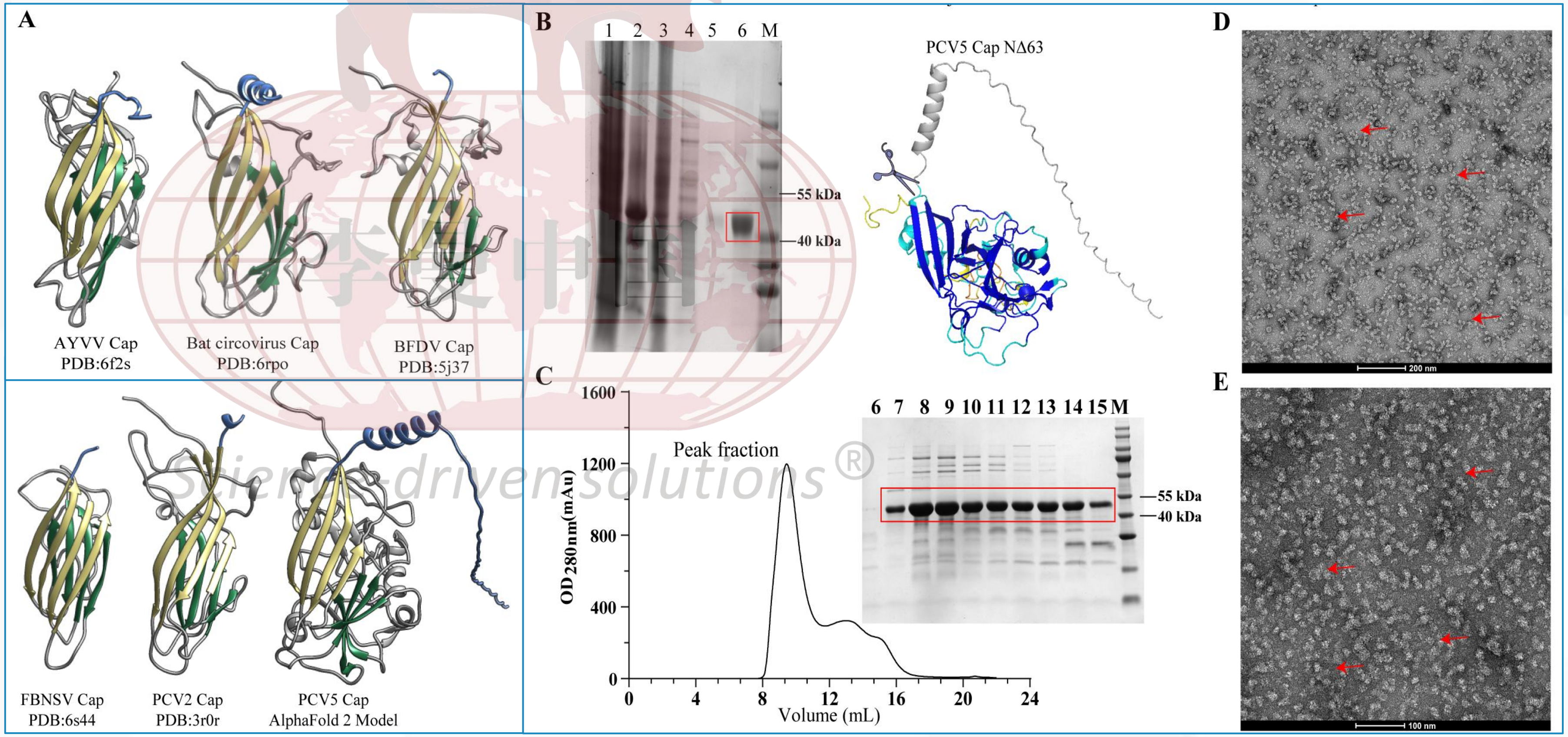


The PCV5 isolation from Intestinal tissue of piglets was performed in Marek’ s disease lymphoma cell line (MDCC-MSB1). After serial generations of the blind passages, PCV5 isolate was obtained in MDCC-MSB1 cells showing no Cytopathic effects (CPE) .

Findings

Expression, purification and assembly of the PCV5 VLPs

- The Cap structure of PCV5 is highly similar to the Cap structure of the published CRESS DNA virus, which is a classic jelly roll. The jelly roll domain consists of two β-sheets, each of which contains four β-strands connected by loop.
- A variant of the PCV5 Cap, lacking the N-terminal 63 residues, was expressed in E.coli, and purified Cap could assemble into VLPs similar to purified PCV5.



Findings

A PCV5 Virus-like particles (VLPs)-based ELISA which was developed

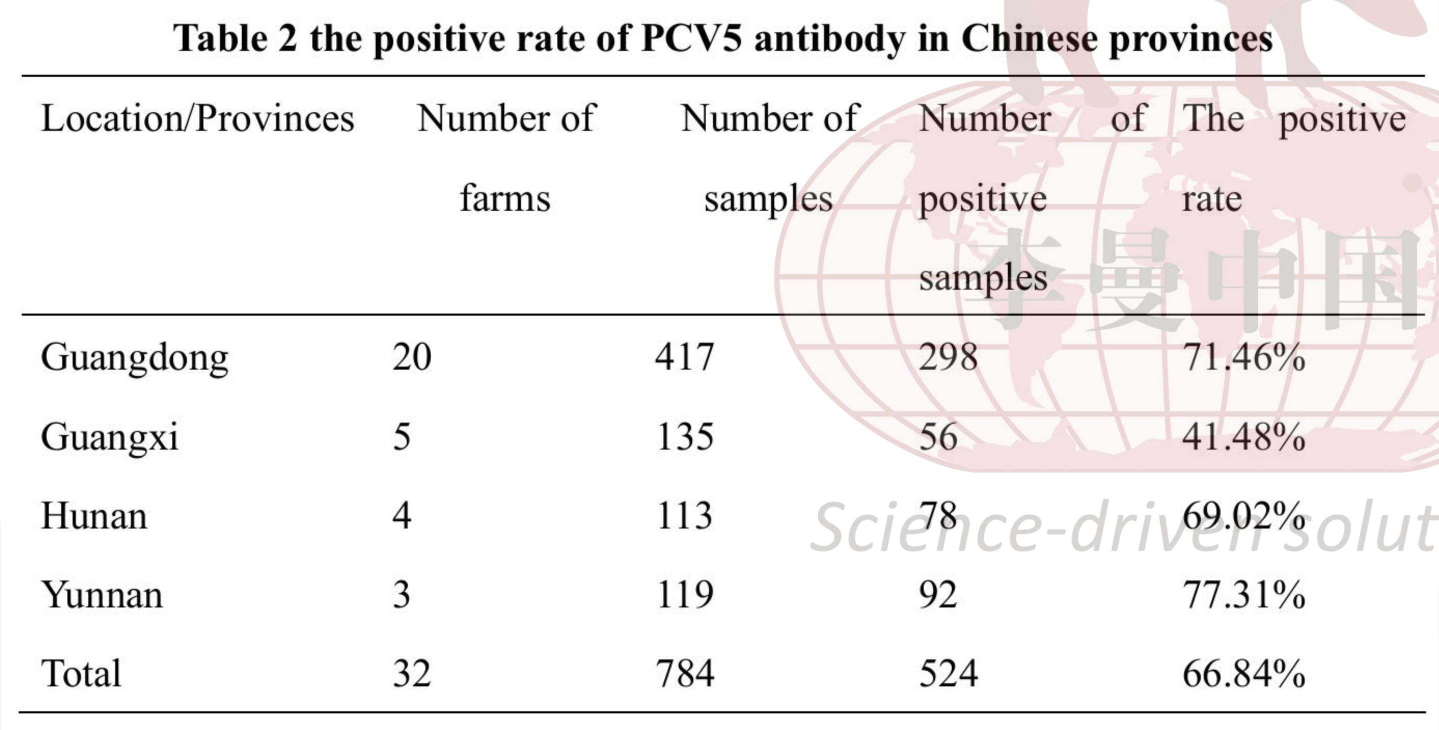
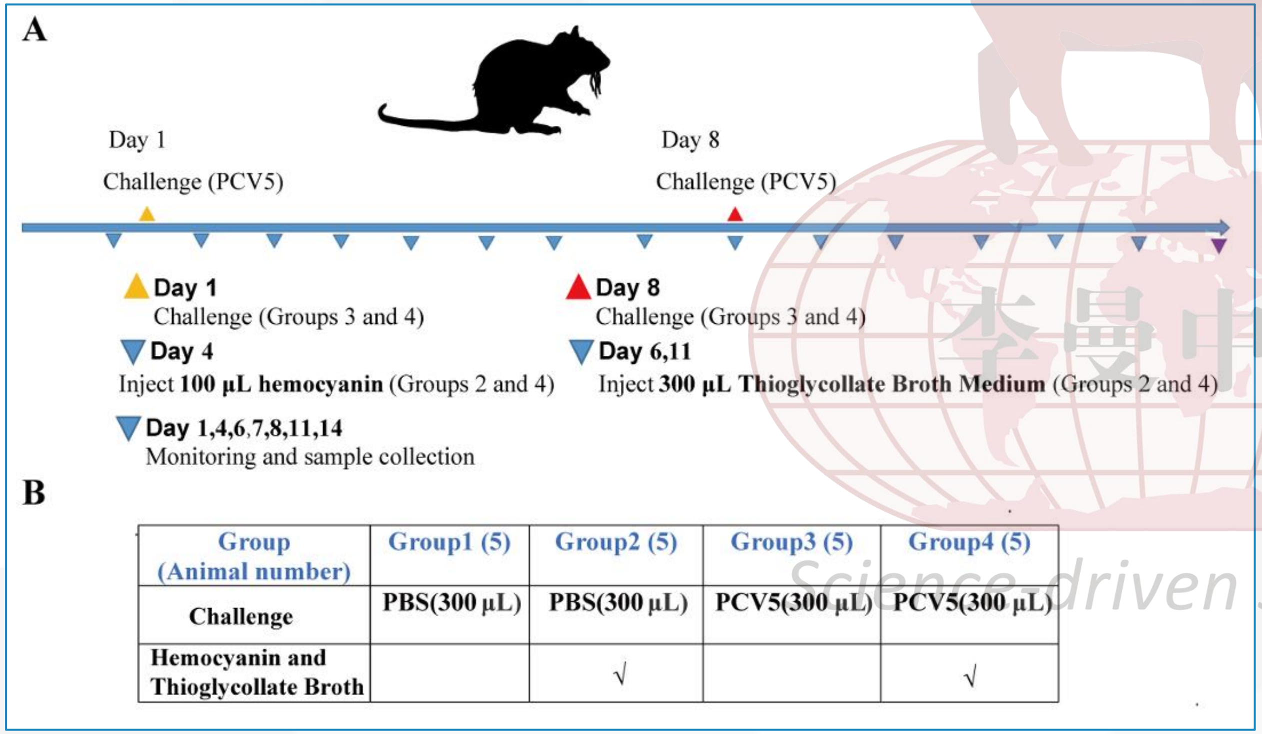


Table 2 the positive rate of PCV5 antibody in Chinese provinces

Location/Provinces	Number of farms	Number of samples	Number of positive samples	The positive rate
Guangdong	20	417	298	71.46%
Guangxi	5	135	56	41.48%
Hunan	4	113	78	69.02%
Yunnan	3	119	92	77.31%
Total	32	784	524	66.84%

Findings

We tried to establish an animal disease model using in mice challenged with PCV5



▲ **Day 1**
Challenge (Groups 3 and 4)

▲ **Day 8**
Challenge (Groups 3 and 4)

▼ **Day 4**
Inject **100 µL hemocyanin** (Groups 2 and 4)

▼ **Day 6,11**
Inject **300 µL Thioglycollate Broth Medium** (Groups 2 and 4)

▼ **Day 1,4,6,7,8,11,14**
Monitoring and sample collection

B

Group (Animal number)	Group1 (5)	Group2 (5)	Group3 (5)	Group4 (5)
Challenge	PBS(300 µL)	PBS(300 µL)	PCV5(300 µL)	PCV5(300 µL)
Hemocyanin and Thioglycollate Broth		√		√

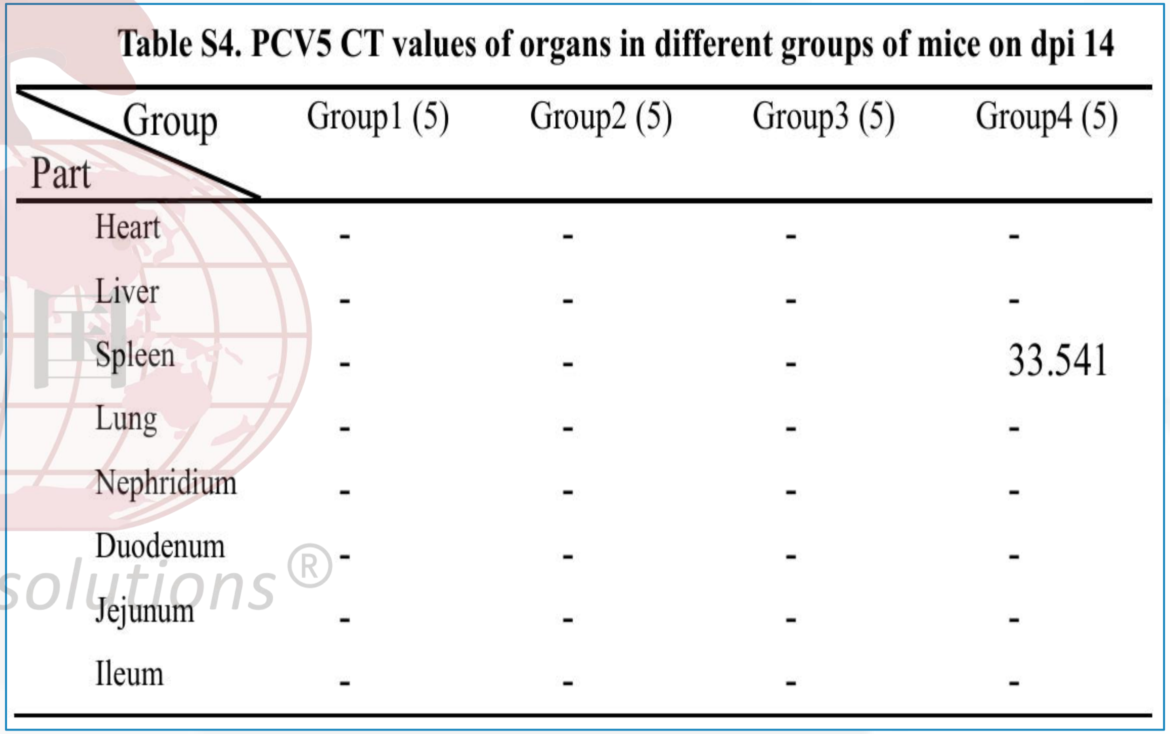


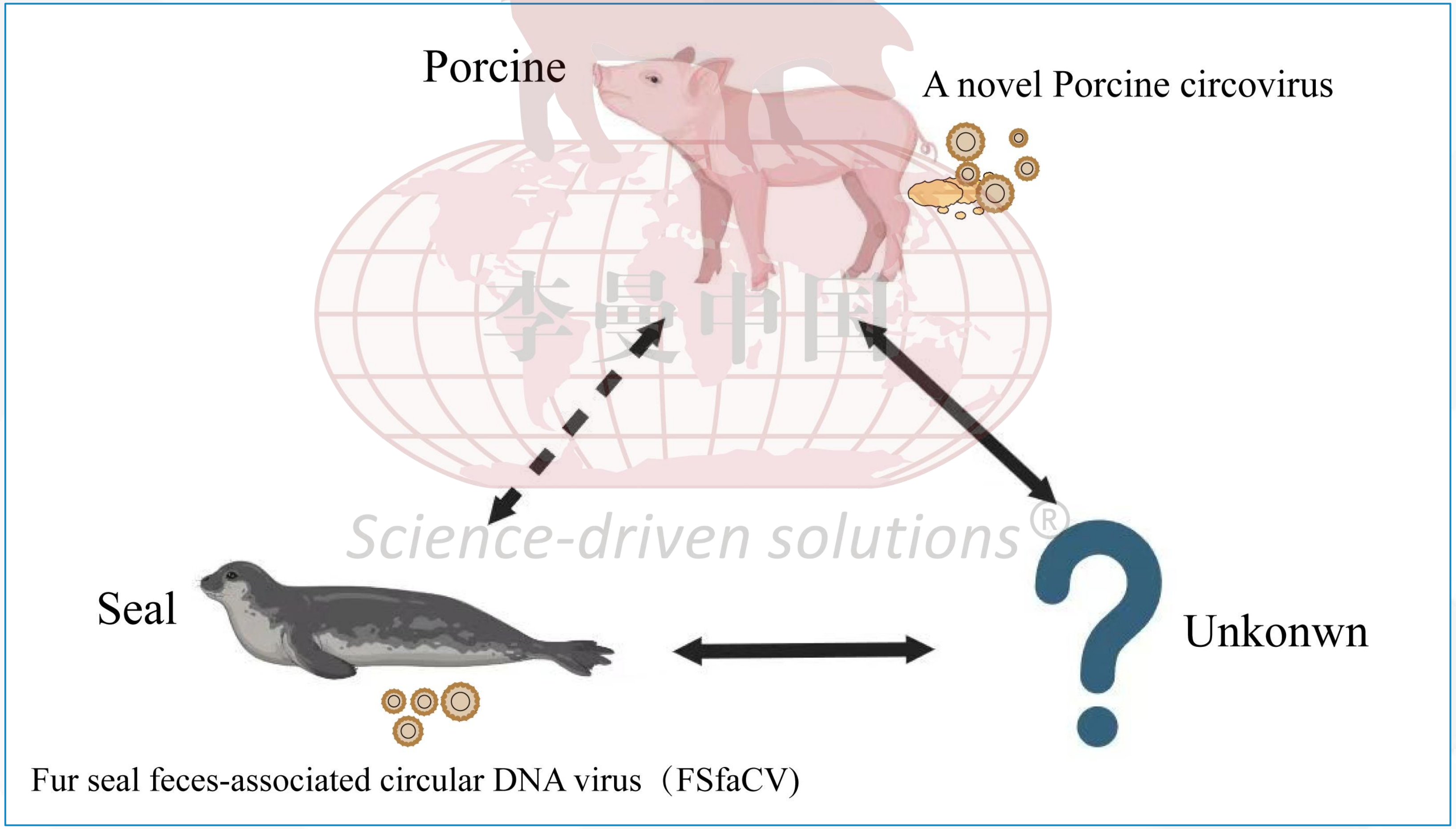
Table S4. PCV5 CT values of organs in different groups of mice on dpi 14

Group / Part	Group1 (5)	Group2 (5)	Group3 (5)	Group4 (5)
Heart	-	-	-	-
Liver	-	-	-	-
Spleen	-	-	-	33.541
Lung	-	-	-	-
Nephridium	-	-	-	-
Duodenum	-	-	-	-
Jejunum	-	-	-	-
Ileum	-	-	-	-

These results indicated that PCV5 is not suitable for establishing a disease model in mice.

Findings

Diagram of infected animals with PCV5



Porcine

A novel Porcine circovirus

Seal

Unkonwn

Fur seal feces-associated circular DNA virus（FSfaCV)

Discussion and Revelation

PCV5 is a novel PCVAD-associated CRESS DNA virus。

What is the pathogenicity of PCV5? Its pathogenic mechanism needs to be further studied, and the synergistic pathogenic mechanism with other diseases to be studied.

What is the prevalence of PCV5? Is there potential for clinical disease makers? As a "pig signal" to guide pig production?