Envisioning the future of disease diagnosis in modern swine production

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The Main Challenges of the pig industry

- Disease
- High Cost
- Location of Farm
- Outmode Facilities
- High Animal Density
- Government Regulations
- Shortage of Professional Man Power

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Correct diagnosis is the first step for disease control and treatment

Pathology is the basis of disease diagnosis

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Comparison the Different Diagnostic Methods

Method	Advantage	Disadvantage
Pathogen isolation	1. Identify the causative agent	1. High time consumption 2. High contamination rate
Molecular diagnosis	1. Fast 2. High sensitivity 3. High specificity	1. High construction and maintence cost 2. Strict environmental control needed 3. Not link to causative agent directly
Serology	1. High specificity 2. Suitable for evaluating the efficacy of immunization and the infection status	1. Limited application in early infection 2. Commercialized kit are limited
Pathology	1. Fast (Gross pathology) 2. Identify the characteristic lesions 3. Differential diagnosis for possible etiology	1. Long term training needed 2. Some lesions are not distinctive for specific pathogen

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What is this disease ?

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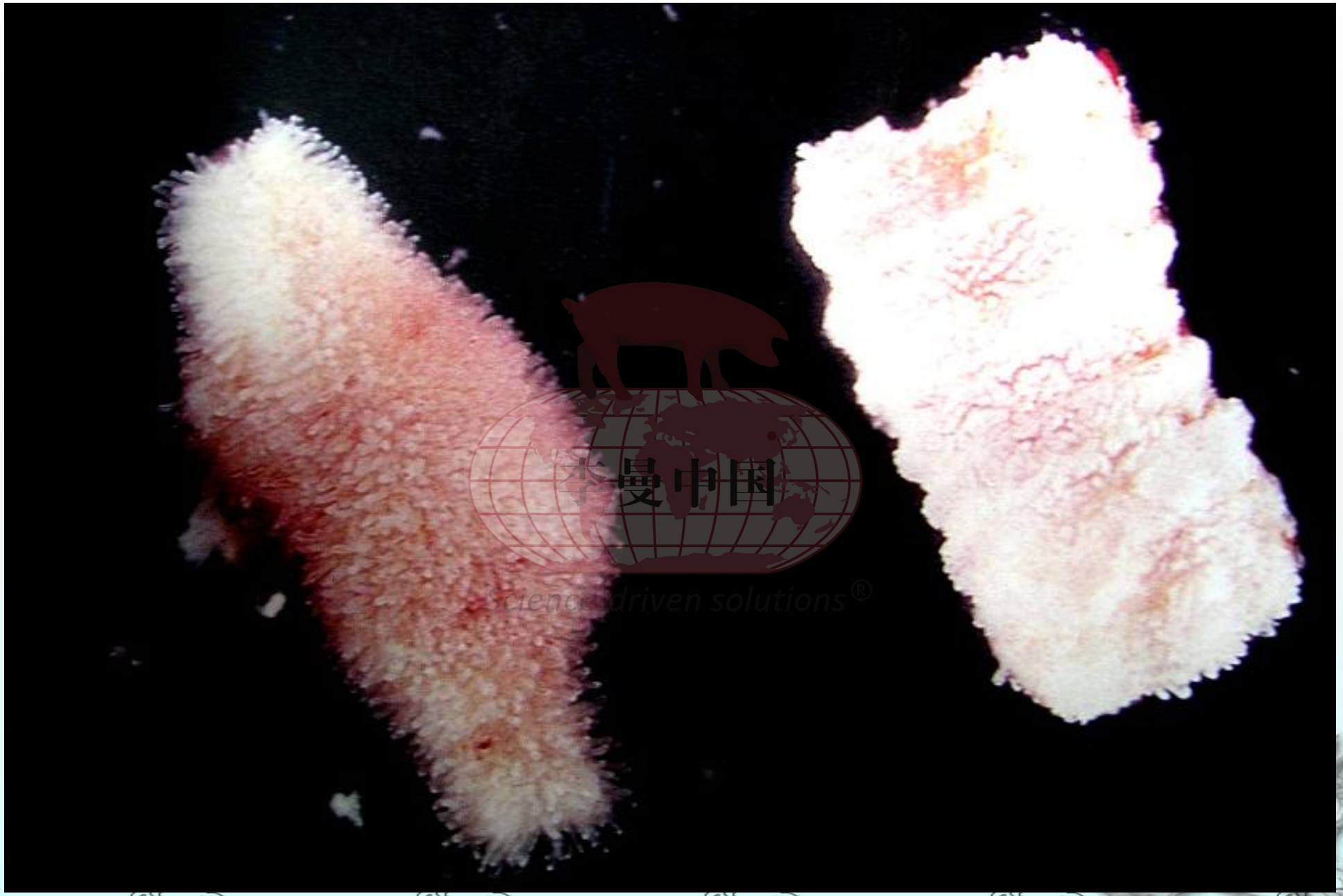
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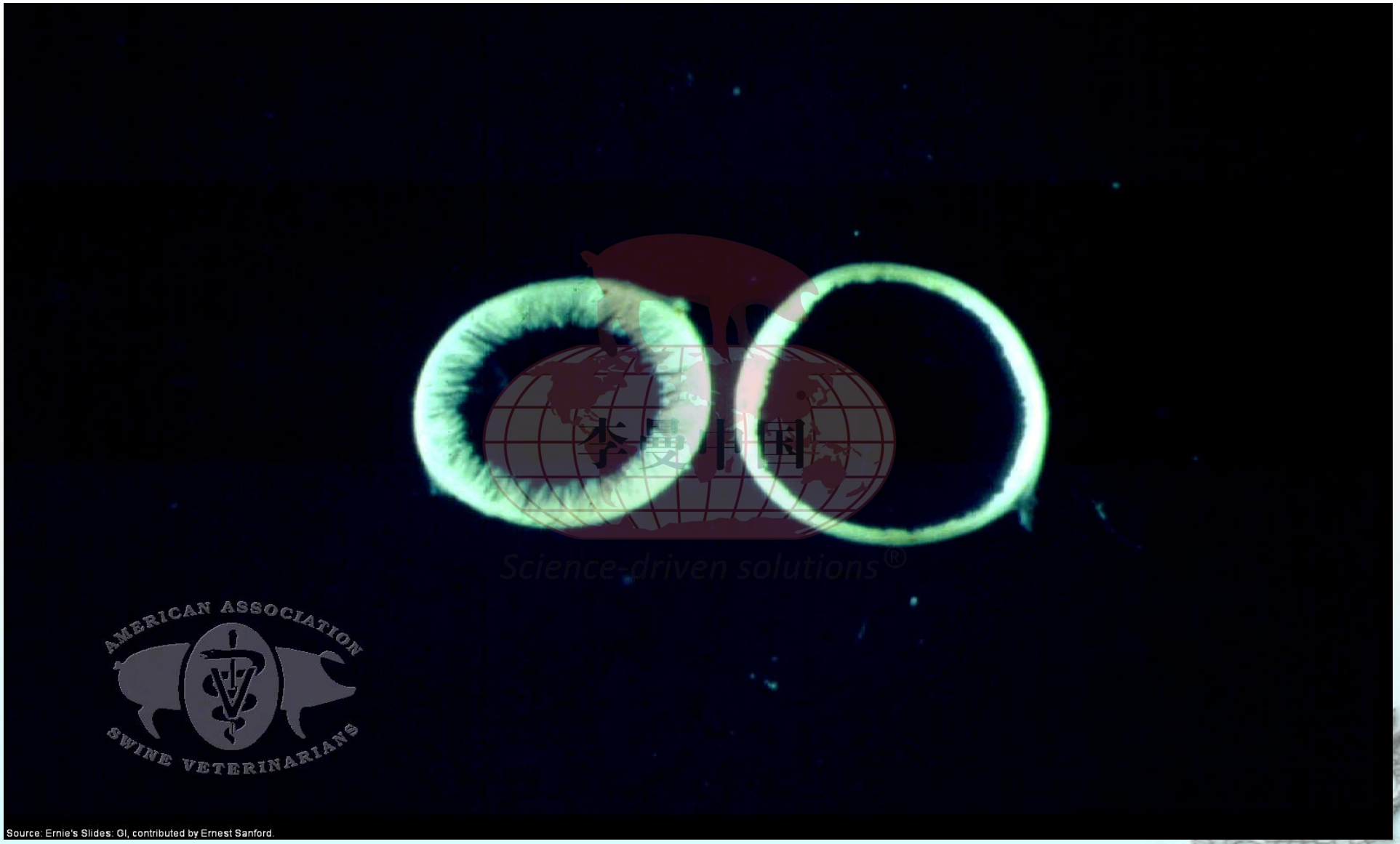
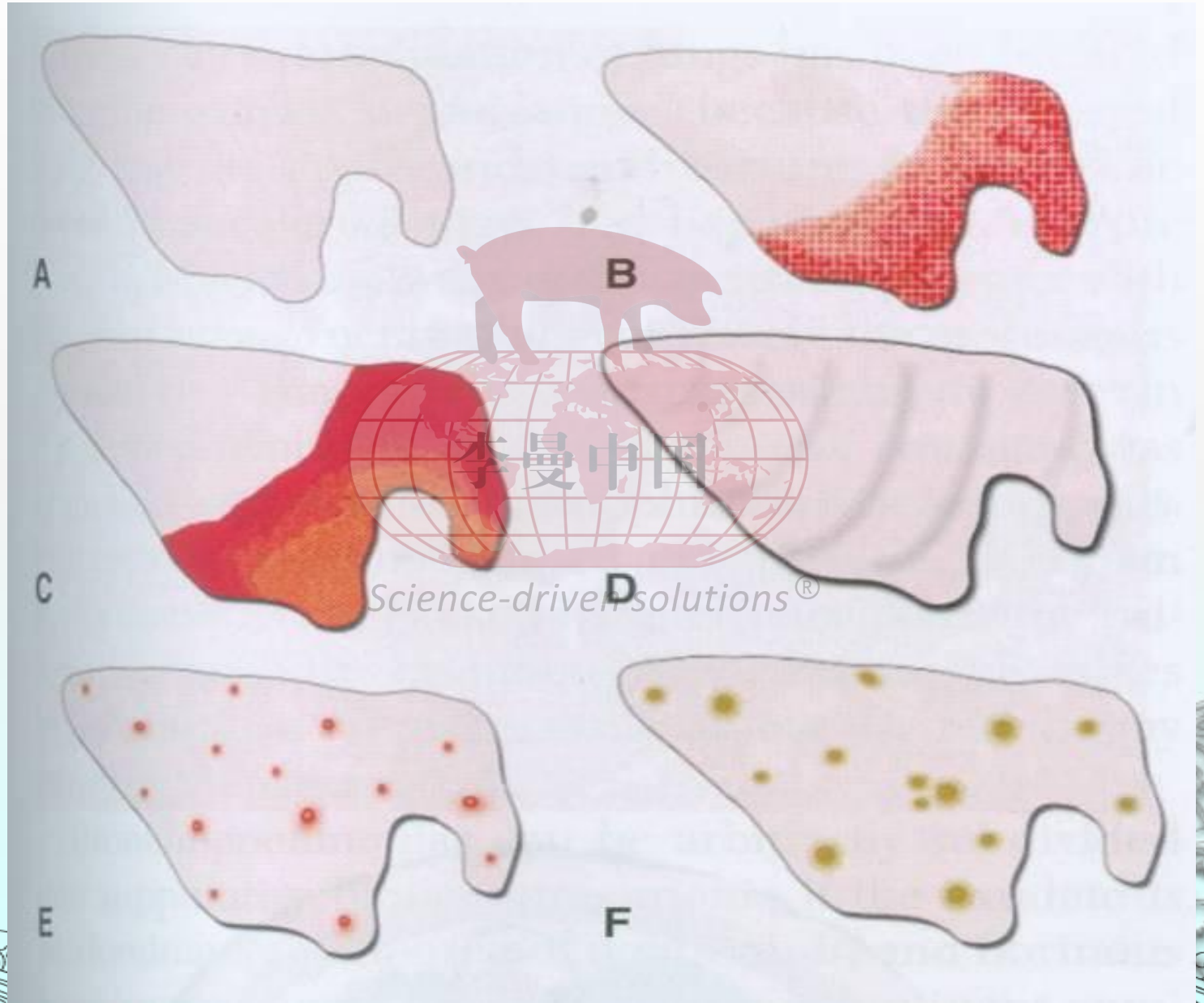


Source: Ernie's Slides: GI, contributed by Ernest Sanford.

Pattern of pneumonia and lung lesions



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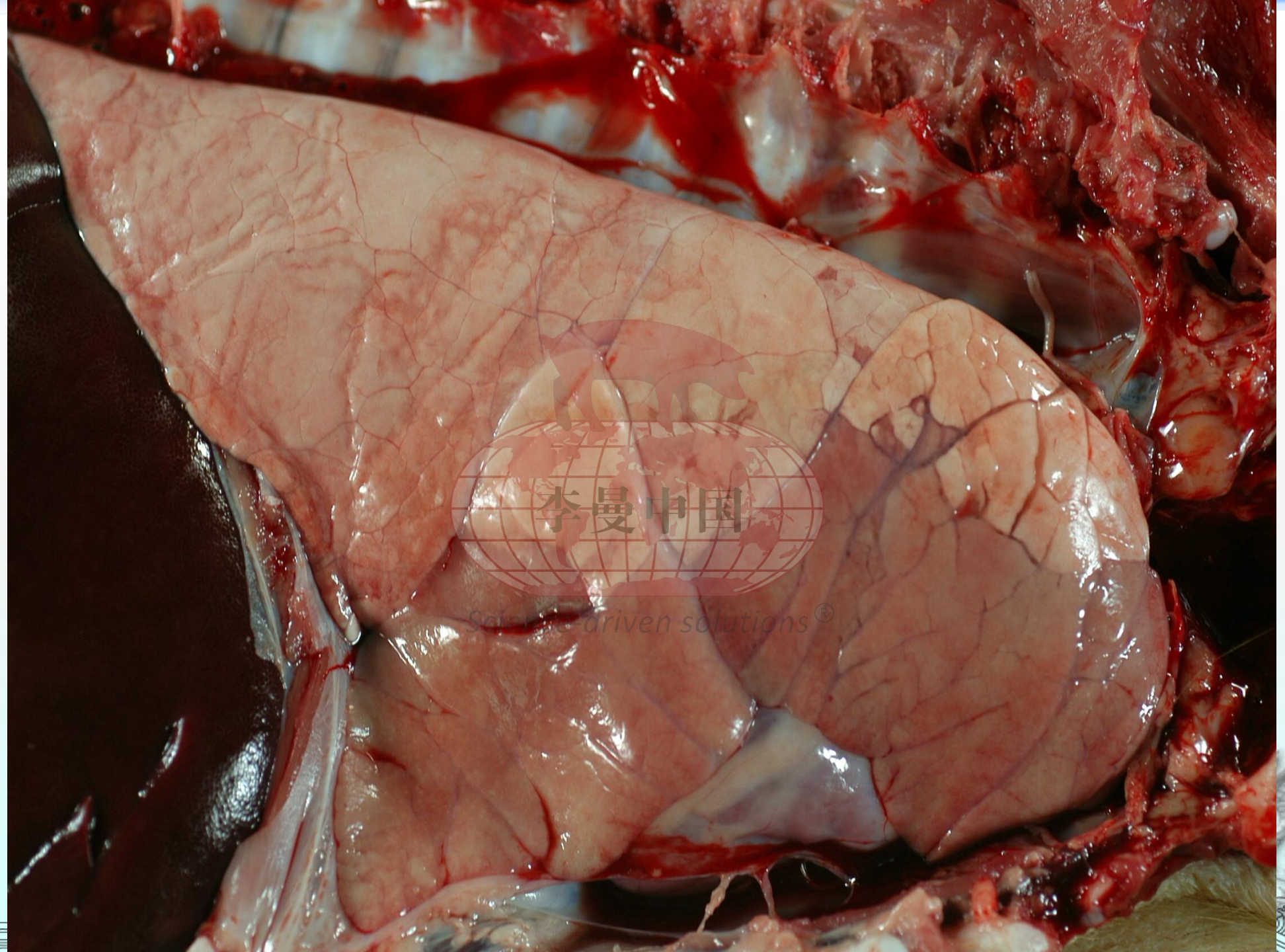
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Fig. 9-52 **Interstitial pneumonia, lung, feeder pig. A,** The lung is heavy, pale, and rubbery in texture. It also has prominent costal (rib) imprints *(arrows)*, a result of hypercellularity of the interstitium and the failure of the lungs to collapse when the thorax was opened. **B,** Transverse section. The pulmonary parenchyma has a "meaty" appearance and some edema, but no exudate is present in airways or on the pleural surface. This type of lung change in pigs is highly suggestive of a viral pneumonia. *(**A** and **B,** Courtesy Dr. A. López, Atlantic Veterinary College.)*

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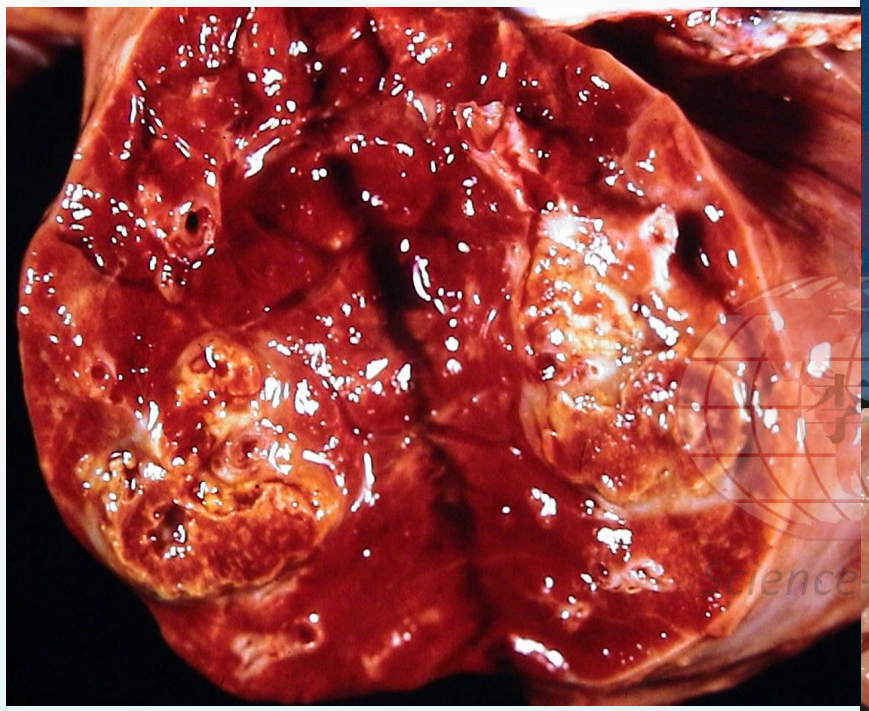

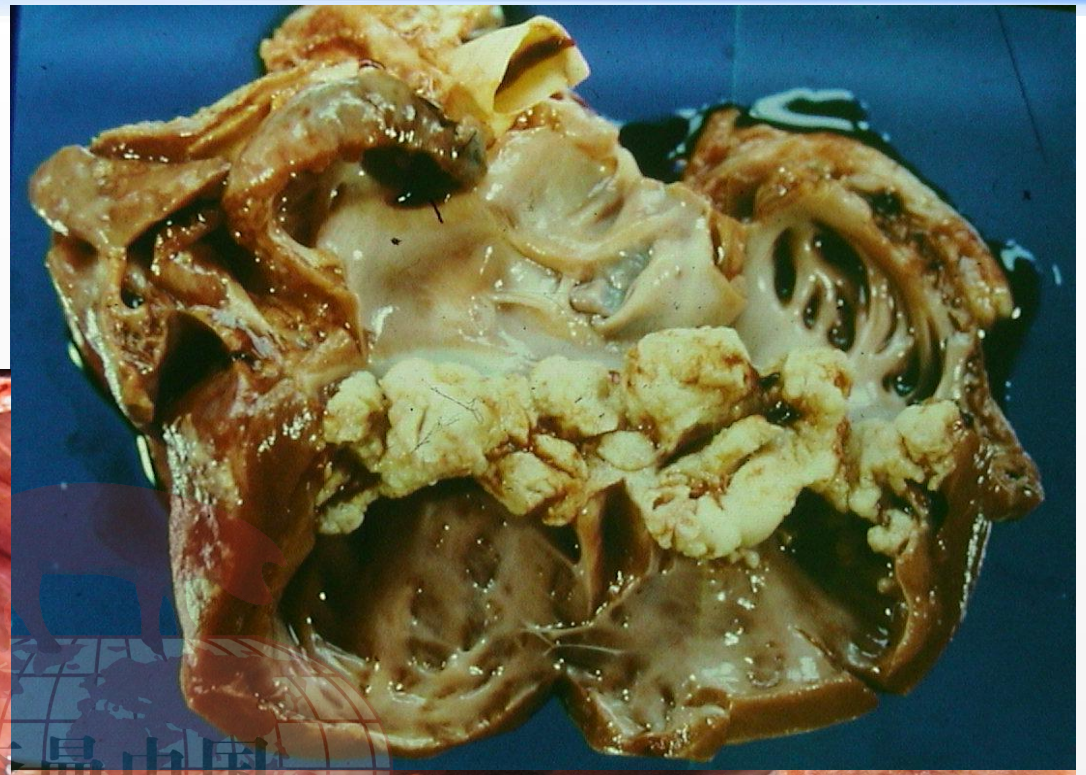

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Differenciated the Duration of Disease ---Acute or Chronic ?

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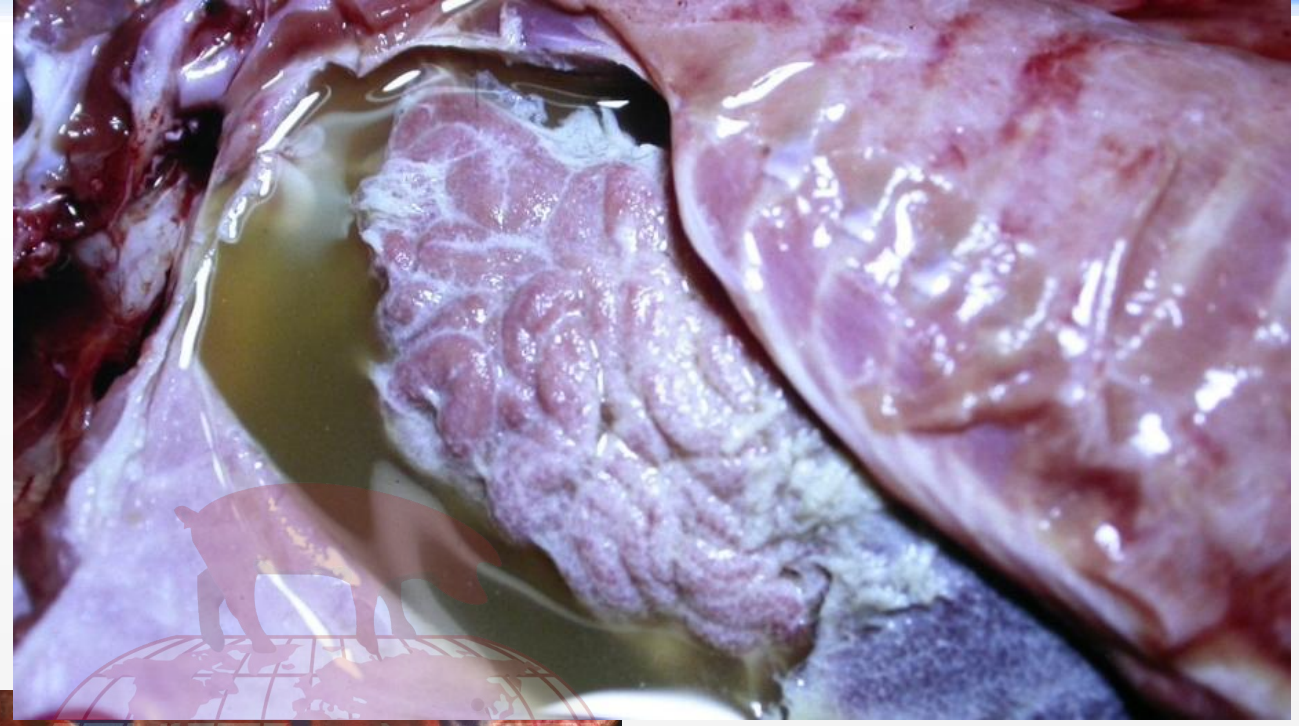

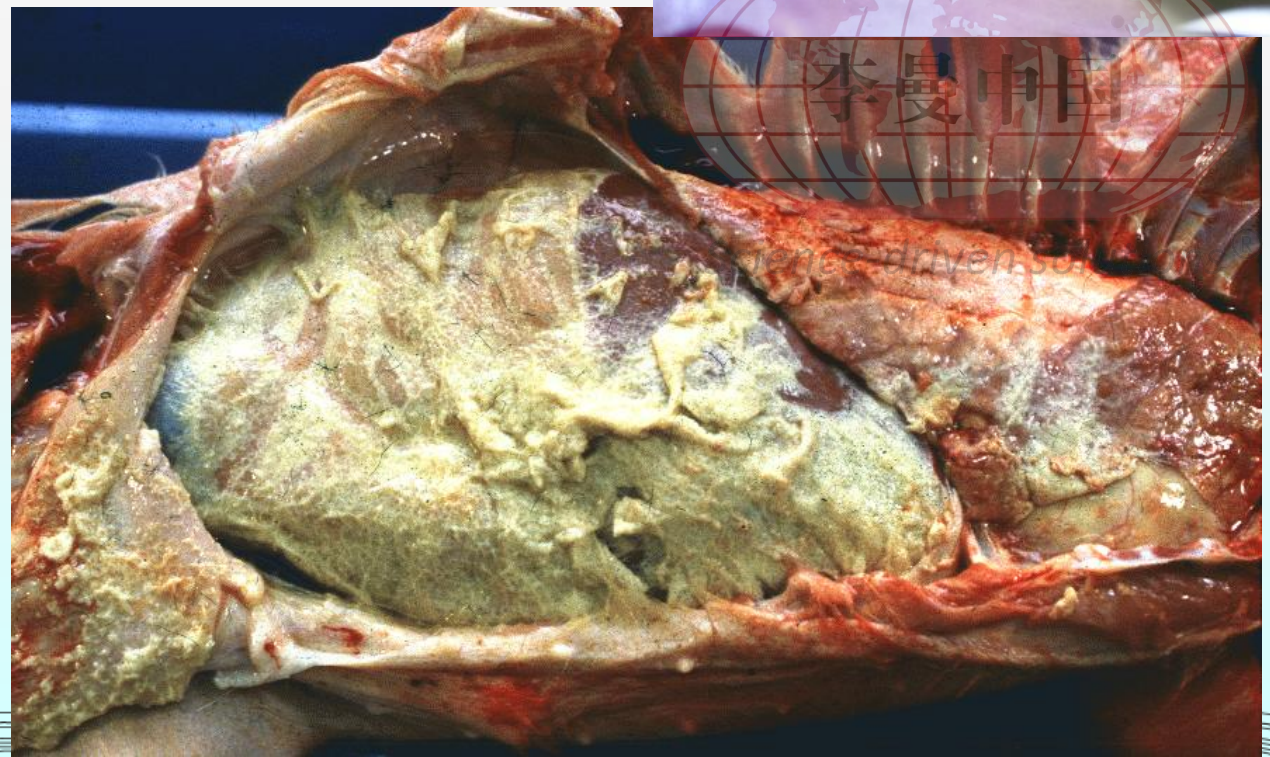

Case 1

Sow: 800 heads

Full-line production system

Suddenly death of grower and finished pigs in a short duration and high mortality. Cyanosis could found in every dead pig.

No medicinal feed addition in this period.

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Erysipelas ?

APP ?

Salmonellosis ?

April 1, 2017

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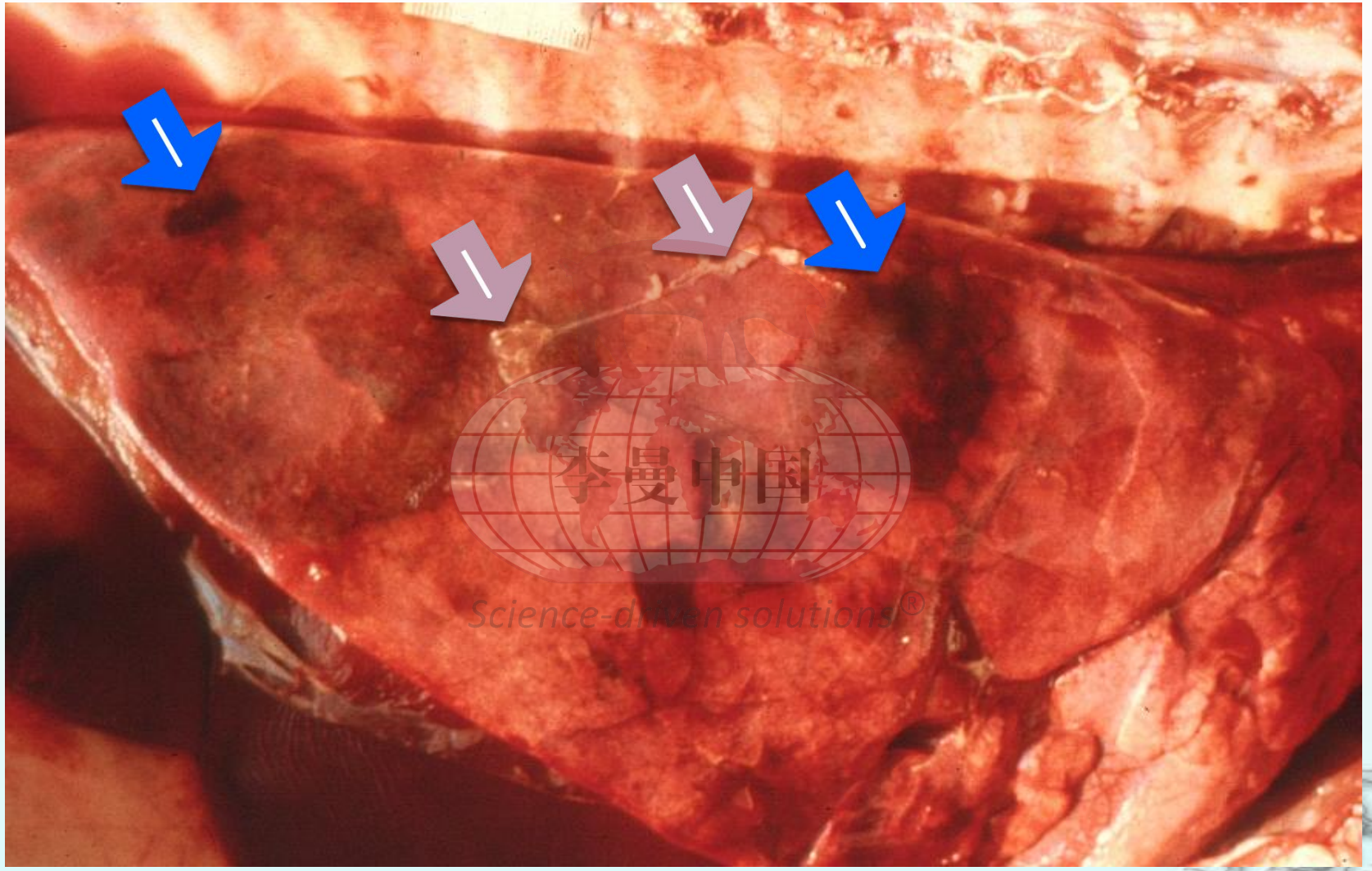
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Emergency treatment

- Ceftiofur i.v. for the pigs in the same pen with no symptom/Feed additive Tilmicosin with 2 kg/Ton
- Separate sick animals for treatment
- Disinfect the pen and surrounding area

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The efficacy of antibiotics against 40 strains of *Atinobacillus pleuropneumoniae*

Antibiotic	Efficacy(%)
Tilmicosin	**91.7**
Ceftiofur	**90.0**
Florfenicol	**87.5**
Cephalothin	**87.5**
Enrofloxacin	**83.3**
Lincomycin/Spectinomycin	67.5
Flumequine	50.0
Gentamicin	39.5
Amoxicillin	32.5
Doxycycline	27.5
Ampicillin	26.3
Oxytetracycline	15.0

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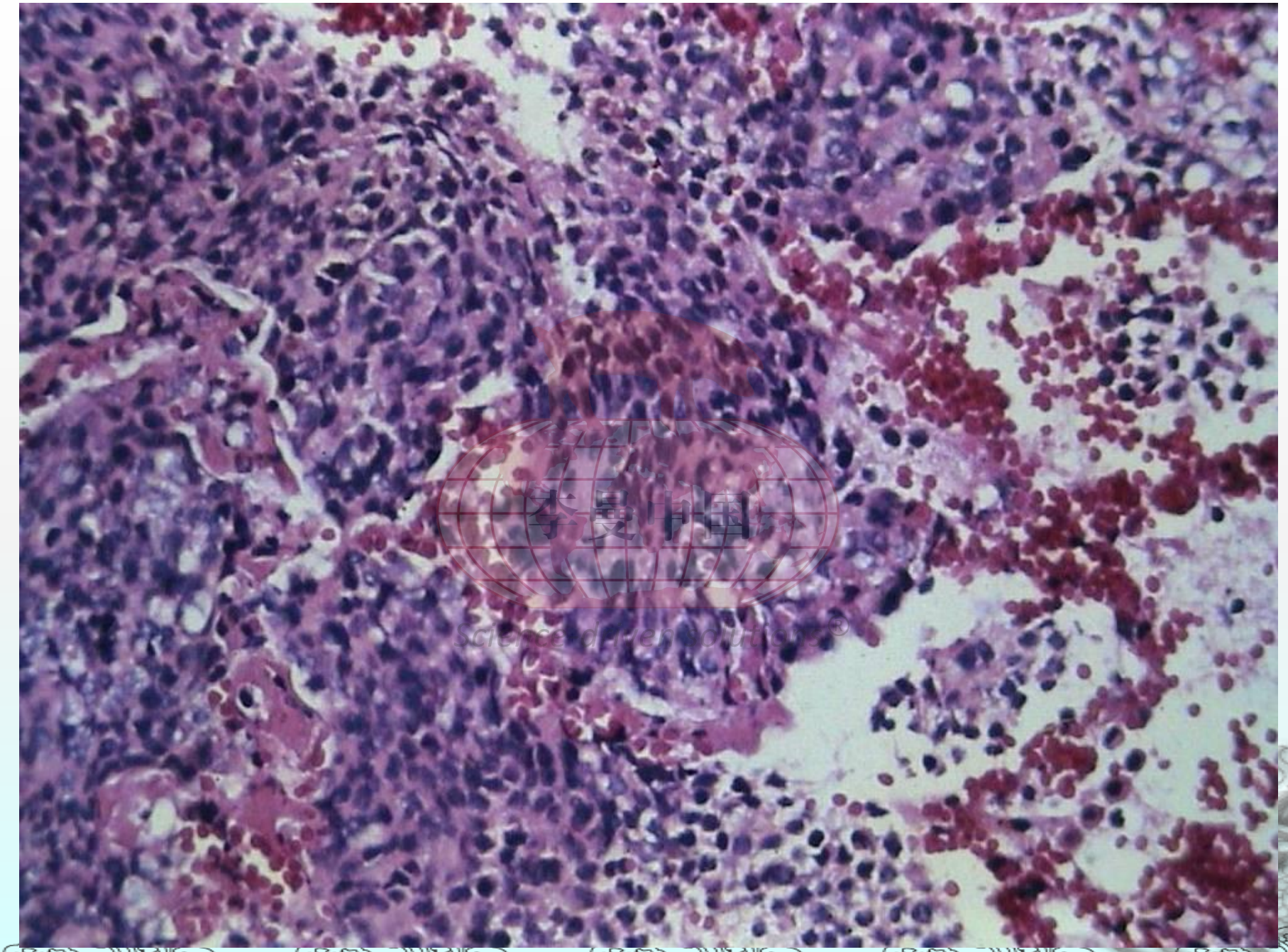

國立屏東科技大學動物疾病診斷中心
藥物敏感性試驗報告

畜主姓名：[redacted]　送檢人：[redacted]　收件人：[redacted]
病例編號：106-658　分離部位：肺臟　送檢日期：106/04/04
細菌鑑定結果：*Actinobacillus pleuropneumoniae*　報告完成日期：106/04/06

藥物名稱	每試錠藥物含量（μg）	藥物敏感性
Trimethoprim/Sulfamethoxazole	25	S
Lincomycin/Spectinomycin	109	S
Amoxicillin	25	R
Ampicillin	10	R
Enrofloxacin	5	S
Flumequine	30	S
Cephalexin	30	I
Cephalothin	30	S
Ceftiofur	30	S
Doxycycline	30	R
Oxytetracycline	30	R
Florfenicol	30	R
Gentamicin	10	R
Spiramycin	100	R
Tilmicosin	15	S
Streptomycin	10	R
Kanamycin	30	R
Apramycin	40	R
Colistin Sulphate	10	I

效果：R: Resistant (具抗藥性) < I: Intermediate (具中等感受性) < S: Susceptible (具感受性)

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Follow up

- Good results for controlling the outbreak by using antibiotics
- App vaccine were recommended for application in the farm

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Outbreak again

– Nov 27, 2017

– Nov 27, 2017

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國立屏東科技大學動物疾病診斷中心

豬病診斷總報告

畜主姓名：	[redacted]	病例編號：	D106-794	送檢日期：	2017/11/27
送 檢 人：	[redacted]	獸 醫 師：	[redacted]	報告完成日期：	2017/12/06
飼養模式：	一貫場	飼　料：	自配料	飼養規模：	母豬 800 頭
動物品種：	豬（LYD）	年　齡：	16 週齡	檢體狀態：	活體
場　址：	[redacted]	畜主/送檢人聯絡電話：	未提供		

現行免疫計畫：

	母豬	廠牌	仔豬	廠牌
豬瘟（CSF）			13 週齡	
假性狂犬病（PR）			8、10 週齡	維克（活毒）
豬萎縮性鼻炎（AR）			1、5 週齡	
豬生殖與呼吸綜合症（PRRS）			3 週齡	貿王
豬第二型環狀病毒（PCV2）			4 週齡	百靈佳
黴漿菌性肺炎（SEP）			1、3 週齡	
放線桿菌症（AP）			9、12 週齡	

藥物治療計畫：

針　劑：無。

飼料添加：無。

(一) 病歷：

該場為飼養規模 800 頭母豬且餵飼自配料之一貫化商業豬場。本週場內 16 週齡（70-80 kg）肥育豬大量死亡，該批豬隻曾於 7-10 週齡注射放線桿菌疫苗。本次疾病爆發病程約 1 週，發生率約 7-8%，致死率約 12%；類似情形於 3、4 月份時亦有發生。

(二) 肉眼病變：

豬隻虛弱不願站立，耳尖輕微發紺。剖檢後可見雙側尖葉、心葉呈現嚴重梅干色斑駁樣；右側橫膈膜葉臟層胸膜有些微纖維素附著，並有局部廣泛性暗紅色出血病灶；左側橫膈膜葉亦可見多發局部相同病灶區（圖 2、）。脾臟破裂並形成凝血塊（圖 5）。雙側腎臟表面及橫切面皆可見多發局部至局部廣泛性白色斑塊（圖 6、圖 7）。

其餘組織及臟器均無顯著之肉眼病理學變化。

圖 1

圖 2

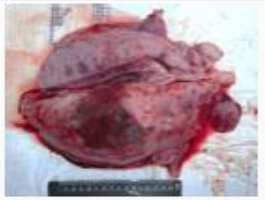

圖 3

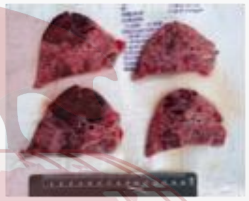

圖 4

圖 5

圖 6

圖 7

(三) 組織病變：

肺臟：胸膜可見纖維素附著，小葉間隔嚴重水腫擴張（圖 8、圖 9）；血管壁壞死出血，肺泡間質細胞增生，且有多量單核炎症細胞浸潤，其後壞死並有少量嗜中性球浸潤（圖 10、圖 11、圖 12、圖 13）；支氣管、細支氣管、肺泡腔內可見多量嗜中性球、壞死細胞碎片蓄積（圖 14）。

肝臟：可見多發局部壞死灶，並有單核炎症細胞浸潤（圖 15、圖 16、圖 17）；門脈三角區微膽管增生（圖 18）。

脾臟：瀰漫性嚴重出血（圖 19、圖 20、圖 21）。

腎臟：腎小管多發局部上皮細胞脫落，間質區域瀰漫性大量單核炎症細胞浸潤（圖 22、圖 23、圖 24、圖 25）。

其餘組織及臟器均無顯著之組織病理學變化。

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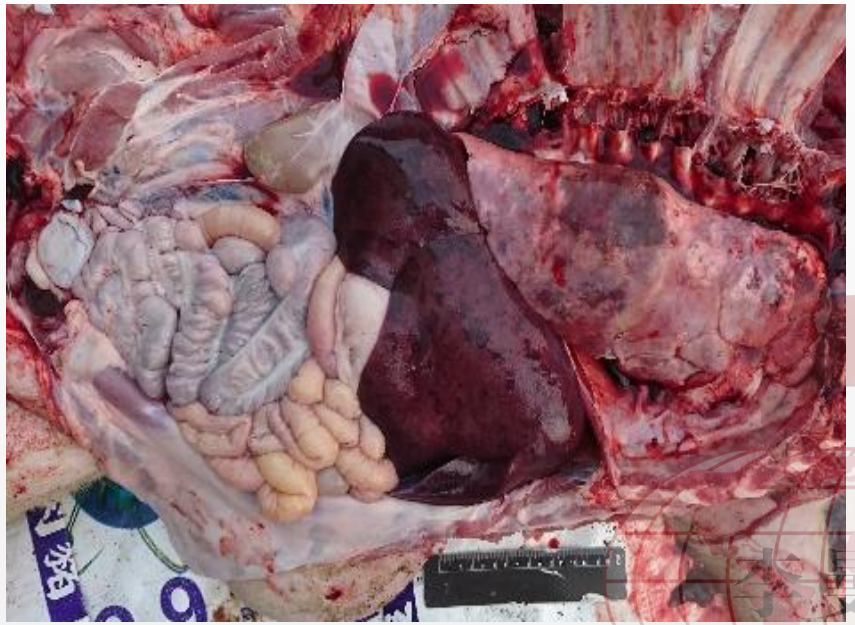
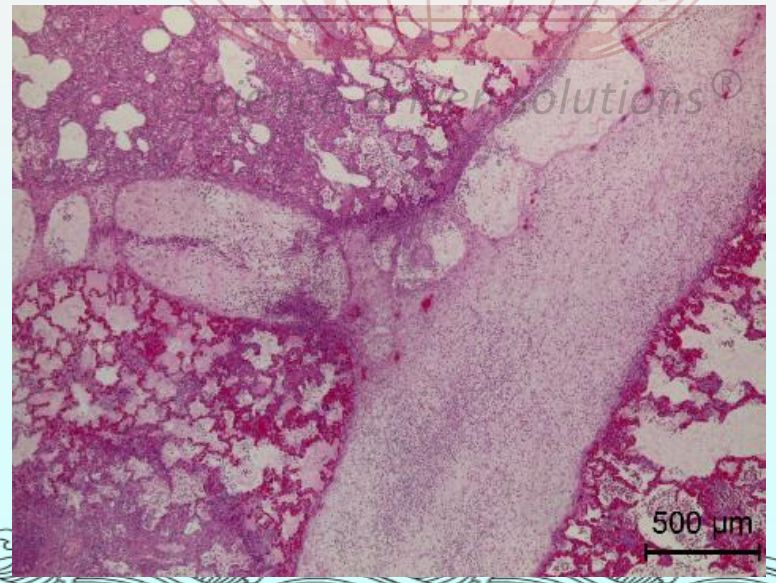


黃麴毒素
不高於100ppb
主要原料
CM


500 μm

國立屏東科技大學動物疾病診斷中心
豬病診斷總報告

(六) 分子生物學檢測結果：

檢測項目	檢體	結果	檢測方式
Porcine circovirus type 2 (PCV2)	血清	2.06×10^4	qPCR
Haemophilus parasuis	肺臟橫膈膜葉	−	PCR
Actinobacillus pleuropneumoniae	肺臟橫膈膜葉	+	PCR
Mycoplasma hyorhinis (Mhr)	肺臟尖心葉	+	qPCR
Mycoplasma hyopneumoniae (Mhp)	肺臟尖心葉	−	qPCR

*核酸定量結果，單位 copies/μL

PCV2 病毒血症檢測介於 10^3 至 10^4 copies/μL 可能出現豬環狀病毒相關疾病(Porcine circovirus associated disease, PCVAD）臨床症狀。高於 10^4 copies/μL 則與 PCVAD 臨床症狀具相關性。

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(七) 最終診斷：

1. 壞死性出血性纖維素性胸膜肺炎（疑似由放線桿菌 *A. pleuropneumoniae* 所引起）
2. 豬第二型環狀病毒（PCV2）感染症
3. 化膿性支氣管性肺炎
4. 壞死性肝炎
5. 脾臟破裂（疑似死後外力撞擊）

PCV2 viral load and specific antibody detection

12 週齡編號	S/P 值	Titer	判讀	16 週齡編號	S/P 值	Titer	判讀
12w-1	2.51	6329	+	16w-1	0.17	332	−
12w-2	1.42	3366	+	16w-2	0.05	86	−
12w-3	2.00	4925	+	16w-3	0.08	146	−
12w-4	2.42	6068	+	16w-4	0.18	345	−
12w-5	2.41	6050	+	16w-5	0.09	171	−
12w-6	1.27	2983	+	16w-6	0.09	161	−
12w-7	2.44	6113	+	16w-7	0.11	202	−
12w-8	1.33	3150	+	16w-8	0.96	2203	+
12w-9	2.22	5525	+	16w-9	0.79	1767	+
12w-10	1.01	2330	+	16w-10	0.10	176	−
平均值	1.90 ± 0.58	4684 ± 1557		平均值	0.26 ± 0.33	559 ± 763	
陽性率	100%			陽性率	20%		

週齡	PCV2 Quantity (copies/μL)	週齡	PCV2 Quantity (copies/μL)
12W-1	3.2×10^{2}	16W-1	4.2×10^{2}
12W-2	0	16W-2	4.8×10^{3}
12W-3	0	16W-3	1.4×10^{4}
12W-4	6.4×10^{1}	16W-4	6.3×10^{2}
12W-5	5.3×10^{1}	16W-5	3.0×10^{0}
12W-6	0	16W-6	3.3×10^{4}
12W-7	2.3×10^{1}	16W-7	0
12W-8	0	16W8	4.7×10^{4}
12W-9	7.5×10^{1}	16W-9	7.9×10^{6}
12W-10	0	16W-10	6.2×10^{4}
陽性率	50%	陽性率	90%
陽性平均值	7.1×10^{1}	陽性平均值	7.0×10^{3}

PCV2 病毒血症檢測介於 10^{3} 至 10^{4} copies/μL 可能出現豬環狀病毒相關疾病（Porcine circovirus associated disease, PCVAD）臨床症狀；高於 10^{6} copies/μL 則與 PCVAD 臨床症狀具相關性。

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- Replace with another PCV2 vaccine ！
- Optimize the vaccination process

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PCV2 viral load and specific antibody detection

12 週齡編號	PCV2 ELISA			PCV2 qPCR
	S/P	Titer	判讀	
12w-1	3.16	8137	+	0
12w-2	1.14	2649	+	0
12w-3	1.78	4339	+	0
12w-4	2.44	6137	+	0
12w-5	2.56	6468	+	0
12w-6	2.29	5703	+	0
12w-7	2.01	4957	+	0
12w-8	2.64	6690	+	0
12w-9	2.60	6557	+	0
12w-10	2.84	7231	+	0
平均值	2.35 ± 0.58	5868 ± 1254		
陽性率	100%			0%

16 週齡編號	PCV2 ELISA			PCV2 qPCR
	S/P	Titer	判讀	
16w-1	2.63	6657	+	0
16w-2	1.46	3487	+	0
16w-3	2.76	7024	+	0
16w-4	2.33	5818	+	0*
16w-5	1.77	4307	+	0
16w-6	2.61	6601	+	0
16w-7	1.96	4800	+	0
16w-8	2.02	4989	+	0
16w-9	2.52	6358	+	0
16w-10	2.21	5500	+	0
平均值	2.23 ± 0.42	5544 ± 890		
陽性率	100%			0%

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Mar. 29, 2018

Case 2

Nursery pigs with fever and sudden paralysis.

Morbidity around 5% and unresponsive to antibiotic treatment.

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Mortality reach 40% after clinical signs displayed.

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Differential Diagnosis

- Suppurative meningoencephalitis
  - *Streptococcus suis*
  - *Heamophillus parasuis*
  - *Mycoplasma hyorhinis*
  - Others
- Non-suppurative meningoencephalitis
  - Pseudorabies
  - Classical swine fever
  - Encephalomyocarditis virus infection
  - Others

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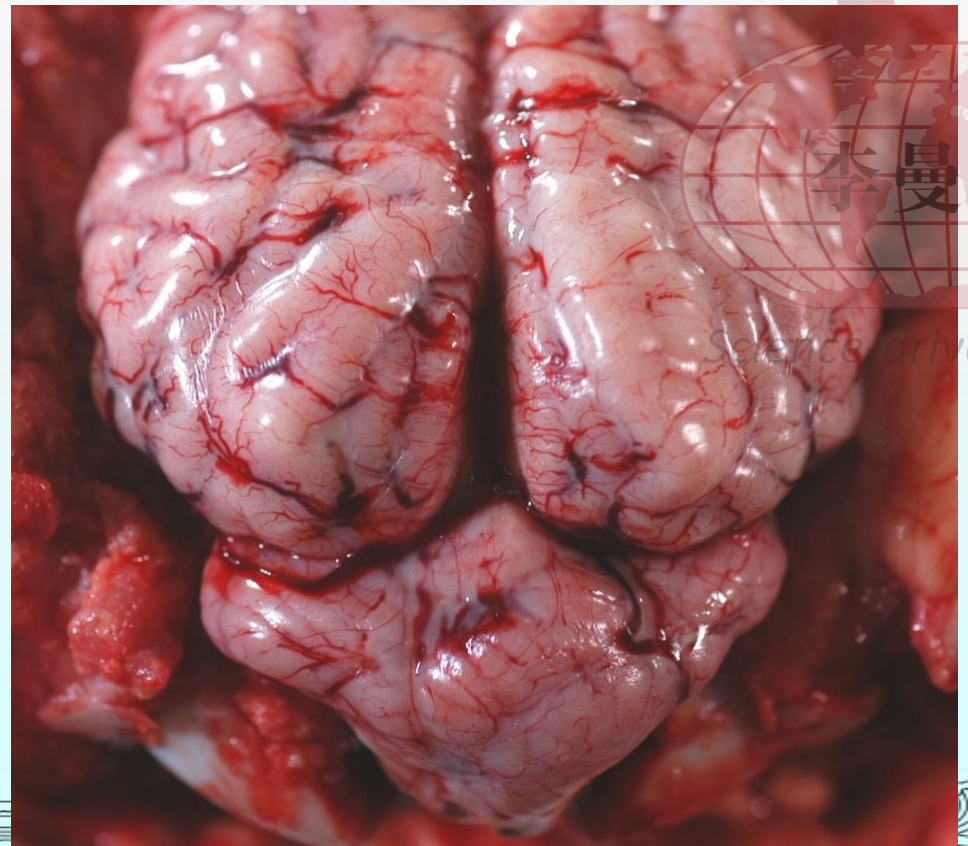
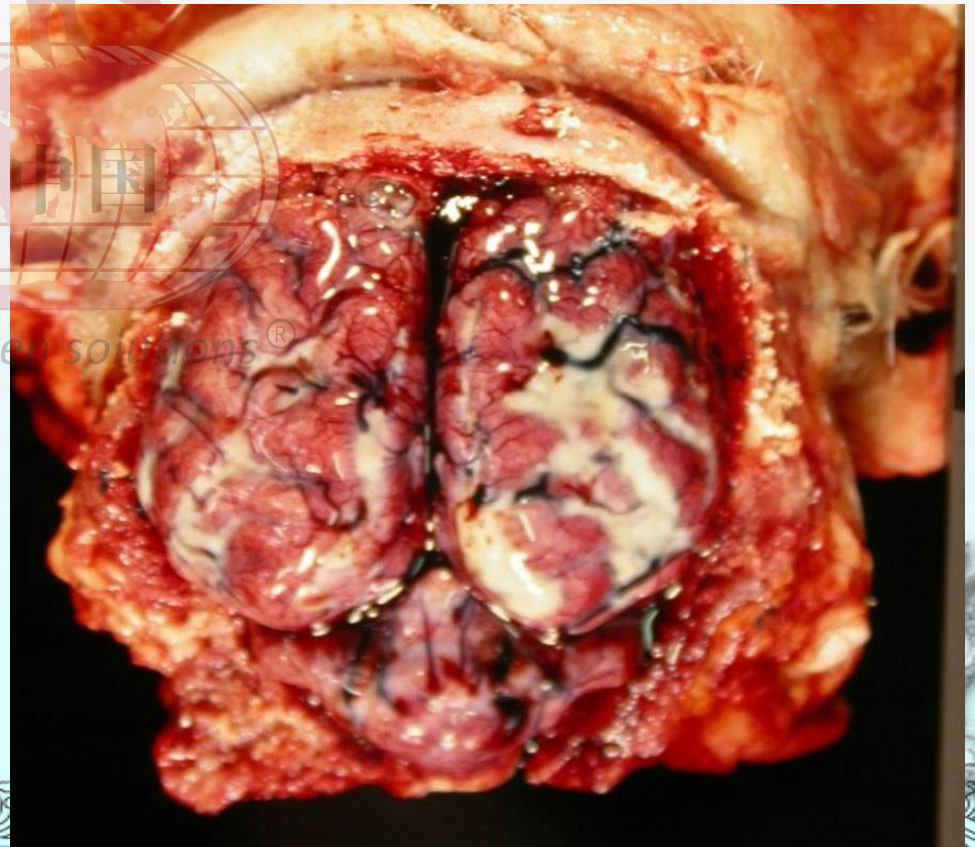
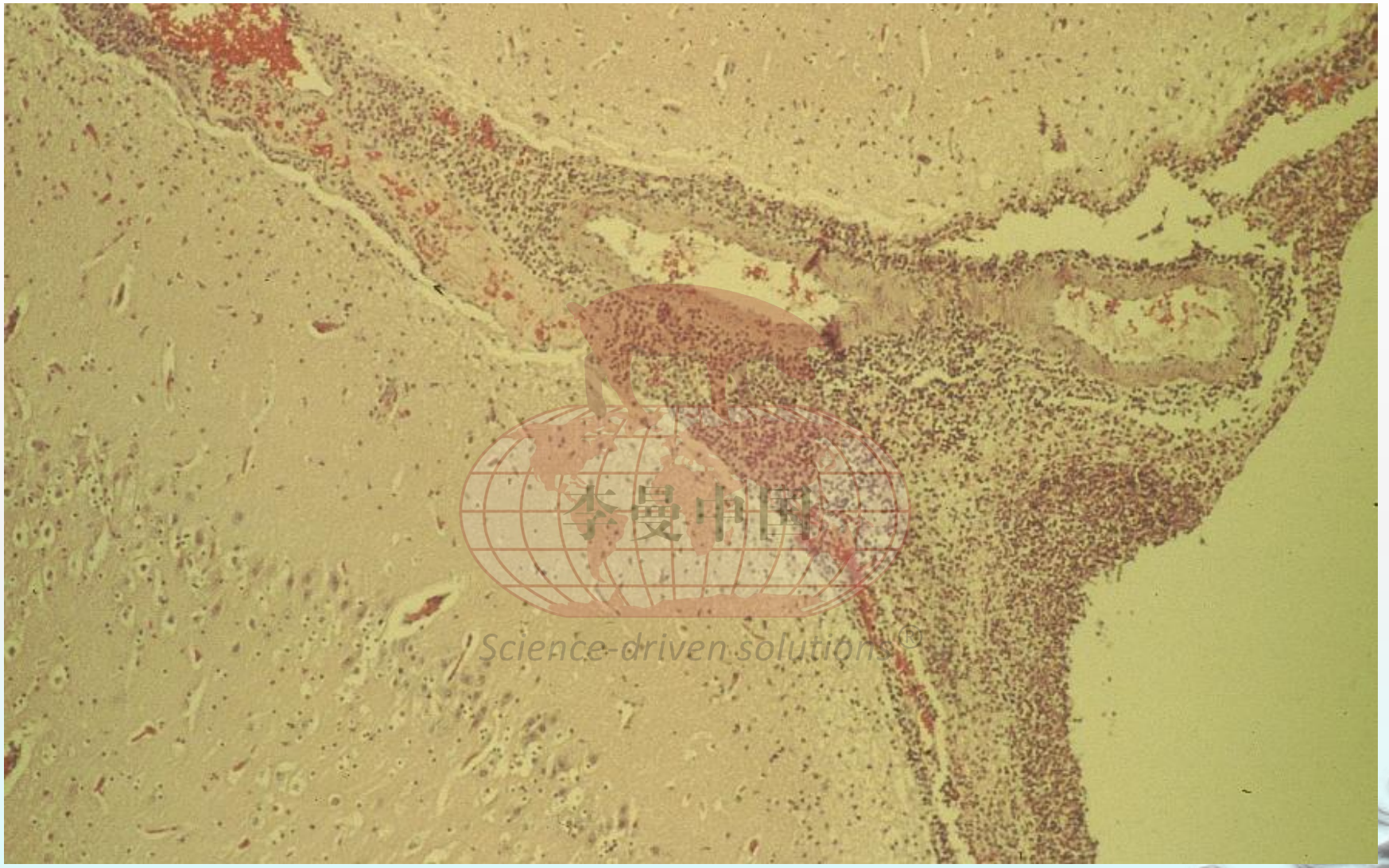
Suppurative Meningoencephalitis



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Treatment

Suppurative Meningoencephalitis

- Bacteria Isolation
- Molecular Detection
- Antibiotic Treatment

Non-Suppurative Meningoencephalitis

- Pathological Differential Diagnosis
- Molecular detection
- Immunization immediately

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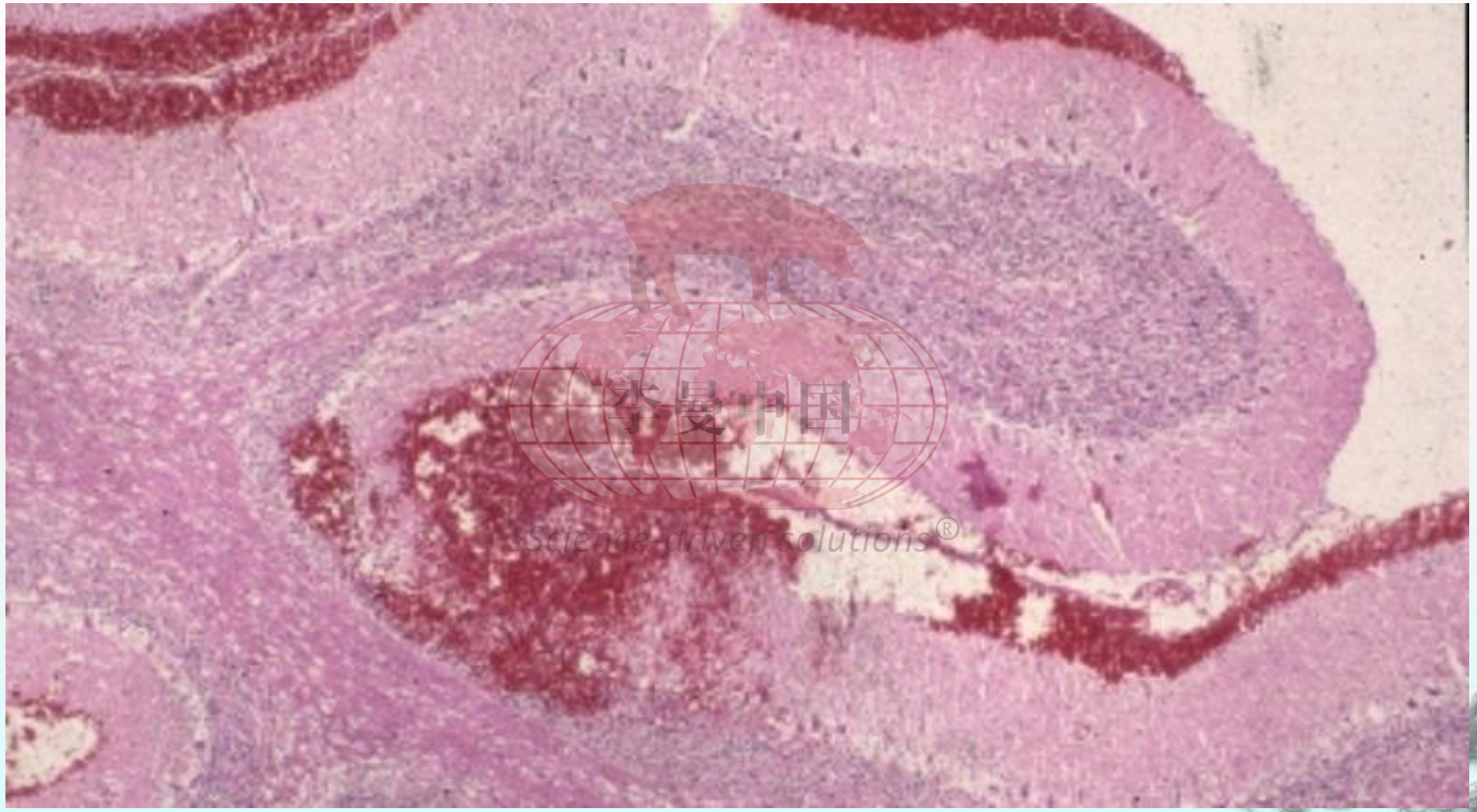
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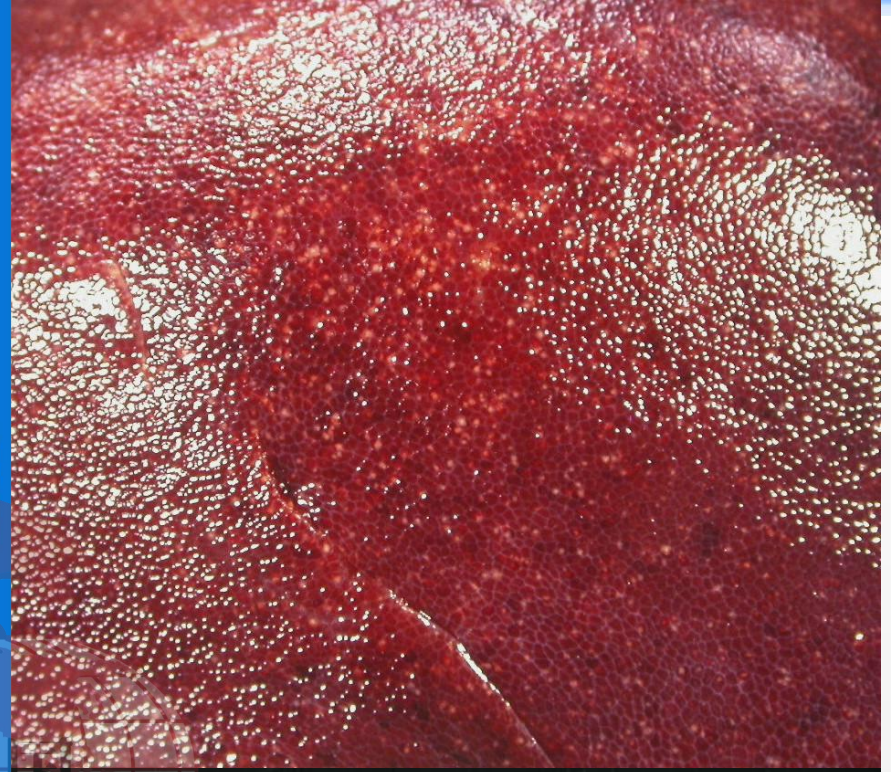

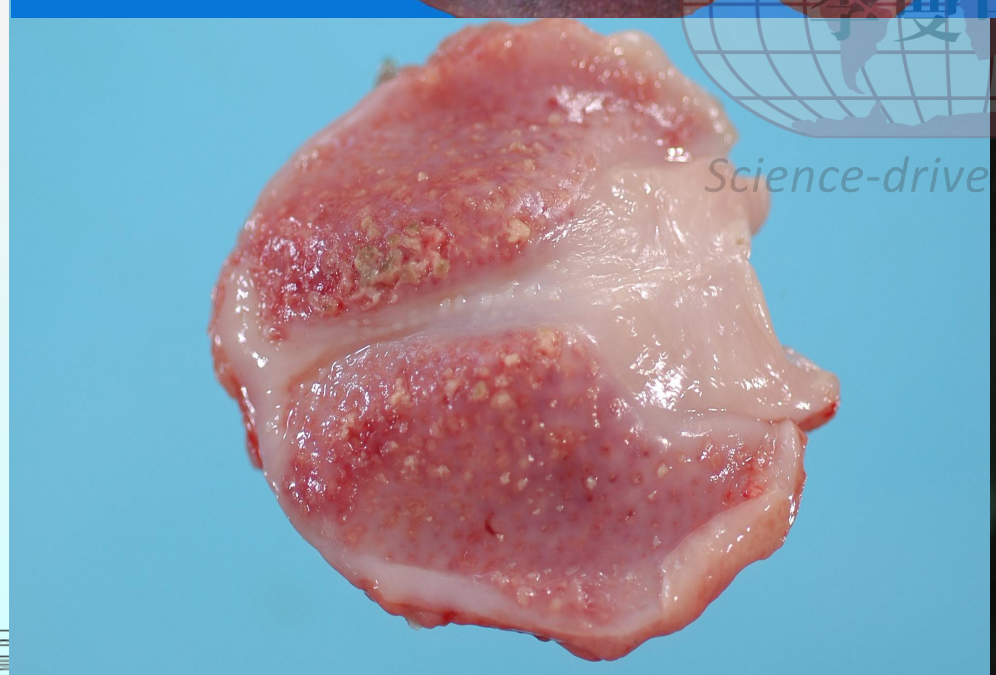

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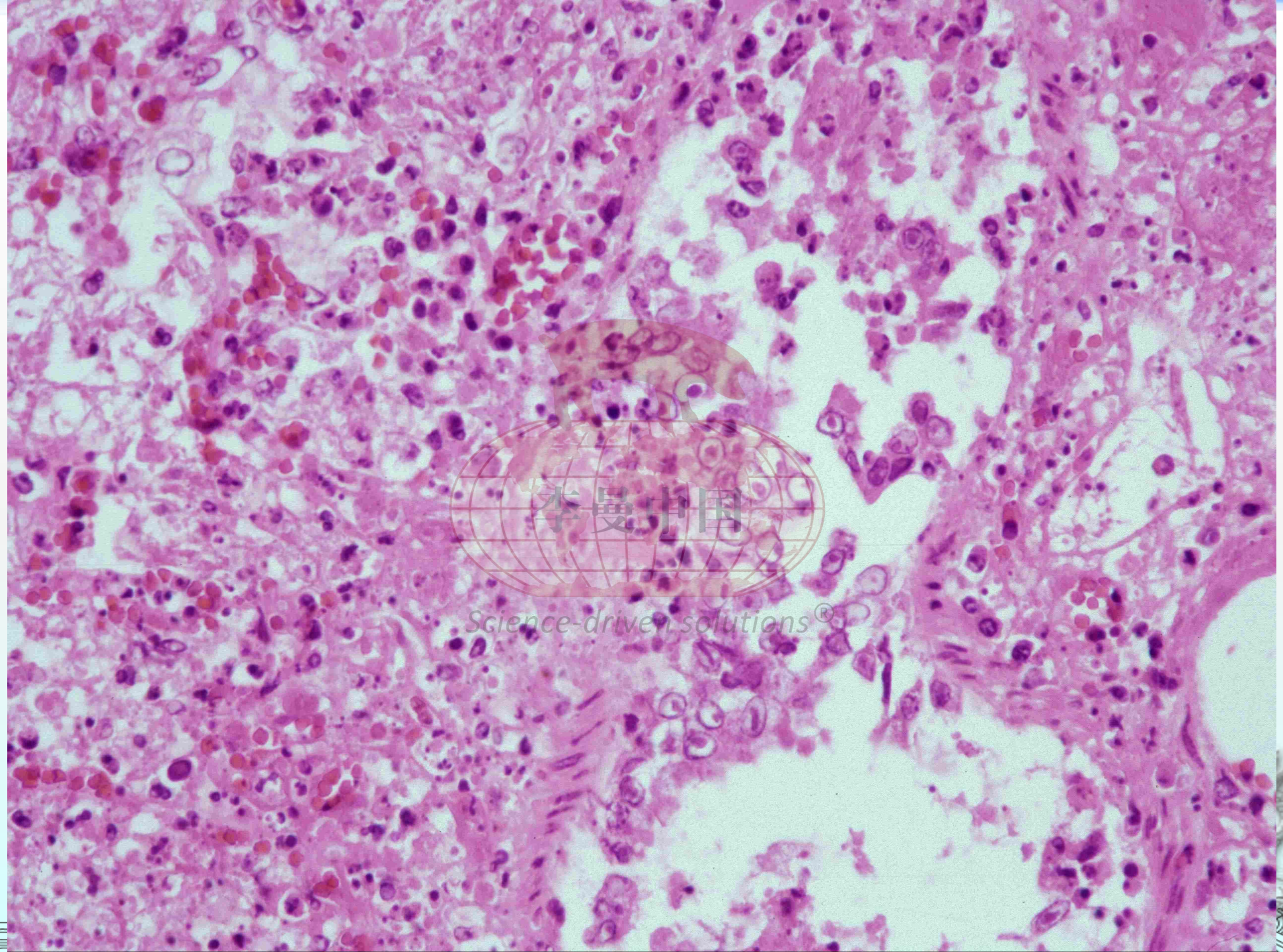
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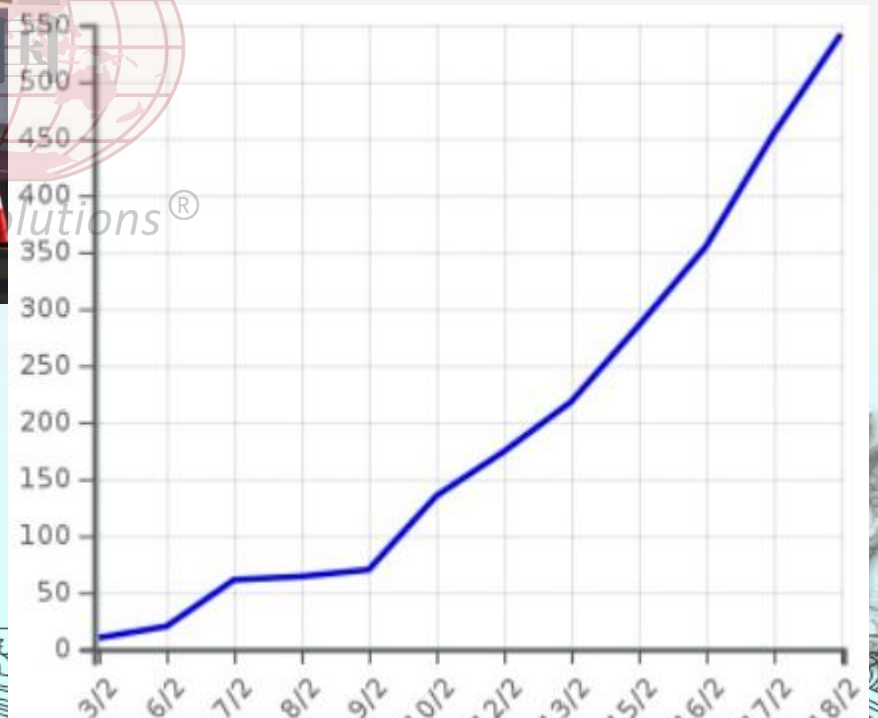
Current diagnostic tools are **time-consuming**



The COVID-19 infected case number in a ship

(Wikipedia, 2020/2/19)

The properties of a new diagnostic kit should include:

- Fast
- Precise
- Portable
- Quantifiable
- Price affordable
- Data shareable

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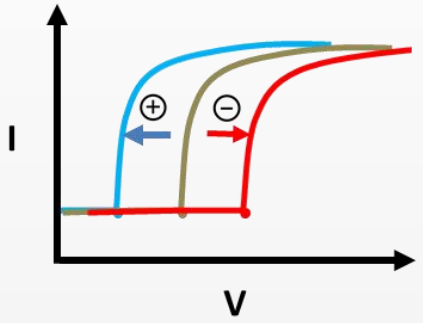
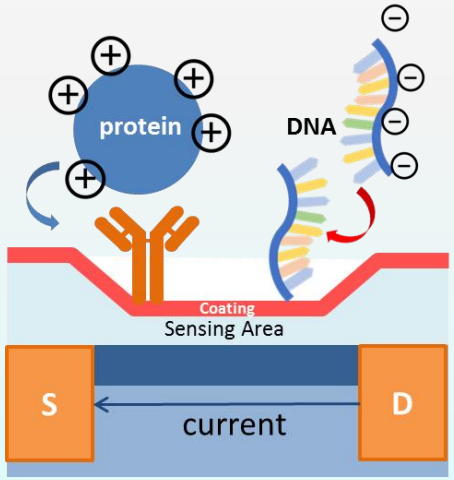
Newly Developed Biosensor system





- The sensor cells can be modified by antibodies or DNA probes
- The biosensor is capable of detecting the electronic signals of both protein and DNA targets
- An Handheld Device is already available

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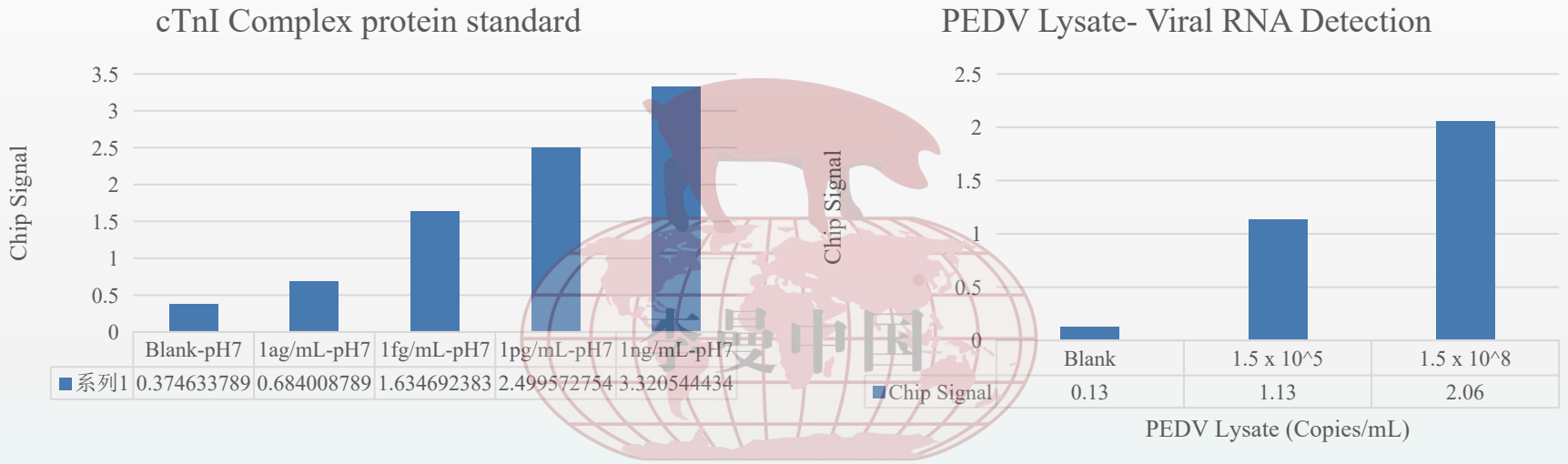
cTnI Complex Protein Standard and PEDV RNA Detection



- The new developed biosensor has been used to detect cTnI (Troponin I) protein in concentrations as low as 1ag/mL.
- The Biosensor is also capable of detecting viral RNA(1.5 x 10^5 copies/mL) in Porcine Epidemic Diarrhea Virus (PEDV) Lysate Samples.

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Thanks for listening

Questions ?