IPVS 2018 DIGESTED

BACTERIA IN VIEW OF IPVS

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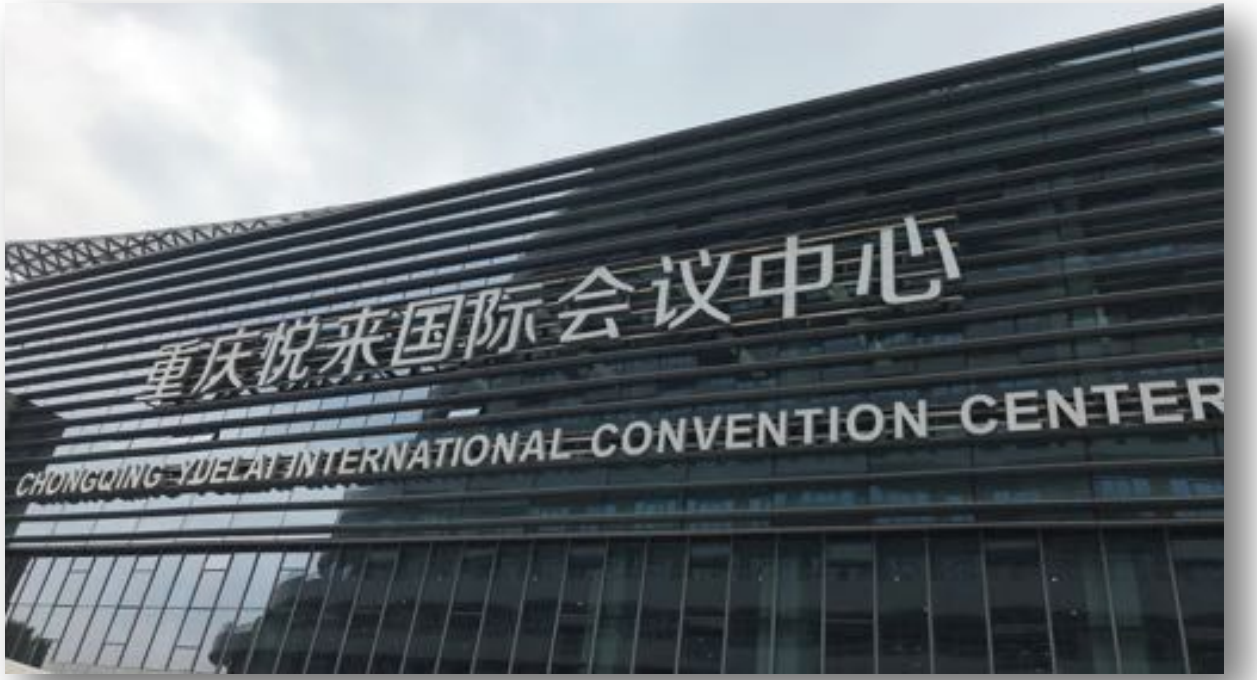

重庆悦来国际会议中心
CHONGQING YUELAI INTERNATIONAL CONVENTION CENTER

Outline talk

- Respiratory Bacteria
- Enteric bacteria
- Antimicrobial Resistance
- Alternative tools for AMR reduction
- Bacterial vaccine

RESPIRATORY BACTERIA

- *Mycoplasma hyopneumoniae*
- *Actinobacillus pleuropneumonia*
- *Haemophilus parasuis*

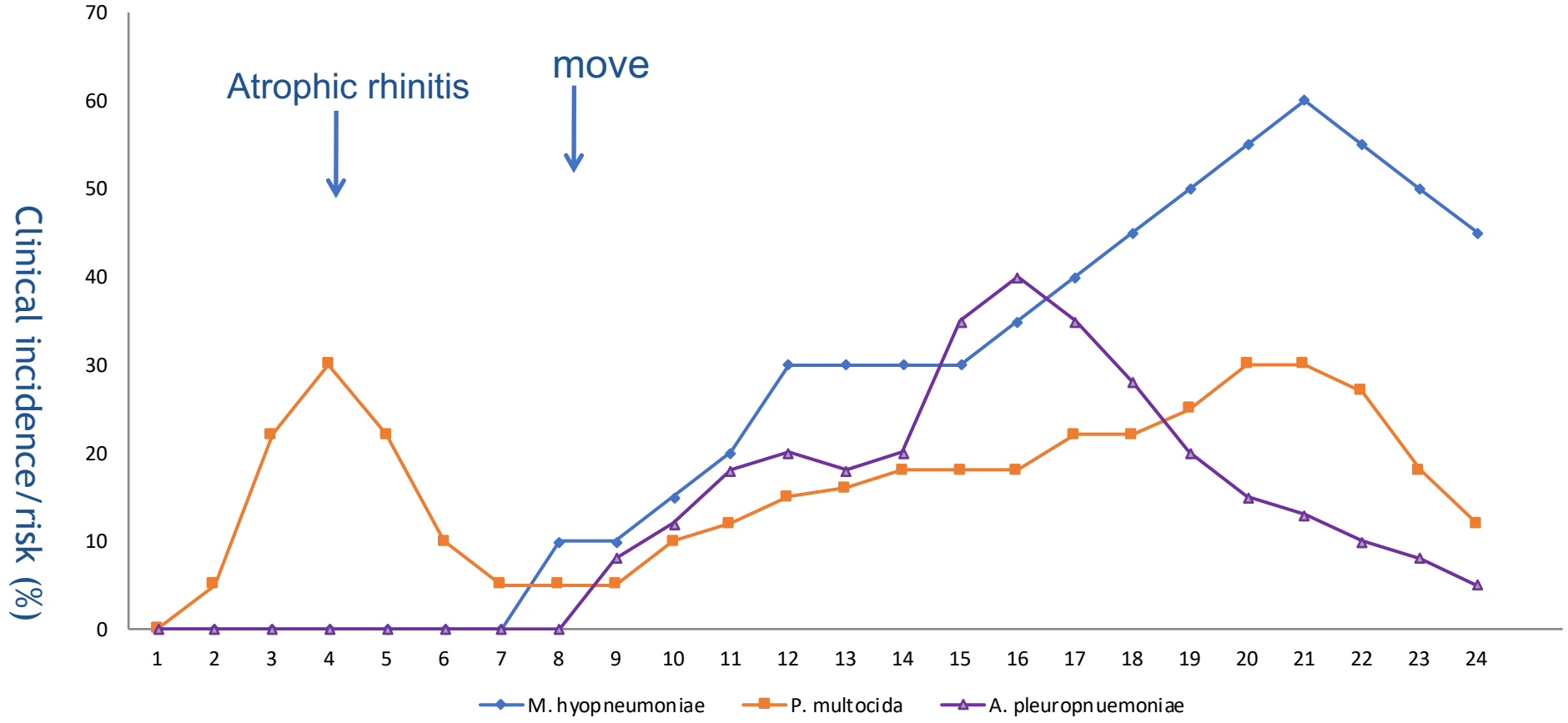
Infection
Epidemiology

Identification and
Detection

Pathogenesis and
pathogenicity

Strategic control and
treatment

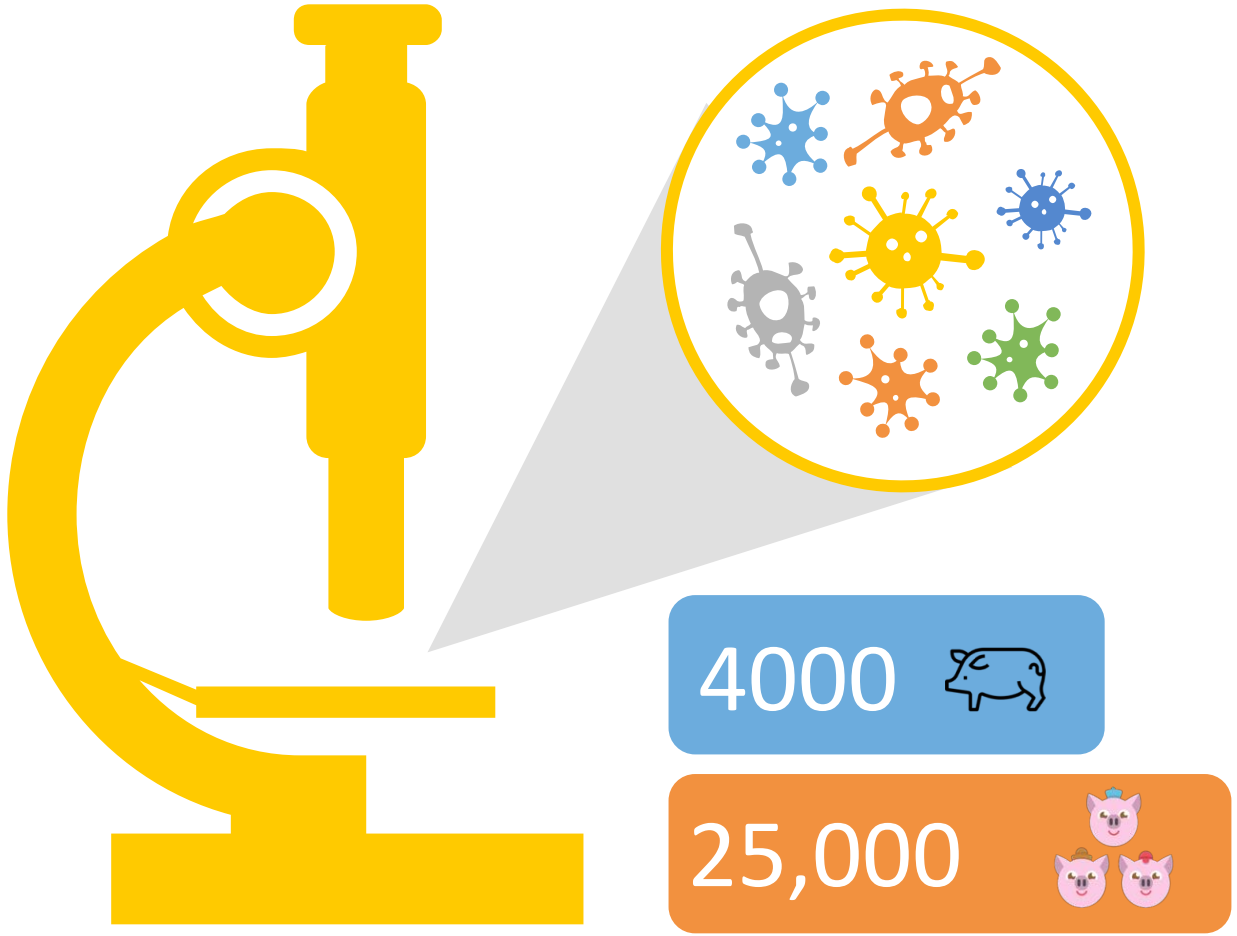
Respiratory disease patterns in swine



Common respiratory and septicemia bacterial pathogens

Respiratory bacteria	Diseases	Age
Pasteurella multocida *Bordetella bronchiseptica*	Atropic rhinitis	1-8 wks
Mycoplasma hyopneumoniae	Enzootic pneumonia	Grower to finisher
Pasteurella multocida	MIRD: mycoplasma-induced respiratory disease	Grower to finisher
Actinobacillus pleuropneumoniae	Pleuro-pneumonia	Grower to finisher
Streptococcus suis	Septicaemia/ MIRD Meningitis, arthritis, peritonitis	2-10 wks
Haemophilus parasuis	Glasser's disease (arthritis, pericarditis, peritonitis)	2-10 wks
Mycoplasma hyosynoviae	Mycoplasmal arthritis	16 wks plus
Erysipelothrix rhusiopathiae	Erysipelas	Grower to finisher and sows

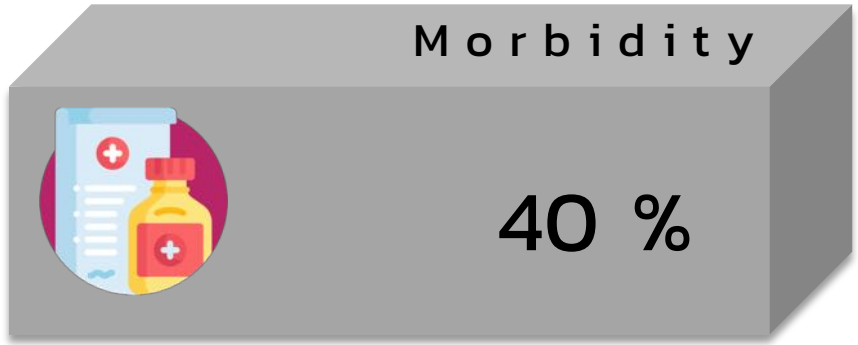
PRDC in a Chinese farm

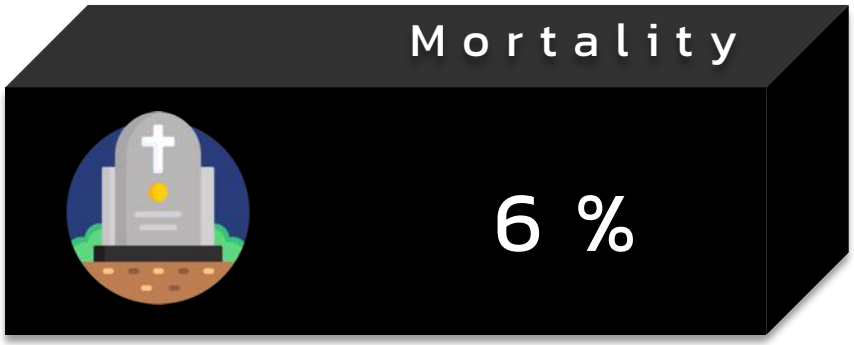


4000

25,000

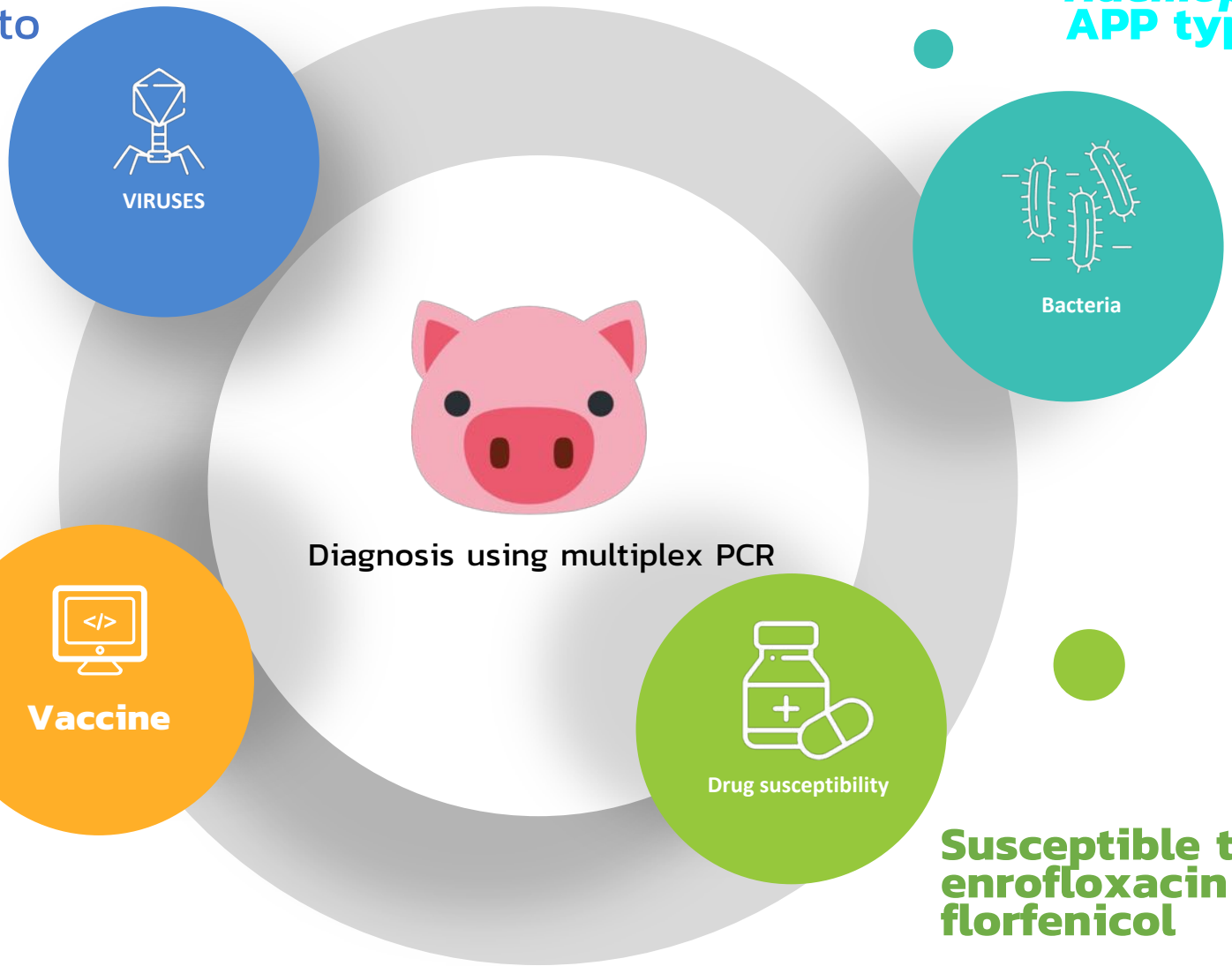
Infection
Epidemiology





PRRS, SIV, PCV2
(PRRS 87% homology to vaccine strain)

***Streptococcus suis, Haemophilus parasuis,* APP type 3**



Susceptible to enrofloxacin and florfenicol

Enzootic pneumonia:

Pathogenesis

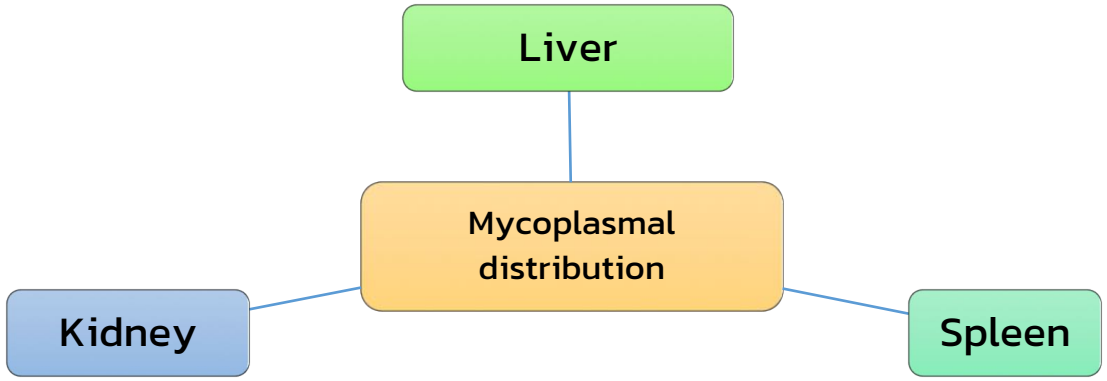
Mycoplasma hyopneumoniae

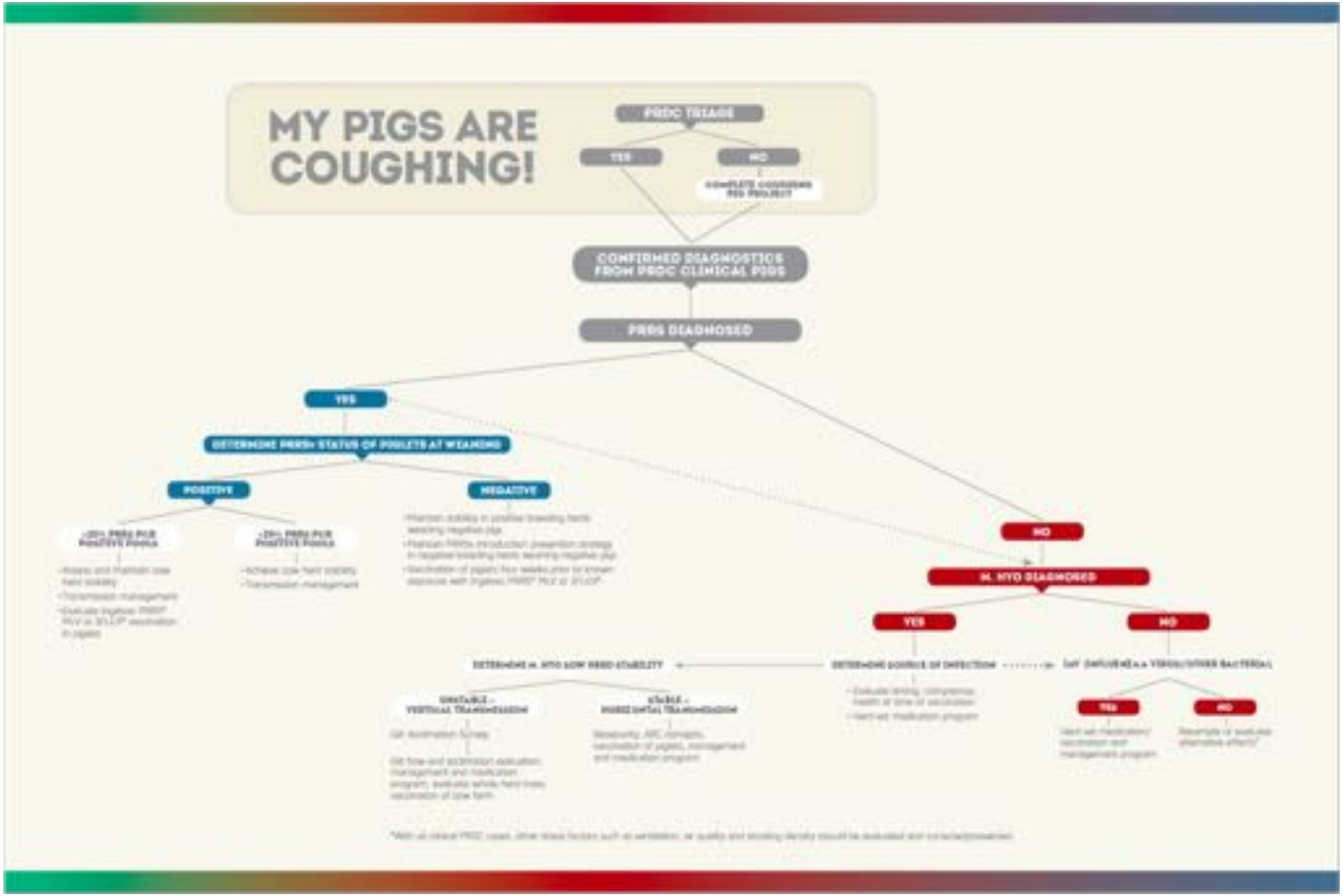
Higher virulent strain

- Higher capacity of host evasion toxic metabolites e.g. H_2O_2
- Toxic metabolites e.g. H_2O_2

Process of adherence

- Adhesin lipoprotein of MH (P97,P102,P159, P97/102)
- Receptor of glycoaminoglycans, plasminogen and fibronectin
- Tangle, clump and split of cilia cells resulting high susceptible to secondary pathogens




MY PIGS ARE COUGHING!
PRRSV TESTING
YES
NO
CONFIRMED DIAGNOSTICS FROM PRRSV CLINICAL PIGS
PRRS DIAGNOSED
YES
DETERMINE PRRSv STATUS OF DOG/ETS AT WEANING
POSITIVE
NEGATIVE
NO
M. HYO DIAGNOSED
YES
NO
DETERMINE M. HYO LOW HERD STABILITY
DETERMINE DEGREE OF INFECTION
SAT (MYCOPLASMA VIRAL/OTHER BACTERIAL)

Enzootic pneumonia:

Mycoplasma hyopneumoniae

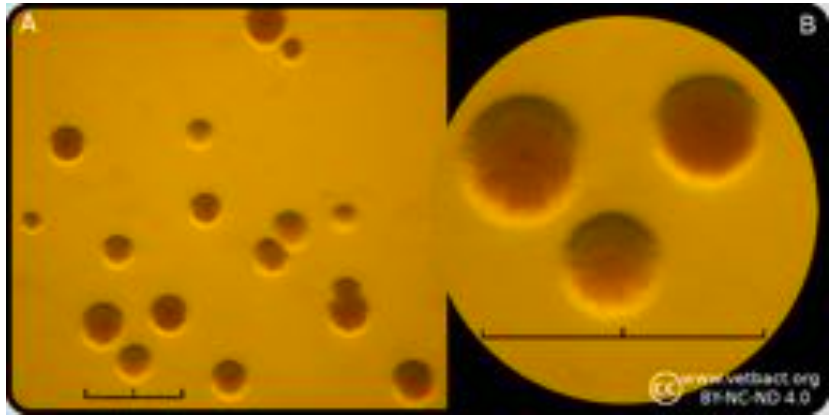
Diagnosis

High sensitivity

Lung, tonsil and synovial fluid

Low sensitivity

Oral fluid and nasal swab



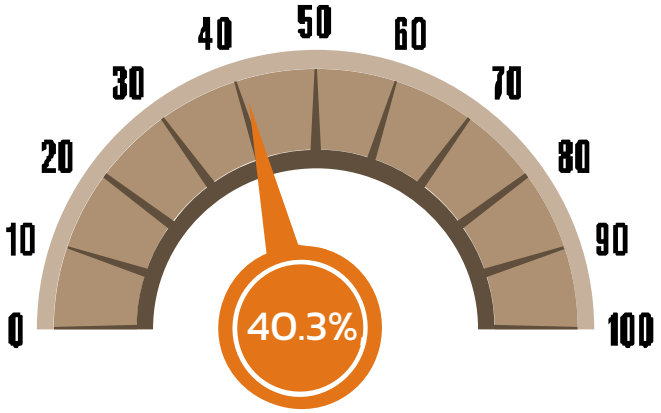
By ELISA test: Tracheal swab and still be recommended > laryngeal swab

Seropositive was not relevant to PCR detection

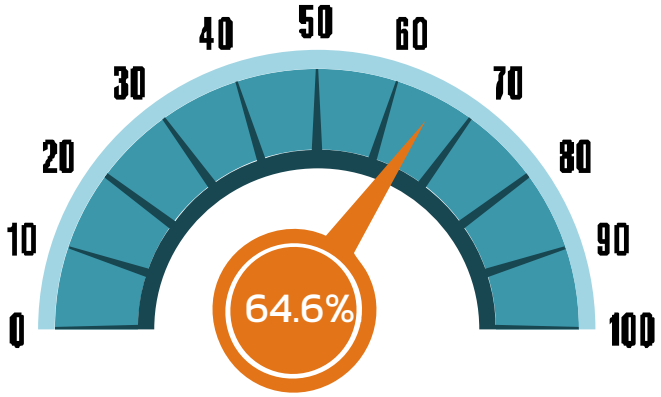
Enzootic pneumonia:

Mycoplasma hyopneumoniae

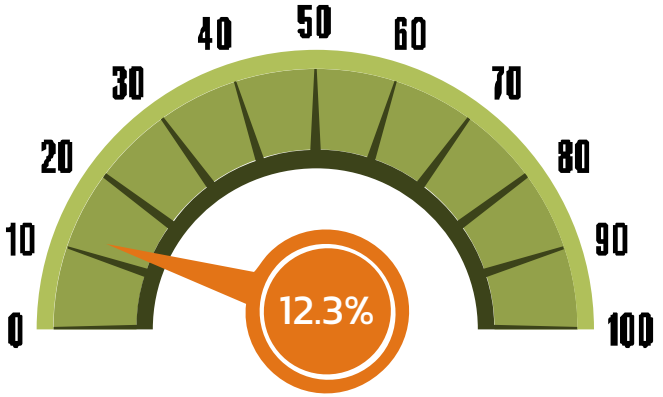
Diagnosis



M. hyopneumoniae



M. hyorhinis



M. hyosynoviae

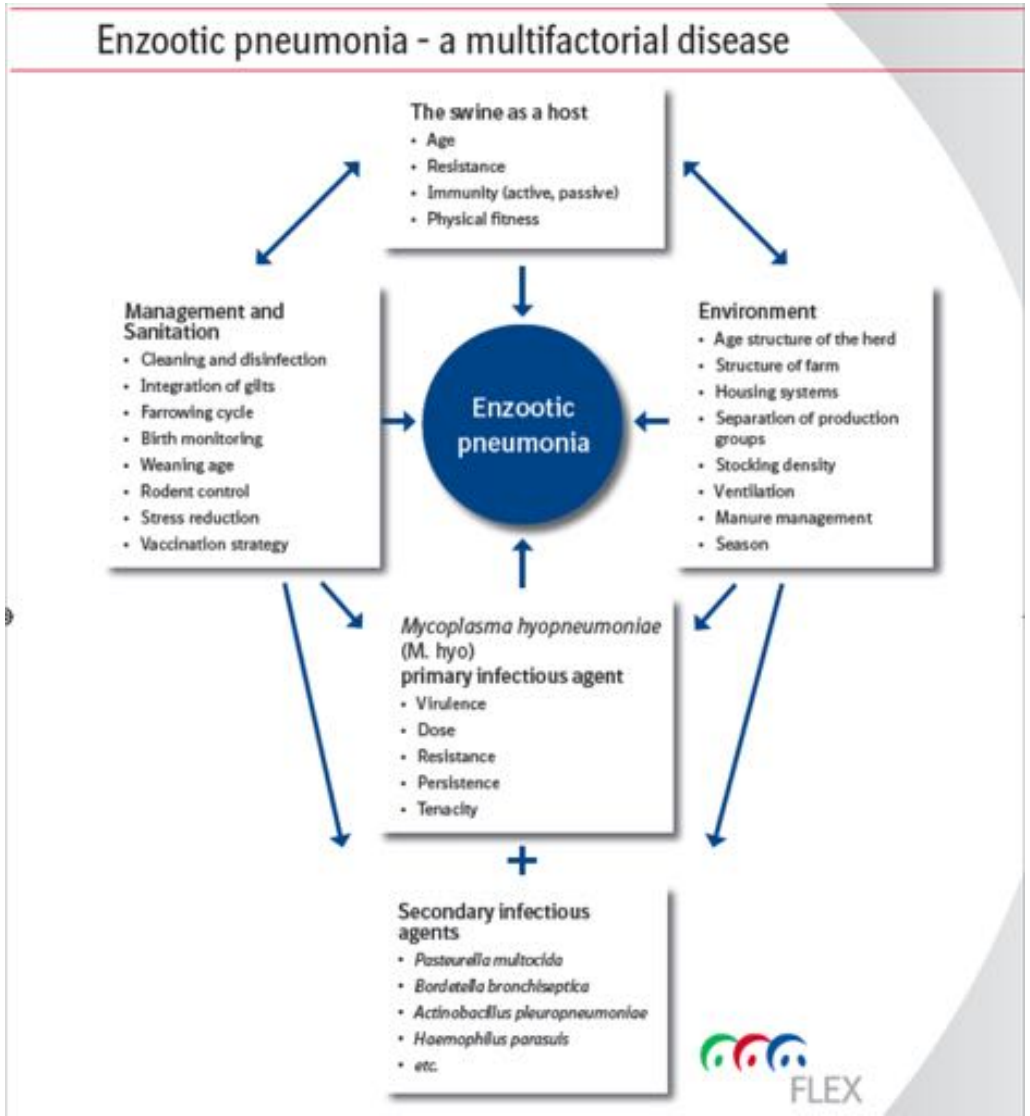
Direct PCR (DP) VS Culture prior to PCR (CPP)

M. hyopneuomoniae, By DP gave higher detection than CPP.

Enzootic pneumonia:

Mycoplasma hyopneumoniae

Managements

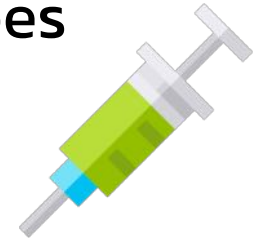


- **All-in/all-out production, stability of herd immunity, stock density, biosecurity**
- **Gilt acclimatization VS vaccine? (Garza-Morono et al, 2018)**
- **Two shot vaccines performed better lung score than one shot. (large scale study in China)**

Enzootic pneumonia:

Mycoplasma hyopneumoniae

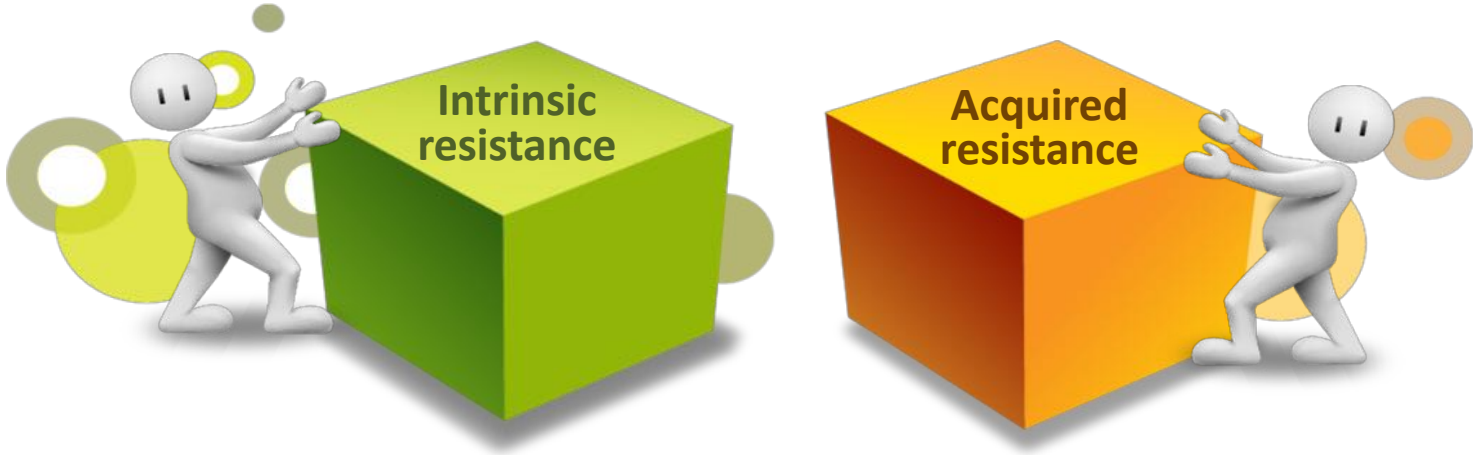
Vaccinations

Vaccine types 	Inactivated
	adjuvanted whole-cell
	recombinant vaccines (only experiment) (IM, ID needle-free)
Outcomes 	Lung lesion
	Reduce clinical sign
	Improve performance
	reduce number of the number of organism in respiratory tract
	Low number of infected piglets

Enzootic pneumonia:

Antibiotic Treatment

Mycoplasma hyopneumoniae



Polymyxin
Beta-lactam
Sulfonamide
Trimethoprim
14-membraned macrolide

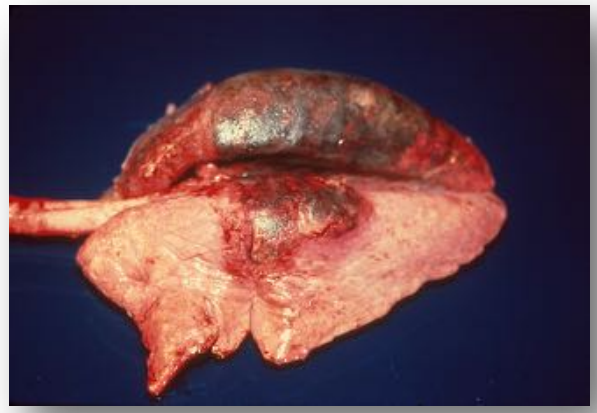
tetracylines,
16-membraned macrolides
(tylosin, tilmicosin),
lincosamides
fluoroquinolones

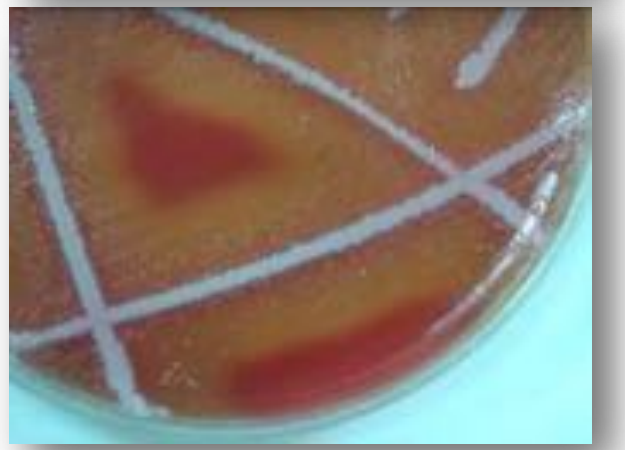
Treatment on the primary or secondary infection?

Arthritis and pleuropneumonia:

Biology and Diagnosis

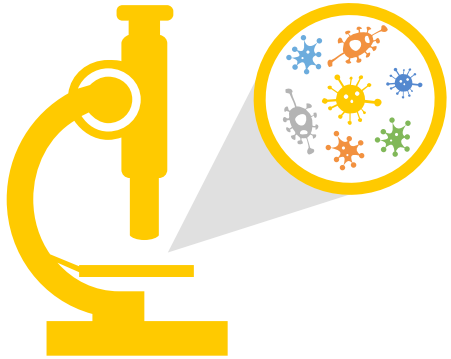
Actinobacillus pleuropneumoniae





- 2 biotypes (NAD+,–) 18 serotypes: APX toxin
- Multiple serotypes infection e.g. 1/9/11 or 3/6/8/15 and Serotype 2,9/11,1,5

In Thailand: serotypes 1-9-11 and 5a, 3-6-8, and 5a, 1-9-11, 3-6-8, 5a and 4-7



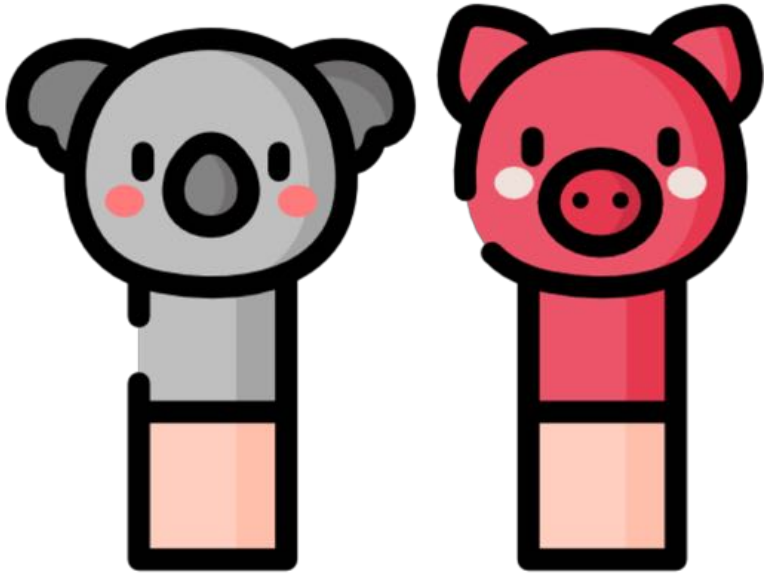
Diagnosis
in subclinical infection : ELISA using LPS based
in acute infection : isolation

Arthritis and pleuropneumonia:

Actinobacillus pleuropneumoniae

Boar and Sow

- APP in semen during AI process by real time PCR
- Semen contamination: APP, *A. seminis*, *Haemophilus*, *Brucella*
- Semen from sero-positive boar was negative by PCR



- Vaccination boosting for sows after fallowing must serotype specific
- Maternal immunity prolongs until 8 week age piglets.

Arthritis and pleuropneumonia:

APP to PRDC

Actinobacillus pleuropneumoniae

APP developing to PRDC upon

- Rapid temperature change
- High relative humidity
- Insufficient ventilation

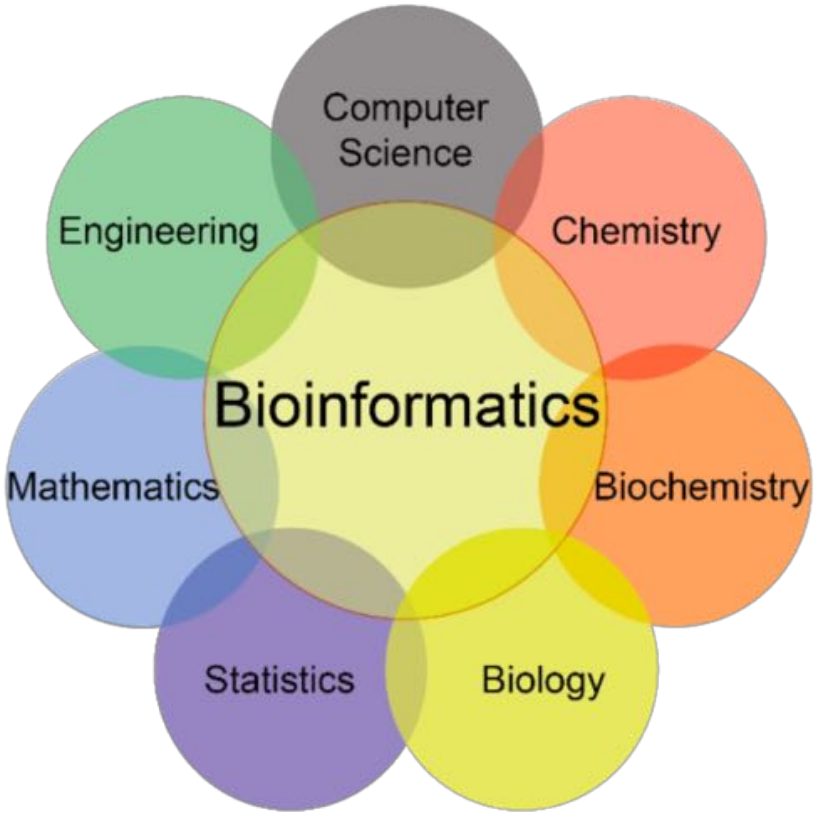
Arthritis and pleuropneumonia:

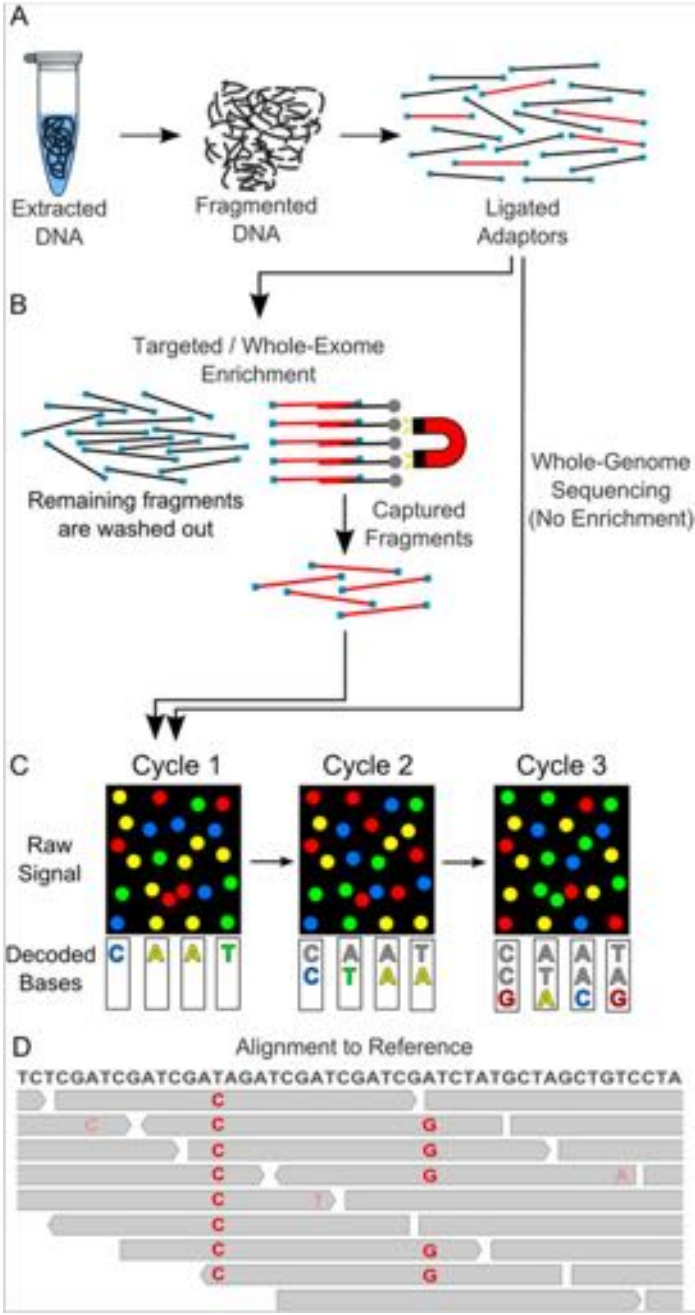
Actinobacillus pleuropneumoniae

Bioinformatics

Application of genomic data for host susceptibility detection

- Tool: Next Generation Sequencing
  - Three functional SNP on three chromosome as a marker





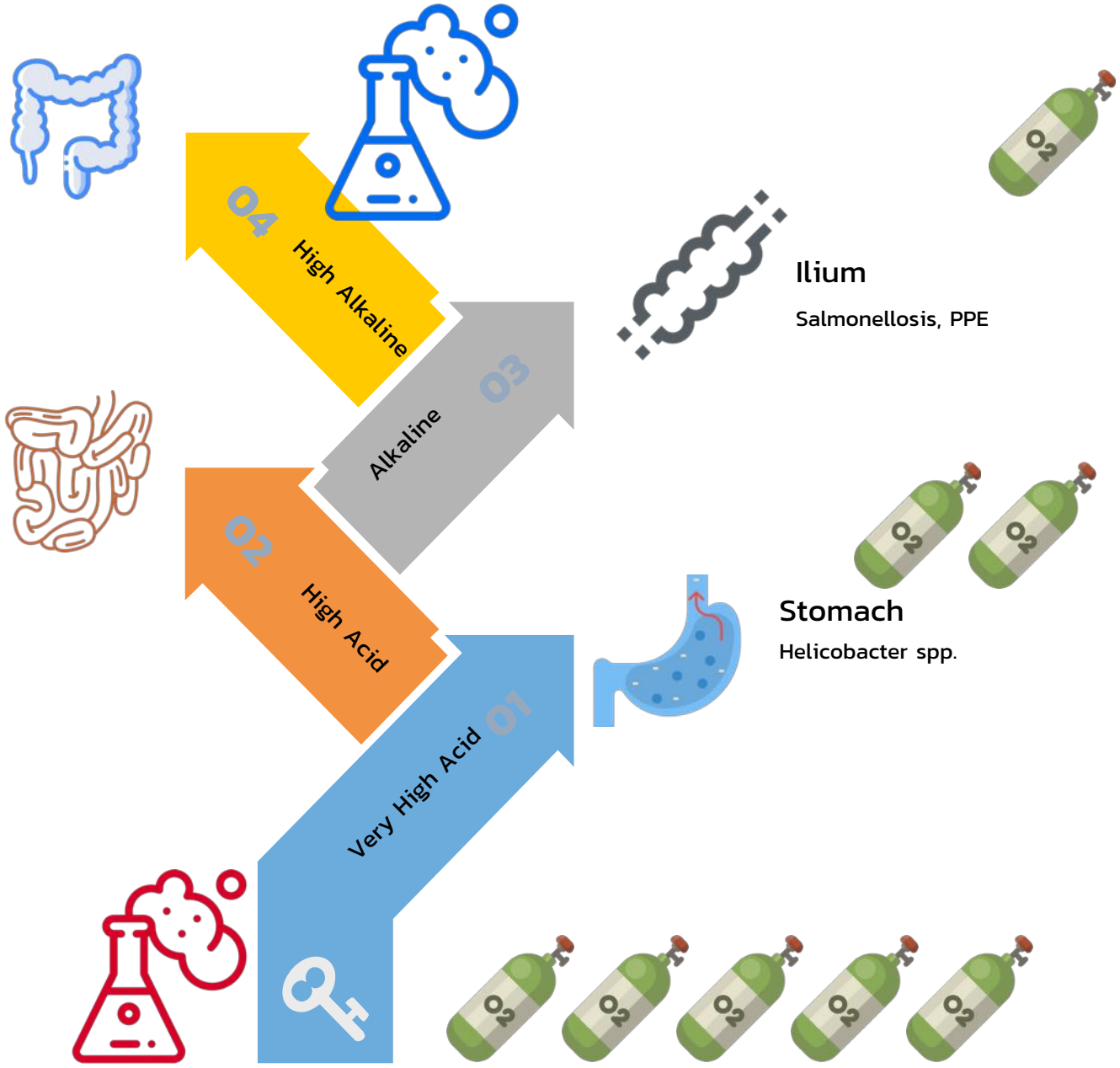
Porcine Enteric Bacteria

Colon, rectum

PPE, Swine dysentery, Porcine intestinal spirochetosis

Duodenum, jejunum

Colibacillosis



Ilium

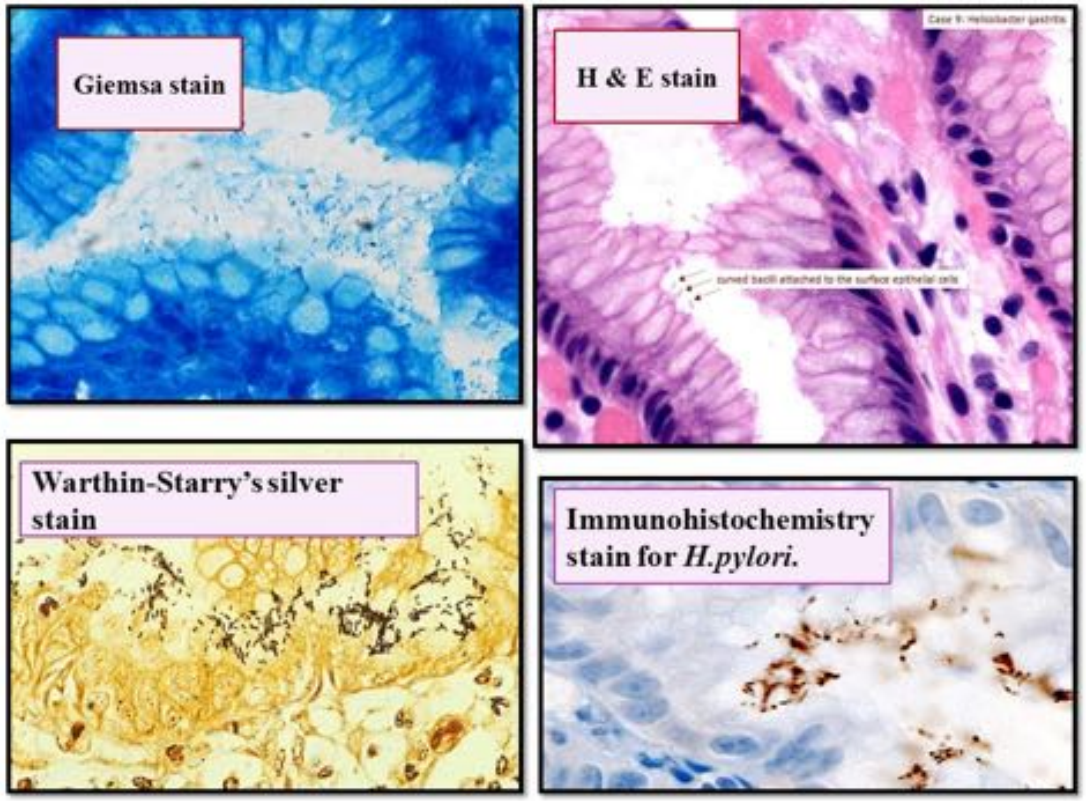
Salmonellosis, PPE

Stomach

Helicobacter spp.

Gastric Ulcer:

Biology and Detection *Helicobacter suis*



- Zoonotic pathogen
- At pyloric mucosa
- 90 samples at slaughterhouses

Positive PCR only	Both Positive	Positive silver stain only
23-50%	3-6%	10-13%

PCR for 16S rRNA

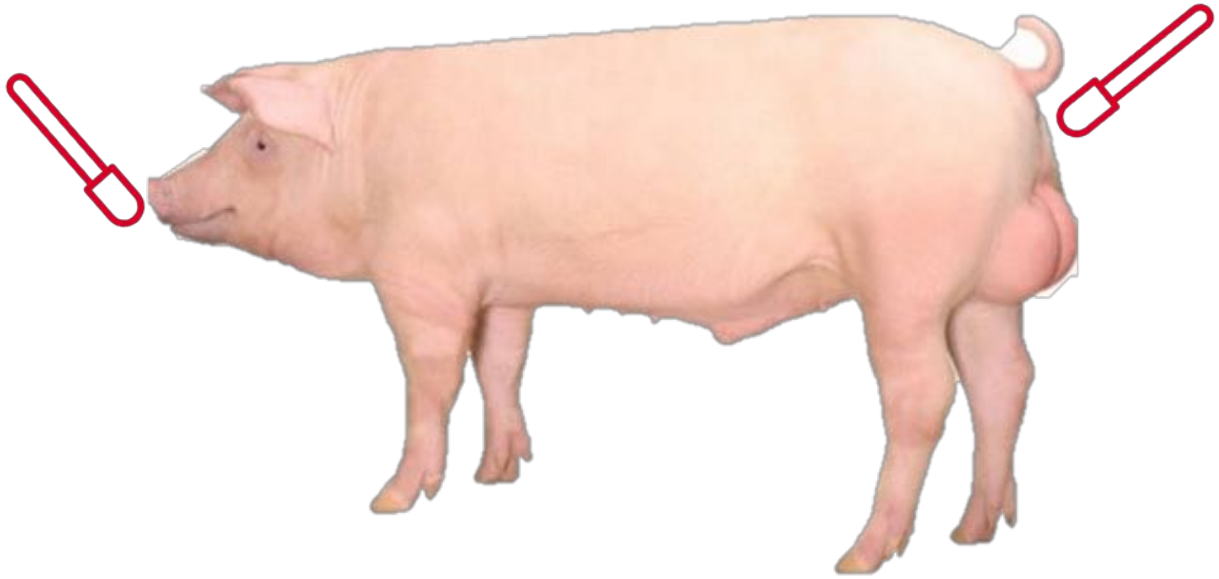
Histopathology ??

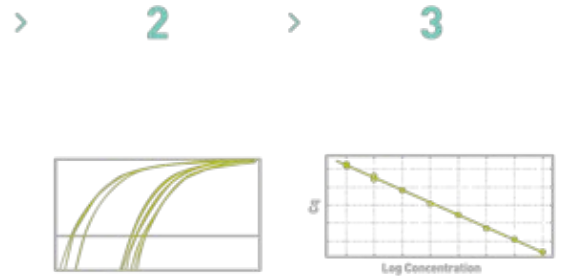
Edema Disease:

Detection

Enterohemorrhagic *Escherichia coli*

Verotoxingenic *E. coli* (VTEC)



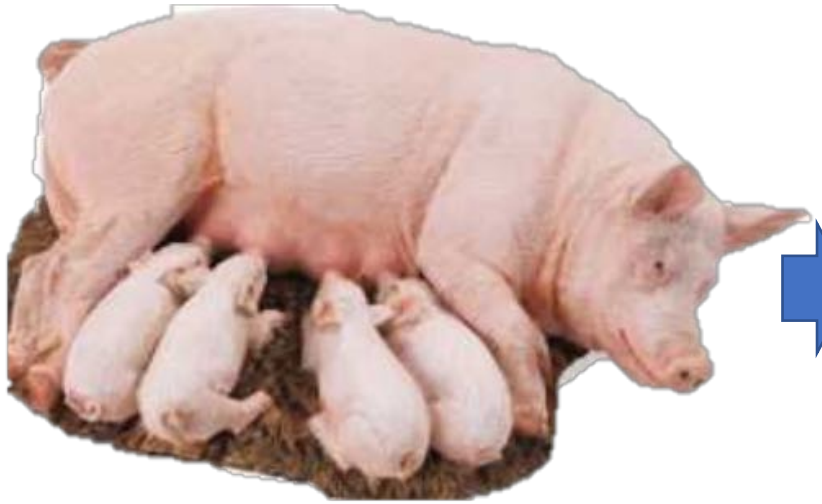


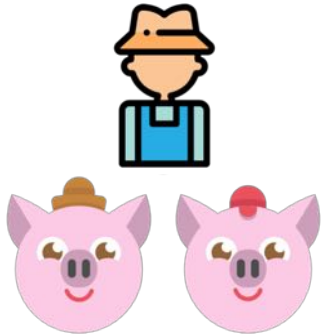
- qPCR detected VTEC positive in both liquid form and fixed in FTA
- Oral fluid and rectal swab showed no significant difference.
- OF was more sensitive (pen)

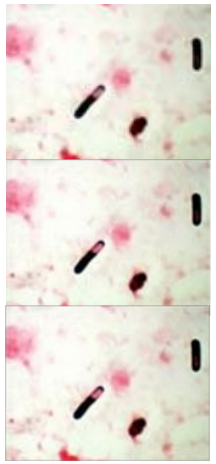
Clostridial enteritis:

Biology and typing

Clostridium difficile







- Vaccination strategy failure
- Sucking piglets with diarrhea, only in positive sows.
- Ribotyping
  - type 078 was found in humans and pigs on 15 farms and type 045 in a farmer and his pigs on 1 farm. (Keessen al et, 2013)
  - Czech republic: 10 ribotypes mostly 078 that contained A, B, binary toxins.

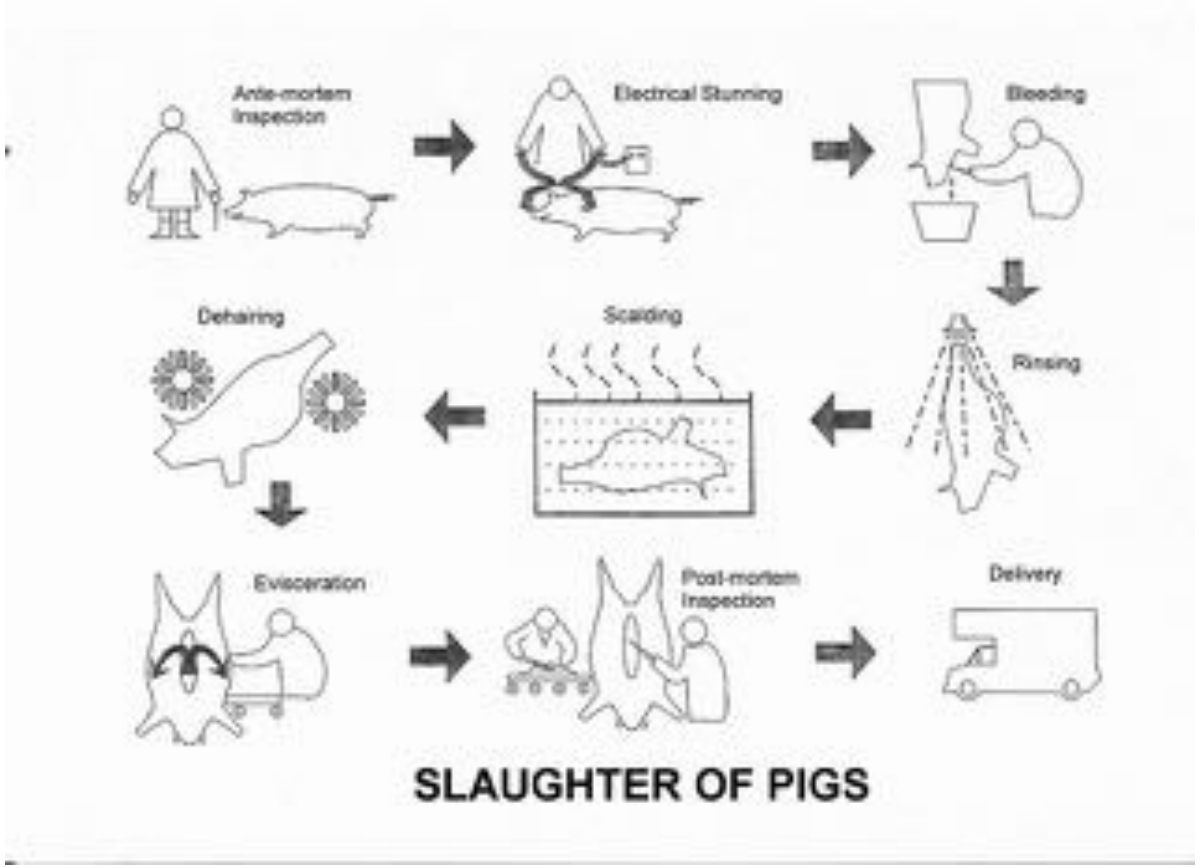
Salmonellosis:

Risk assessment

Salmonella enterica

Key risk points of *Salmonella* contamination in slaughterhouse

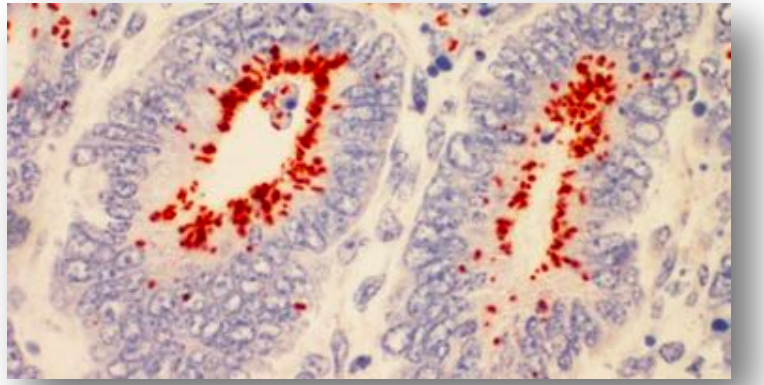
- 25.4% (from 480 samples)
- From 4 slaughtering stage; polishing, rectal drilling , evisceration and splitting
- Post-splitting process was major contamination risk point.

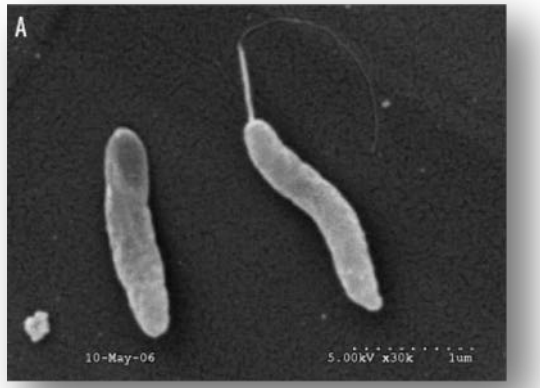


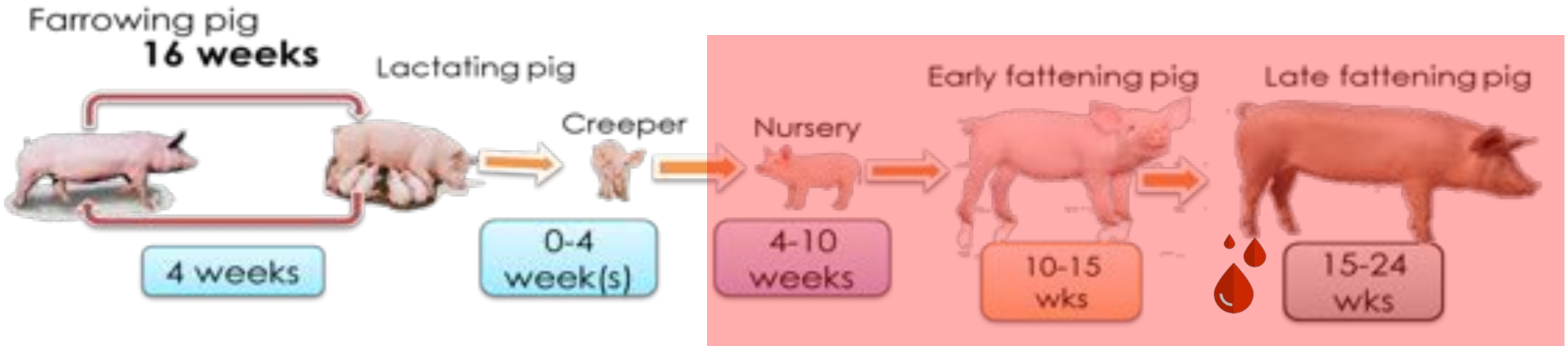
Porcine proliferative enteropathy:

Lawsonia intracellularis

Biology and clinical Sign



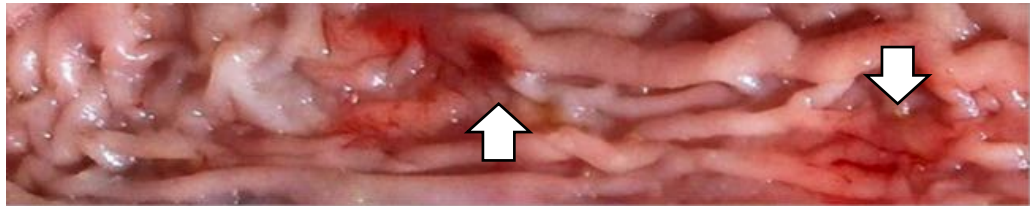




Porcine proliferative enteropathy:

Lawsonia intracellularis

Clinical Sign

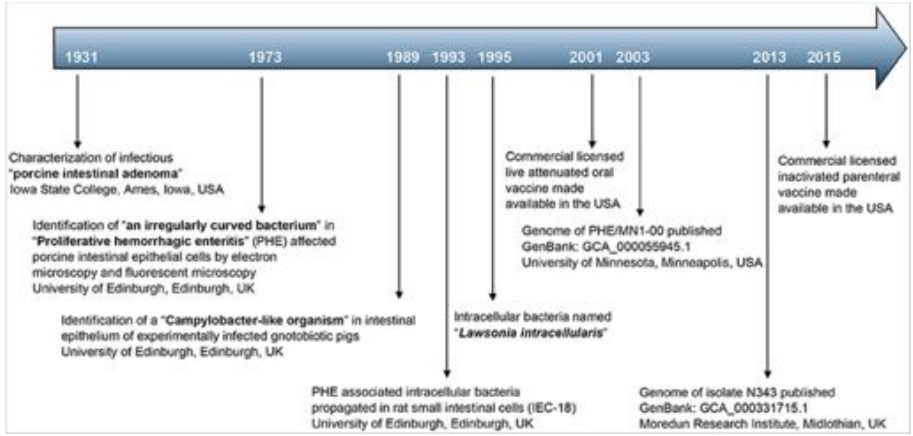


Disease resolving within 7-8 weeks: underweight nursery pigs (PIA)

LI intermittently shed a long production cycle

PHE leads to bloody to dark, tarry diarrhea resulting death in adult

Subclinical cases: result in reduced growth performance



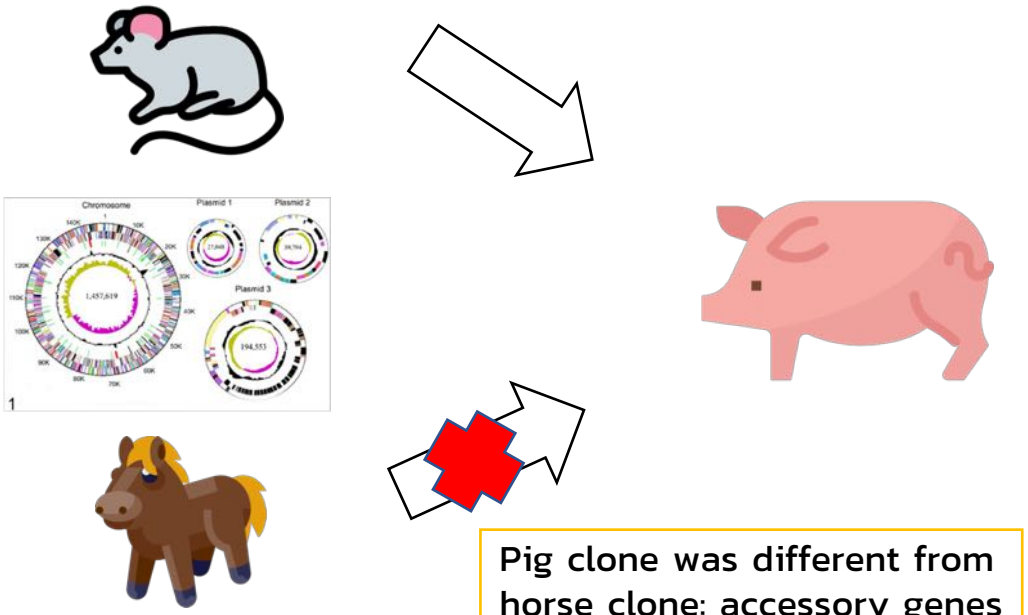
Dedicated to Dr. Gordon Lawson,
The Royal (Dick) School of Vet Studies at Univ of Edinburgh

Porcine proliferative enteropathy: *Lawsonia intracellularis*

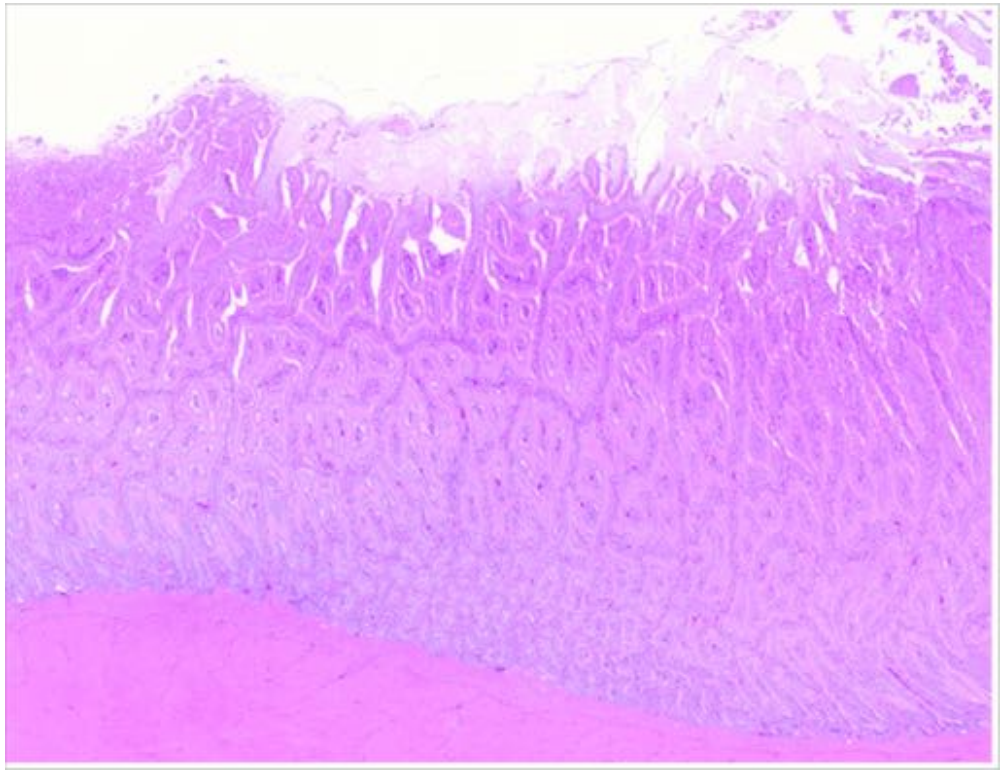
Disease and transmission

Transmission between mouse to pigs but not from horse to mouse

Whole genome LI 1.4 Mbp + prophage as genomic island



Pig clone was different from horse clone: accessory genes represented host specificity



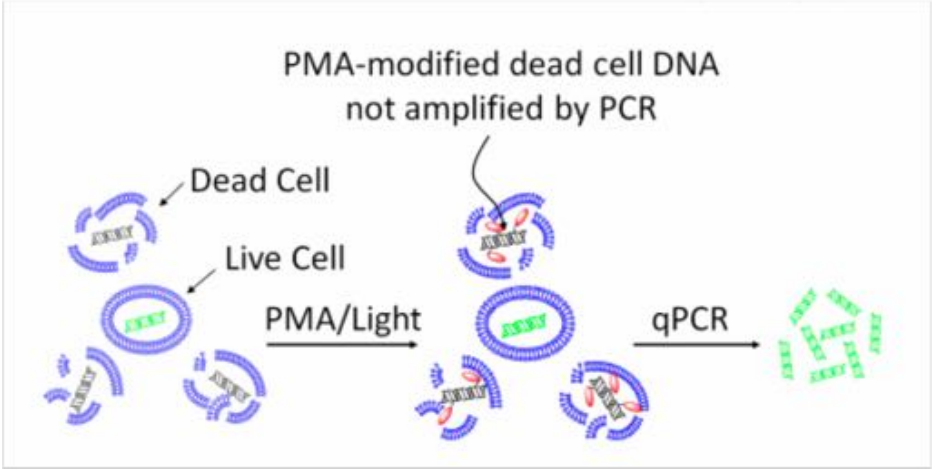
A combination of Notch-1 signaling and disruption of the β-catenin/Wnt pathway may be associated with immature crypt cell proliferation

Porcine proliferative enteropathy: *Lawsonia intracellularis*

Diagnosis

Directed MIC for LI by Propidium monoazide (PMA) – qPCR

PMA is DNA binding dye that inhibits PCR amplification



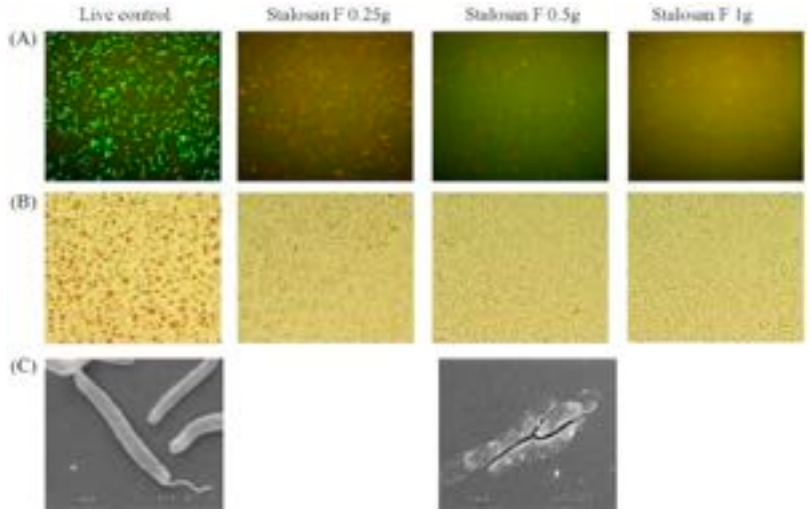
MIC of LI in Thailand

Intracellular LI : susceptible to tiamulin and valnemulin

intermediate to enrofloxacin and tylosin

Extracellular LI: susceptible to valnemulin and carbadox

Intermediate to tiamulin and tylosin



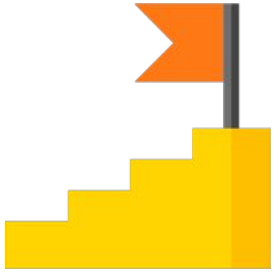
Porcine proliferative enteropathy: *Lawsonia intracellularis*

MISC

Enterisol®

skatole and indole in backfat as parameters

(by-product of microbial breakdown tryptophan)



Li antibody

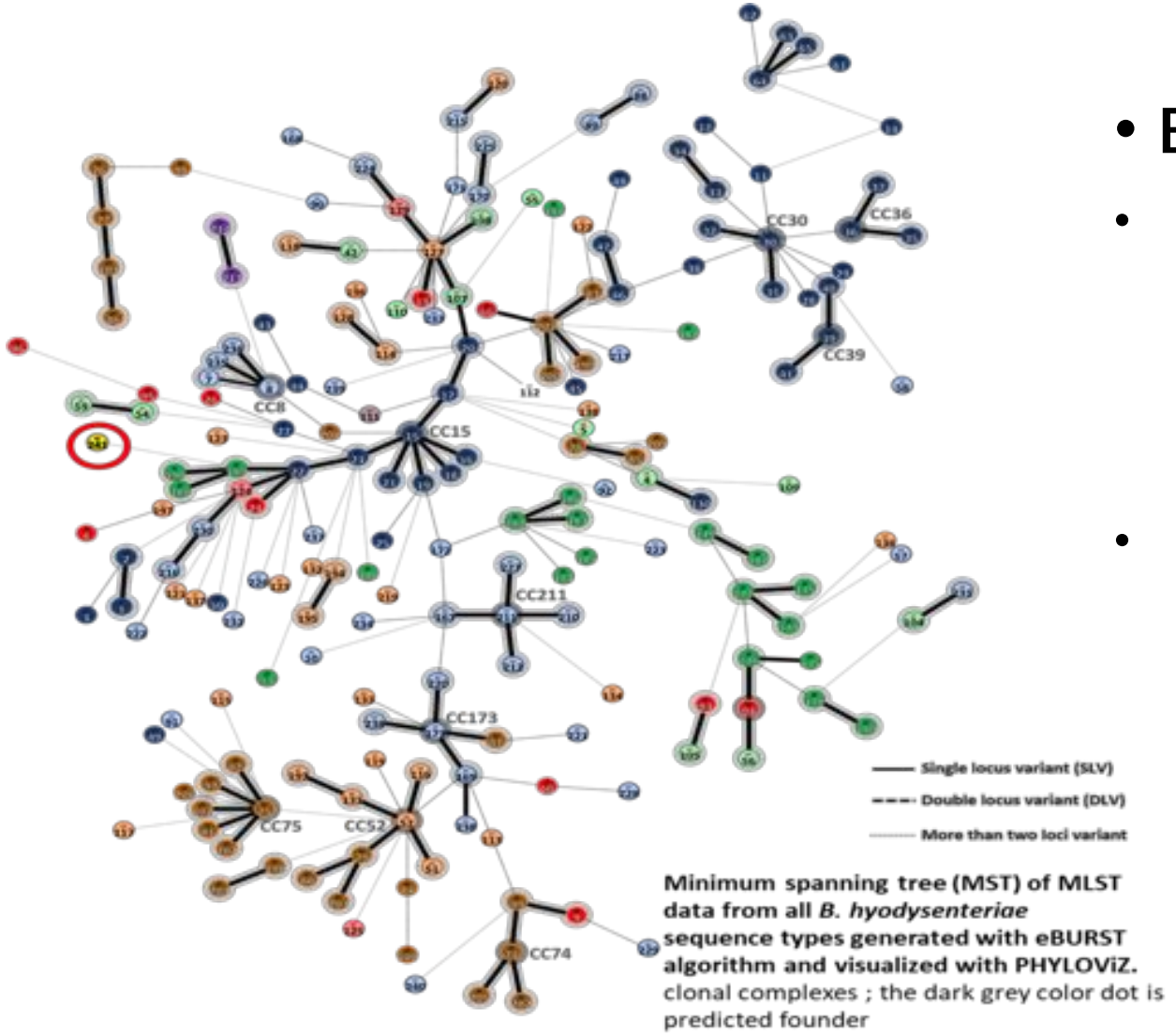
Reduce malodor

Lawsonia still present in farm

Swine Dysentery:

Biology and Gene

Brachyspira hyodysenteriae



Minimum spanning tree (MST) of MLST data from all *B. hyodysenteriae* sequence types generated with eBURST algorithm and visualized with PHYLOViZ. clonal complexes ; the dark grey color dot is predicted founder

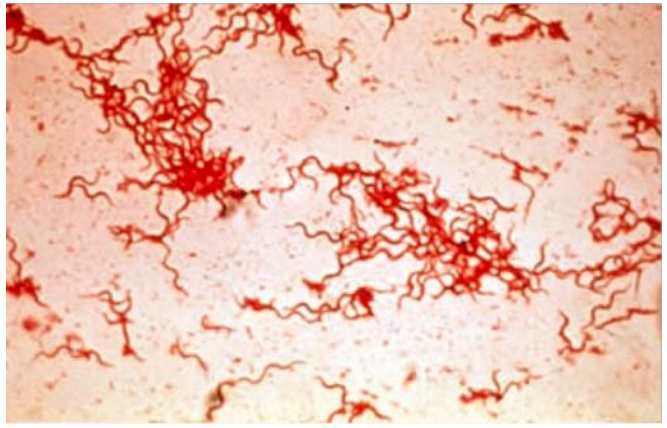
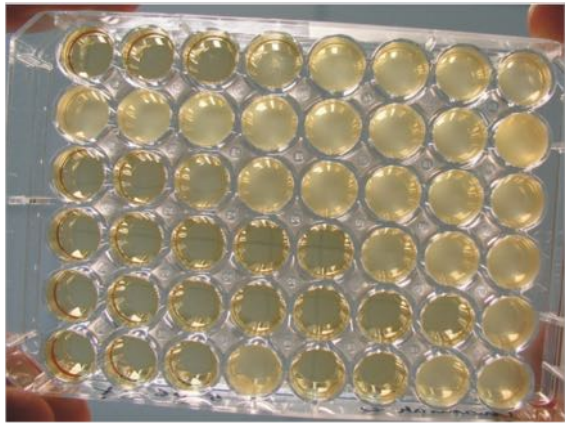
- Bh in Thailand strain CU01
  - The bacterial strain resisted to **tiamulin, tylosin, lincomycin, monensin** and **amoxicillin** but did not resist to olaquindox.
  - A2058T and G2059A mutations on the 23S rRNAgene, associated with , **erythromycin, clindamycin, tylosin** and **lincomycin** resistance, and a mutation at G1058C on the 16S rRNA gene associated with **doxycycline** resistance.

Swine Dysentery:

Biology and Gene

Brachyspira hyodysenteriae



- Pleuromutilin resistant Bh
  - Point mutation on 23s rRNA
  - Tiamulin-valnemulin antibiotic resistant (TVA) gene (505 aa) (Resistance = >2 ug/ml)
  - TVA facilitates development of higher MIC in mutated Bh

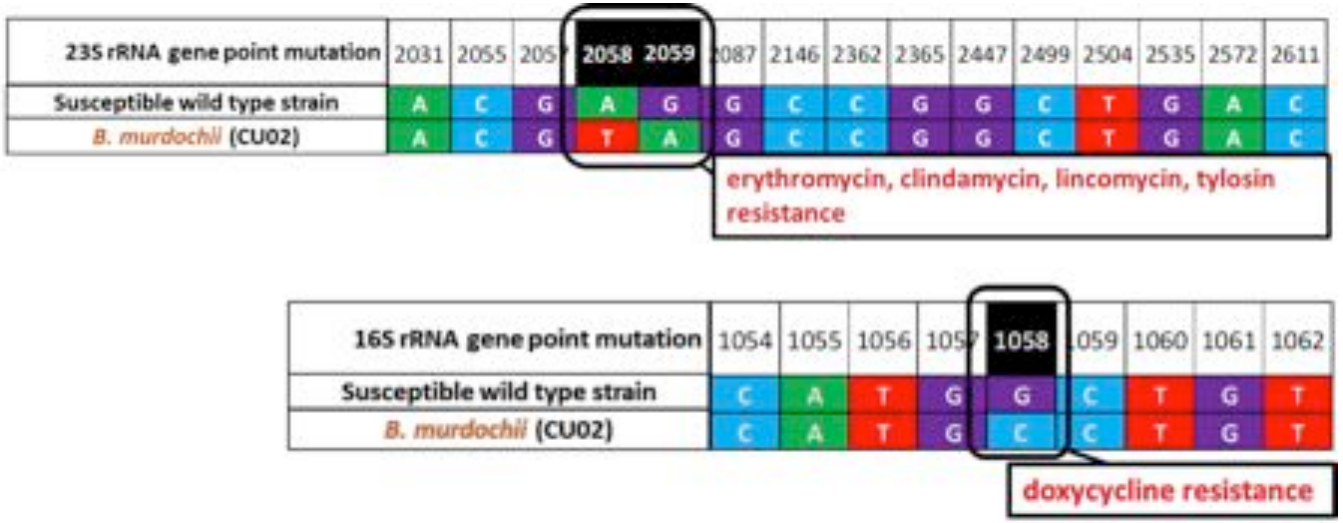
Swine Dysentery:

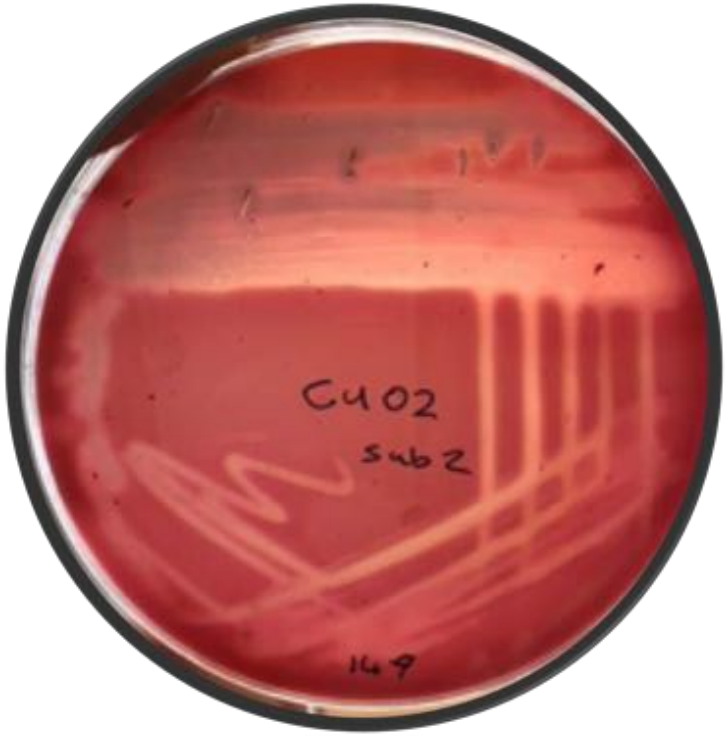
Emerging pathogen

Brachyspira murdochii

The first isolate from a case of porcine colitis in Thailand.

- The bacterial strain resisted to **tiamulin, tylosin, lincomycin, monensin** and **amoxicillin** but did not resist to olaquindox.
- A2058T and G2059A mutations on the 23S rRNAgene, associated with , erythromycin, clindamycin, tylosin and lincomycin resistance, and a mutation at G1058C on the 16S rRNA gene associated with doxycycline resistance.

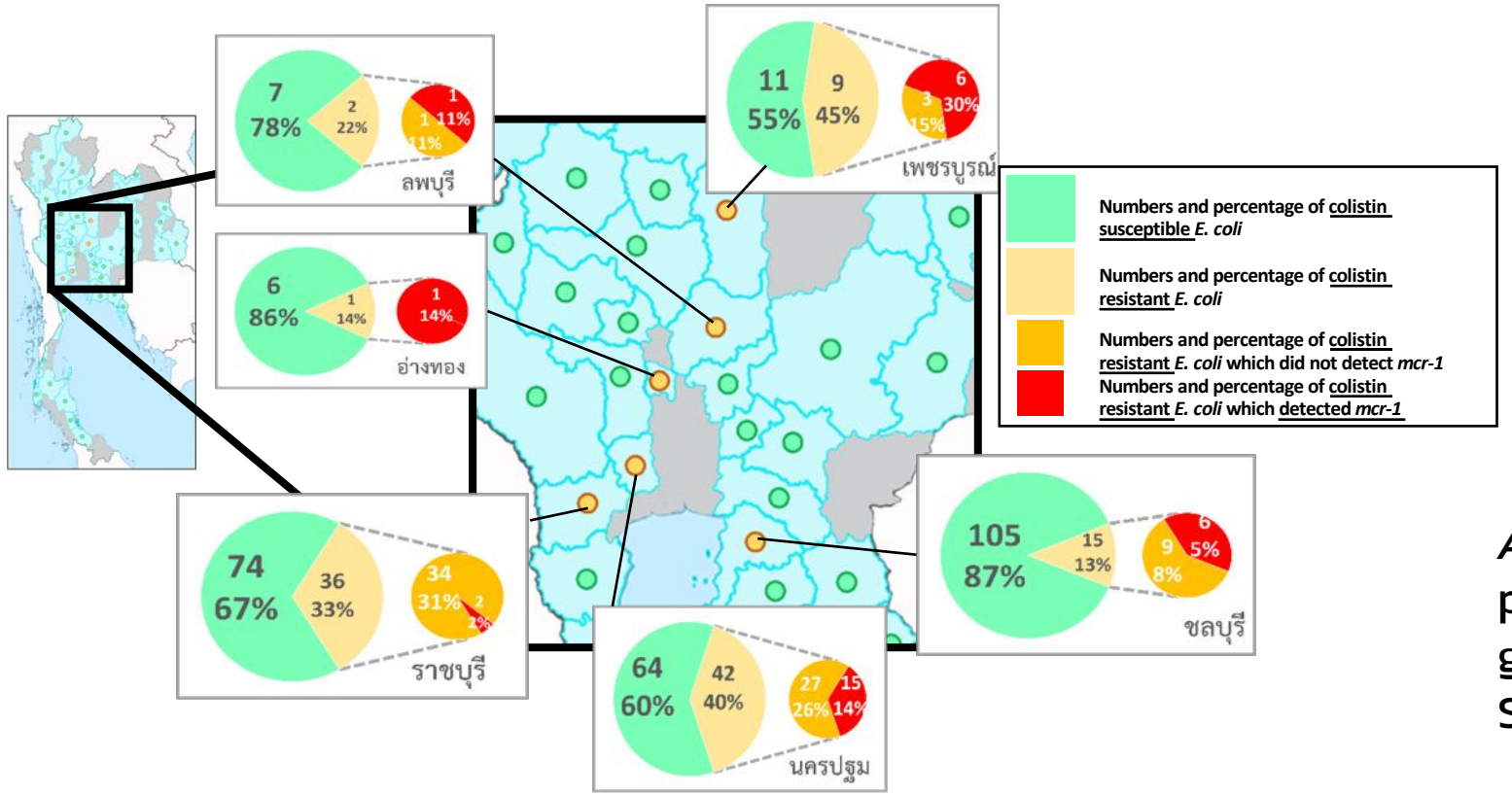




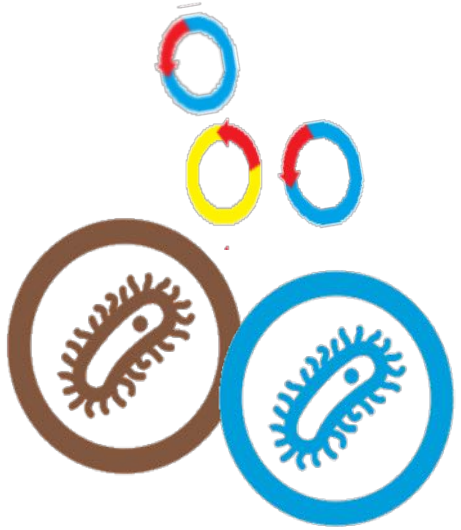
Antimicrobial Resistant Bacteria :

mcr gene family

colistin resistance



- *mcr* 1 and *mcr* 3 were common type in Thailand with transferable ability



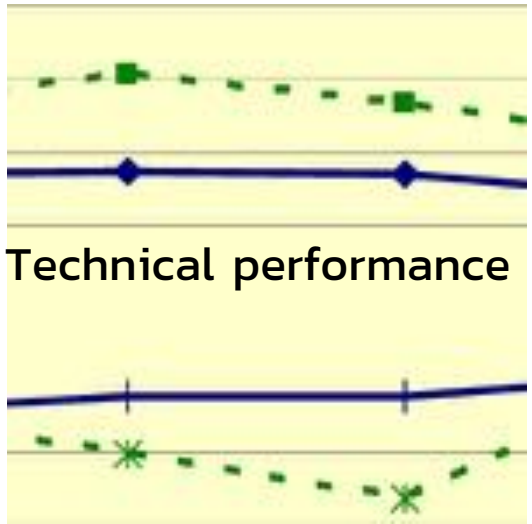
Aeromonas hydrophila in backyard pig feces in China possessed *mcr5* gene (on PI064-2) as well as Salmonella enterica in Germany.

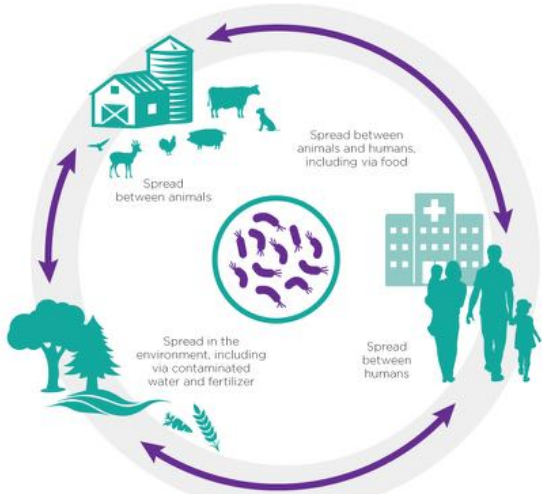
WGS revealed mcr5 gene located on a transmissible plasmid pI064-2

Antimicrobial Resistant Bacteria :

AMR Reduction

Tailor-made coaching





AMU and AMR
(phenotype and genotype)

Alternative antimicrobial :

Enhance antibiotic efficacy

Drug combination

Halquinol				Tiamulin			
Avilamycin		Colistin		Amoxicillin		Doxycycline	
ETEC	S	S	I	HP	S		S
mcr1	S	N/A		APP	S		S
Salmo		S		S. suis	I		I
CP			I	PM	I	*A*	I

Synergist	Indifferent	Antogonist

ETEC = enterotoxigenic *E. coli*,

mcr1 = *E. coli* possessed plasmid mcr-1 gene

Salmo = *Salmonella* Typhimurium

CP = *Clostridium perfringens* Type C

HP = *Haemphilus parasuis*

APP = *Actinobacillus pleuropneumoniae*

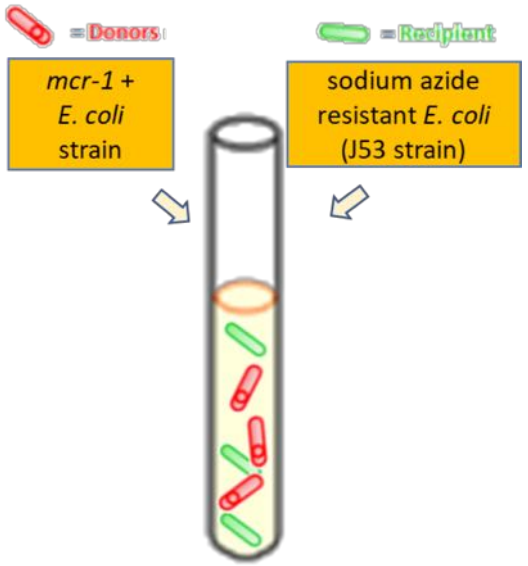
S. suis = *Streptococcus suis type II*

PM = *Pasteurella multocida*

Alternative antimicrobial:

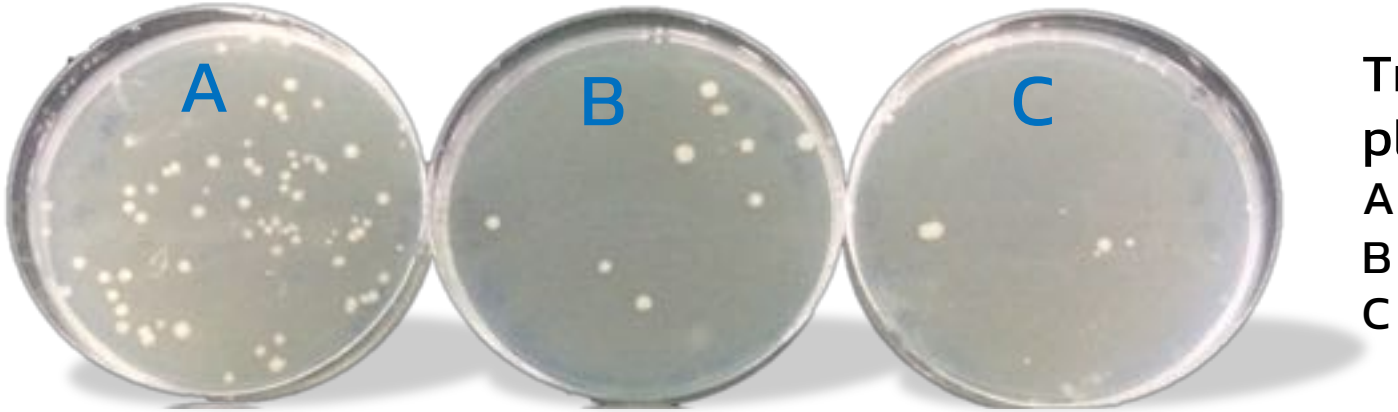
Antibiotic resistance control

Flavomycin®



- Flavomycin® could reduce the conjugative rate of the plasmids mediating **colistin resistance** and **ESBL *E. coli***, in vitro.

- Flavomycin® at 8 μg/ml showed a reduction **up to 10 times** and at 16 μg/ml decreased **up to 100 times** for the plasmid containing ***mcr*-1** and ***bla*** $_{\textbf{CTX-M}}$ **family**.



Transconjugant colonies on LB agar plates
A : control
B : Flavomycin at 8 μg/ml
C : Flavomycin at 16 μg/ml

Alternative tool controlling AMR:

Type and mechanism

Probiotic-Prebiotic

- Multi-strains probiotic Bactosac ® by top dressing for 4 weeks
  - improved colostrum composition (fat, protein and total solid)

- Probiotic induced immunomodulatory responses in intestine in vivo.
  - *L. plantarum*
  - IL-6, TNFa, IL10, NK-kappa-B

- Probiotic lactobacilli changed microbial composition
  - Predominant lactobacilli residing in the jejunum.

Whether Our Local Probiotics can reduce gene conjugation ?

Probiotic strains

Lactobacillus plantarum	*31F*
Lactobacillus plantarum	25F
Lactobacillus plantarum	22F
Pediococcus pentosaceus	77F
Pediococcus acidilactici	72N
**Lactobacillus plantarum*	JCM 1149
**Pediococcus acidilactici*	DSM 20284

* : Reference strains

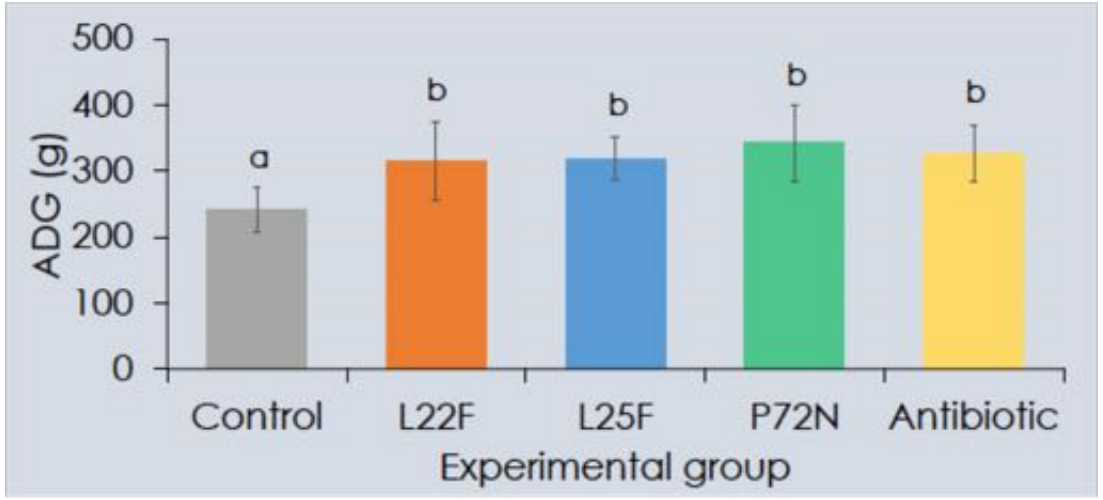
Probiotics:

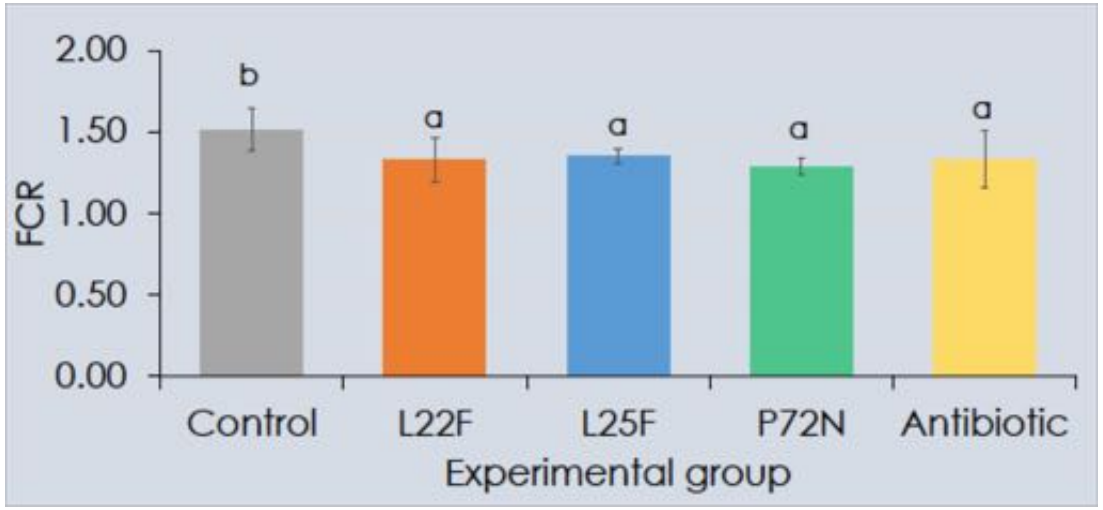
Swine Production & Welfare

Lactobacillus plantarum 22F (L22F), *L. plantarum* 25F (L25F) and *Pediococcus acidilactici* 72N (P72N)

- **The group of Thai LAB strains isolated from healthy pigs that revealed high performance of *in vitro* studies**

- **L22F, L25F and P72N** were supplemented to neonatal piglets to compare their efficacies on growth performance (ADG and FCR) with **antibiotic usage** along nursery period (wk3-wk8).
- **P72N** showed the highest performance in all groups, however there was **no significant difference among 3 LAB strains and antibiotic groups**.
- The using of Thai LAB strains could improve growth performance in pigs equal to antibiotic usage demonstrating the feasibility of using this strains as substitute for antibiotics.





Benefit

Pig's average body weight (BW) at Wk_{22} in each experimental group.

Experimental group	Control	L22F	L25F	P72N	Spraydry	ABO
BW at WK_{22} (kg)	87.46	96.08	99.36	107.03	102.85	100.55
		102.08 (average in 4 groups)				

BW different between probiotic supplement and control group (kg/pig)

102.08-87.46 = 14.62

Price of alive pig at farm ~60 baths/kg

Price different between probiotic supplement and control group (kg/pig)

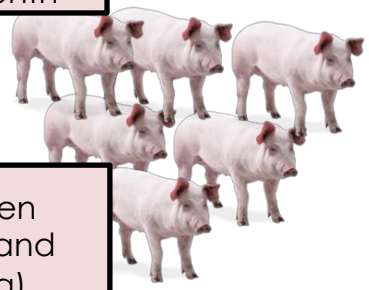
14.62*60 = 877.2

Probiotic expense ~100 baths/pig

Price different between probiotic supplement and control group (kg/pig)

877.2-100 = 777.2

Production power ~100 fattening pigs/month

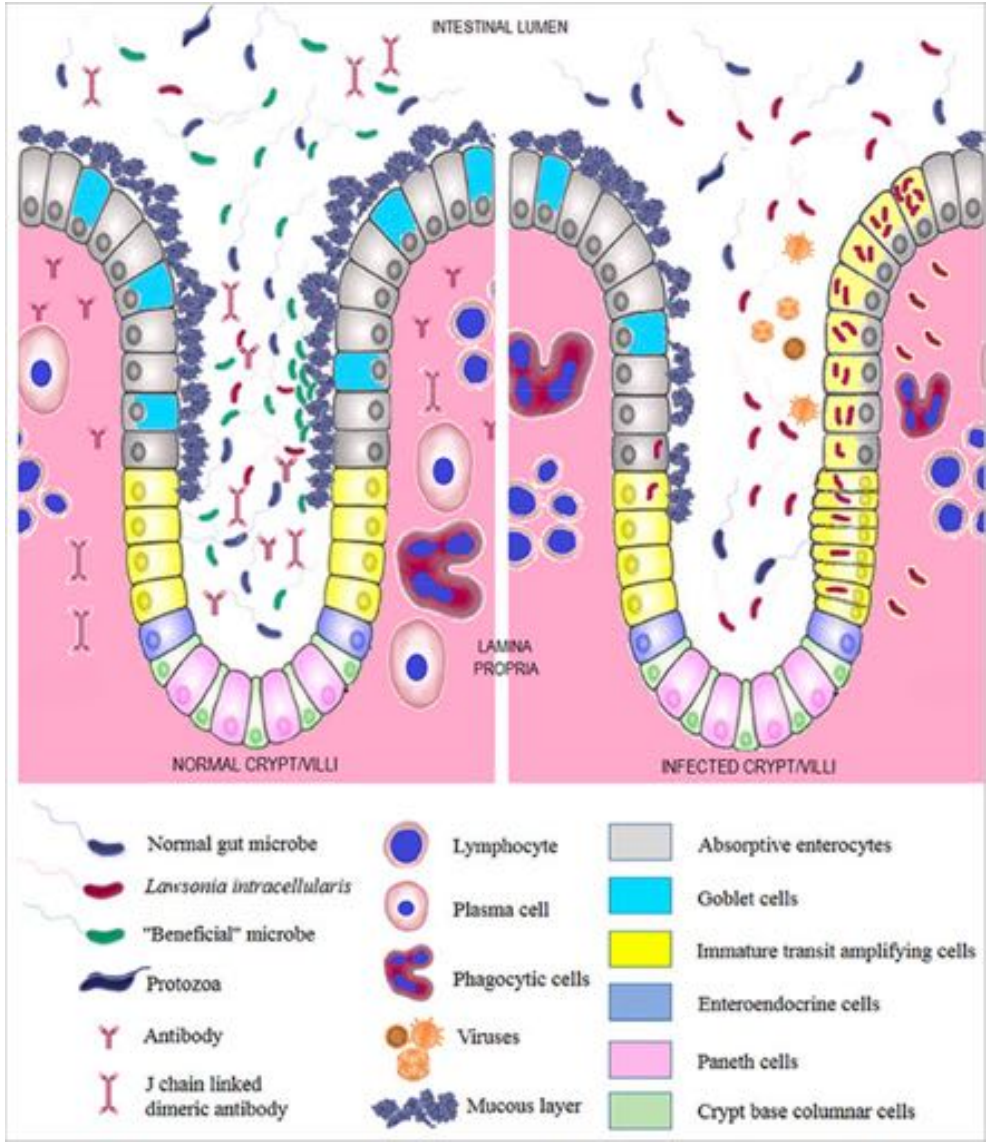


Different income between probiotic supplement and control group (bath/month)

777.2*100 = 77,7200

Probiotic strains

Can The Local Probiotics reduce PED infection?



Probiotics & Antimicro. Prot.
DOI 10.1007/s12602-017-9283-y

Protective Effects of Cell-Free Supernatant and Live Lactic Acid Bacteria Isolated from Thai Pigs Against a Pandemic Strain of Porcine Epidemic Diarrhea Virus

Wandee Sirichokchatchawan[1] · Gun Temeeyasen[2] · Dachrit Nilubol[3] · Nuvee Prapasarakul[1]

Reduced the plasmid conjugation

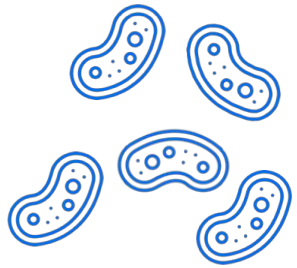
Reduced Salmonella, Lawsonia and PED infection

Live and Cell free supernatant Thai probiotic strains can reduce resistant rate via bacterial conjugation interference

Alternative tool controlling AMR:

Types and Outcomes

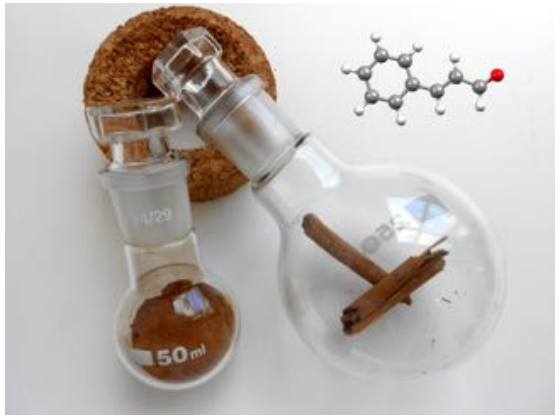
Acidifier and lactic acid bacteria



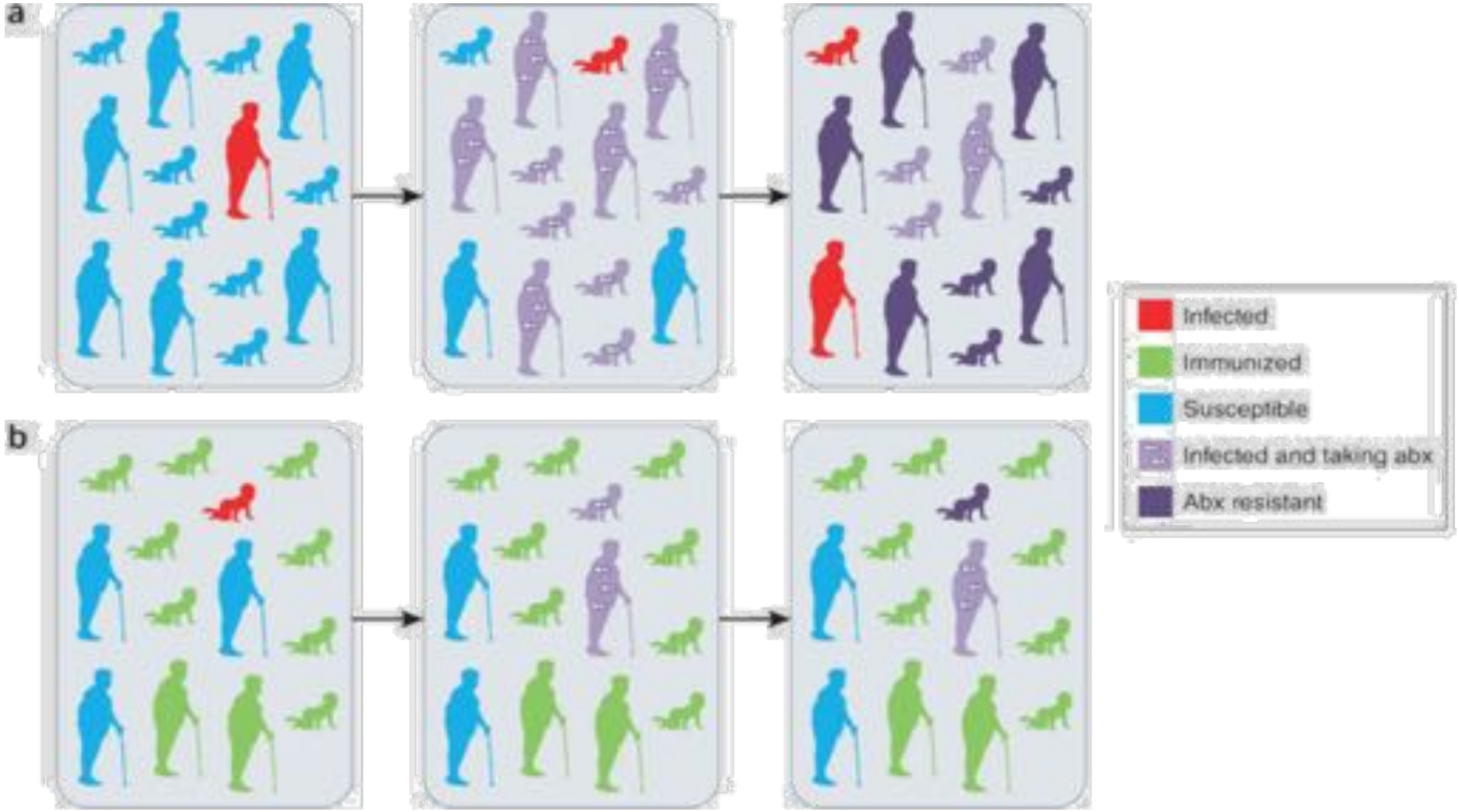
- Organic compounds (formic, acetic, and proprionic), cinnamaldehyde and Permeabilitzing Complex™ (PC)

  (1 kg/ton)
  - Reduce number of *Salmonella* and *E. coli*
  - Increase number of Lactic acid bacteria
  - Rising height of jejunal villi
  - Improved piglet performance

- Cocktail formic and lactic acid (FL) + rye overgrown mycelium (ROM) of *Agaricus sufescens* + mannose hydrolyzed copra meal (CM) = FL-ROM-CM) reduced ESBL/AmpC

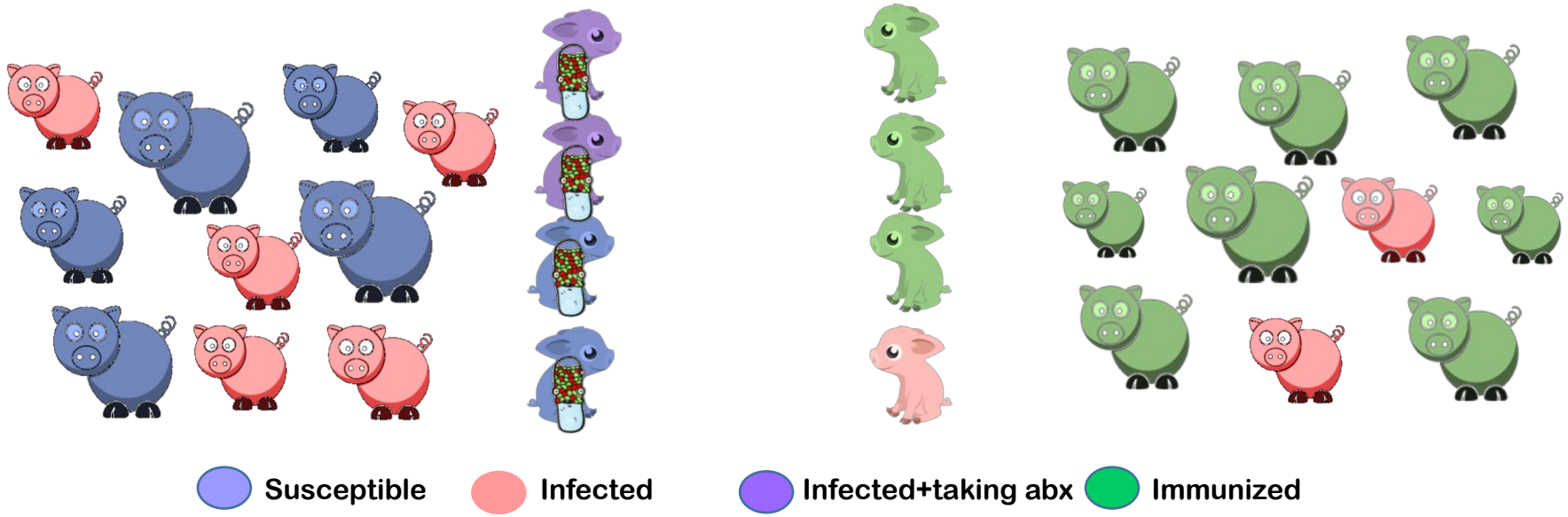


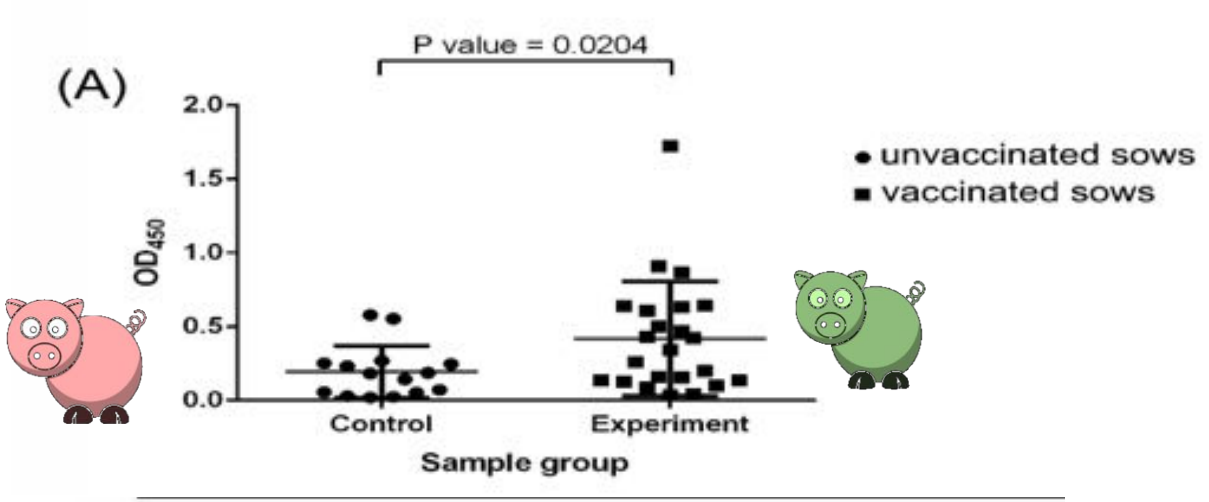
The role of vaccines in preventing bacterial antimicrobial resistance

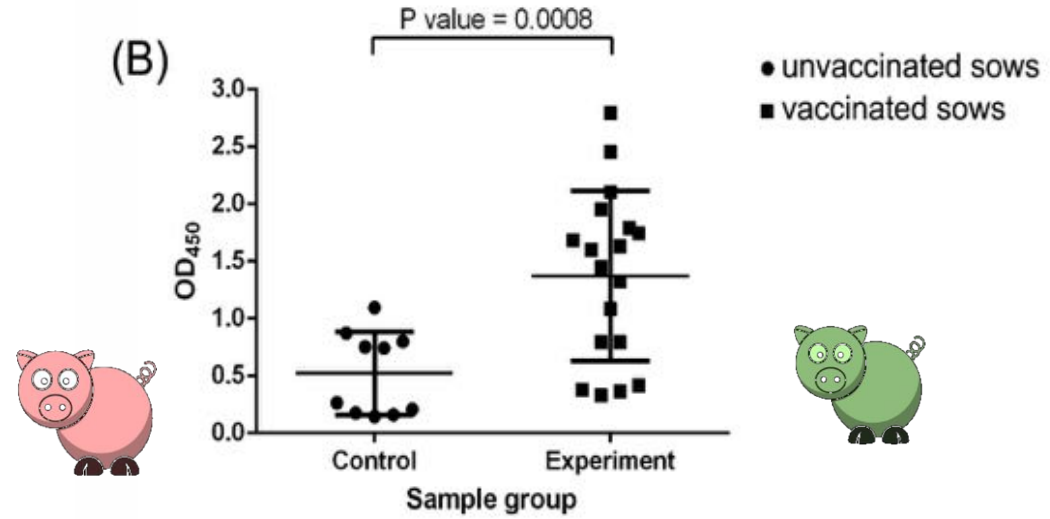


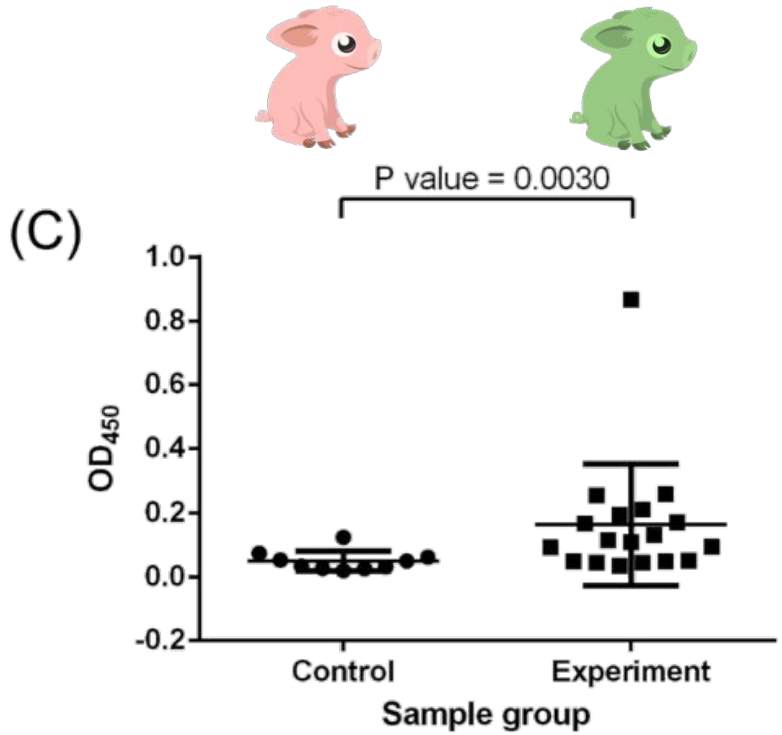
Jansen et al., 2018 Nature Medicine

No.	Gene	สุกรได้รับวัคซีน(69)		สุกรไม่ได้รับวัคซีน(58)		*p*-value
		แม่สุกร	ลูกสุกร	แม่สุกร	ลูกสุกร	
1	k88	9	1	21	6	
		10 (14.49%)		27 (46.55%)		*p*<0.05
2	k99	15	1	18	5	
		16 (23.19%)		23 (39.66%)		*p*<0.05
3	987p	8	2	20	9	
		10 (14.49%)		29 (50.00%)		*p*<0.05
4	f41	10	3	18	9	
		13 (18.84%)		27 (46.55%)		*p*<0.05
5	Ltb	10	1	22	5	
		11 (15.94%)		27 (46.55%)		*p*<0.05







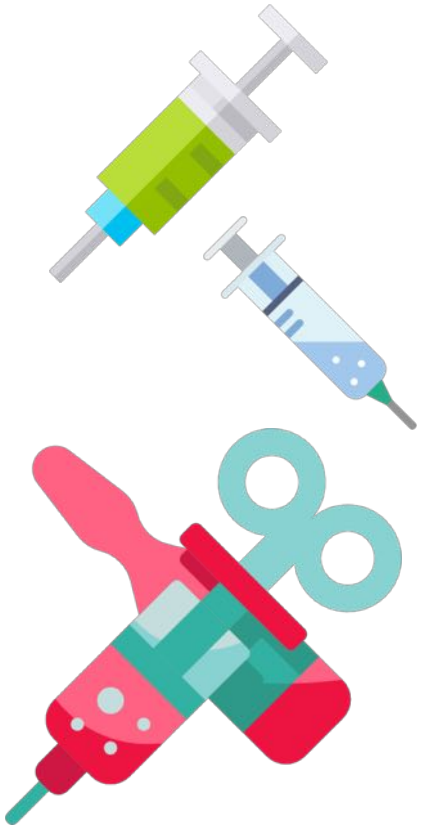


- **Evaluation of IgG levels against *Escherichia coli* heat-labile enterotoxin B subunit in the sera of sows at the farrowing day (A), colostrum (B), and sera of 3 weeks old piglets (C) by ELISA method**

Alternative tool controlling bacterial diseases:

Types and Outcomes

VACCINES



- Hyogen ® one shot VS the other three vaccines
- Hyogen ® combined with Circovac ® had the same outcome with separately vaccination
- ISCOM-P97R1 in Mh live nano-vaccine (dialysis method)
  - P97R1 recombinant protein as adjuvant in vaccine
- PCV + M. hyopneumoniae challenged trial (Zoetis)
- Entericolix ®, coli-clostridia combination (F4/F18)
- Porcilis ® ColiClos (PC); F4ab, F4ac, F6, LT, +F41, +F18 (IM)
  - Purpose: prevention through transfer of antibody via colostrum
- Coliprotec ®; Bivalent E. coli F4/F18 (ETEC) (Oral 21 day)
  - Focus on post-weaning period effect on finisher.
- Multiepitope fusion antigen (MEFA) of ETEC; F18, K88, Lt, STb, Sta, STx2e using non toxic LT mutant as backbone
  - Backbone + adhesin + neutralized various toxin

Summary

01

Epidemiology and Pathogenic characterization

02

Detection using novel diagnostic tools

03

Bioinformatic for evolution and selection analysis

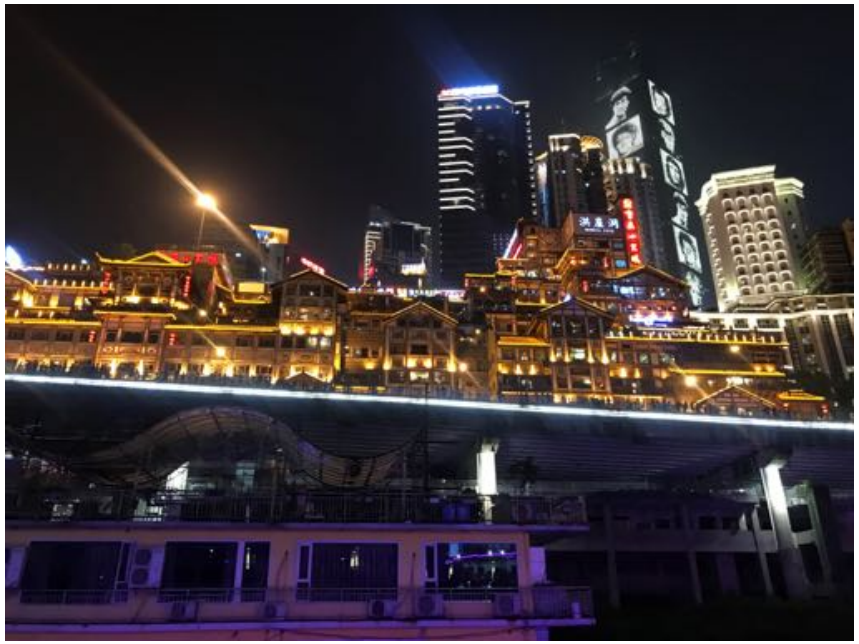
04

AMR and Alternative tool; probiotic, acidifier or herbal

05

Vaccine development and efficacy

Innovation technology for future industry



THANK YOU FOR YOUR ATTENTION

ACKNOWLEDGEMENTS

- Agricultural Research Development Agency (ARDA)
- Research Unit; Diagnosis and Monitoring of Animal Pathogens
- Faculty of Veterinary Science, Chulalongkorn University
- Virbac (Thailand) co. ltd.
- Elanco (Thailand) co. ltd
- Huvepharma (Thailand) co. ltd.
- Zoetis (Thailand) co. ltd
- KMP biotechnology co. ltd.
- CPF co. th