24th International Pig Veterinary Society Congress

8th European Symposium of Porcine Health Management

Pig digest: Bacteriology

24th International Pig Veterinary Society Congress
8th European Symposium of Porcine Health Management
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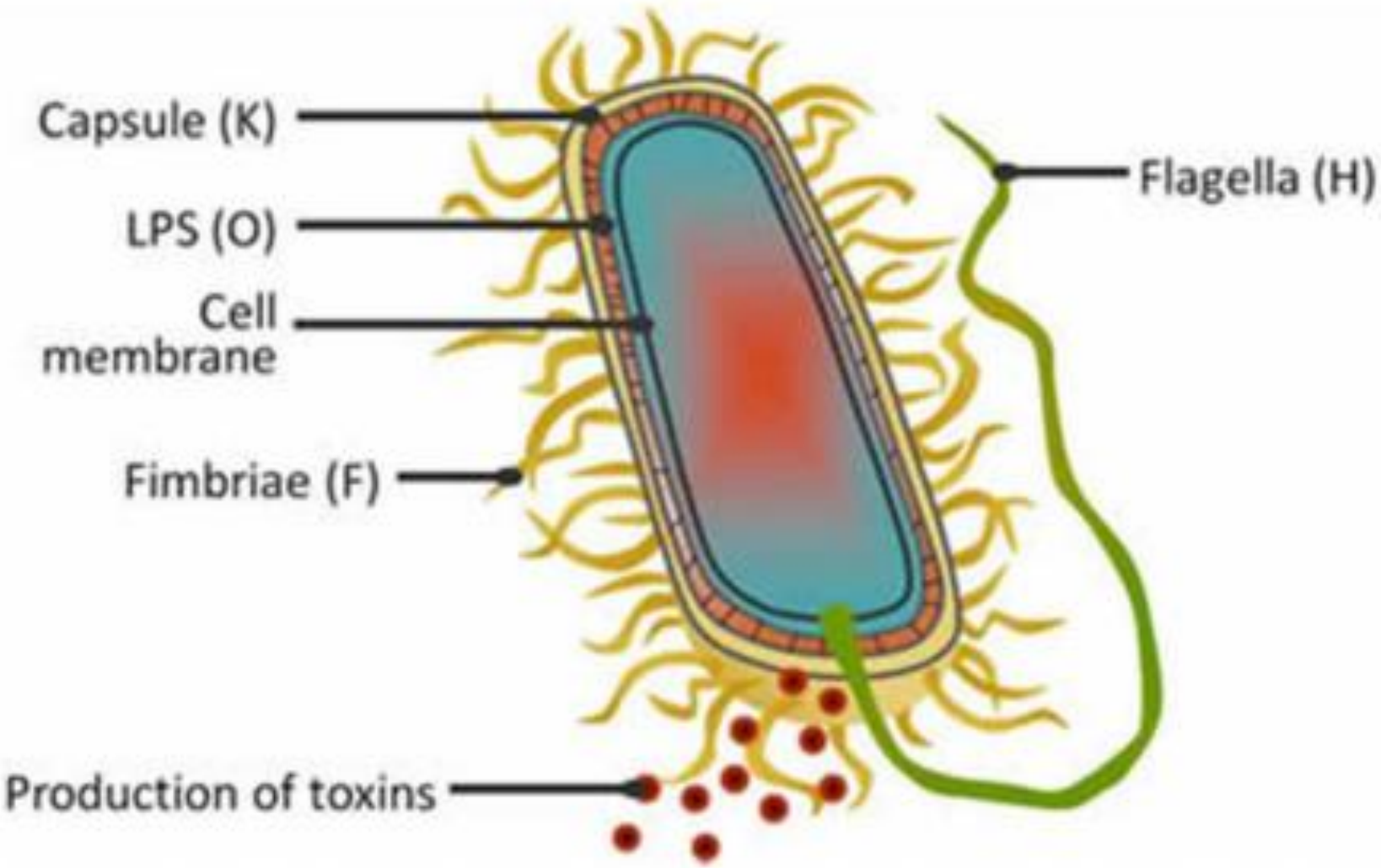
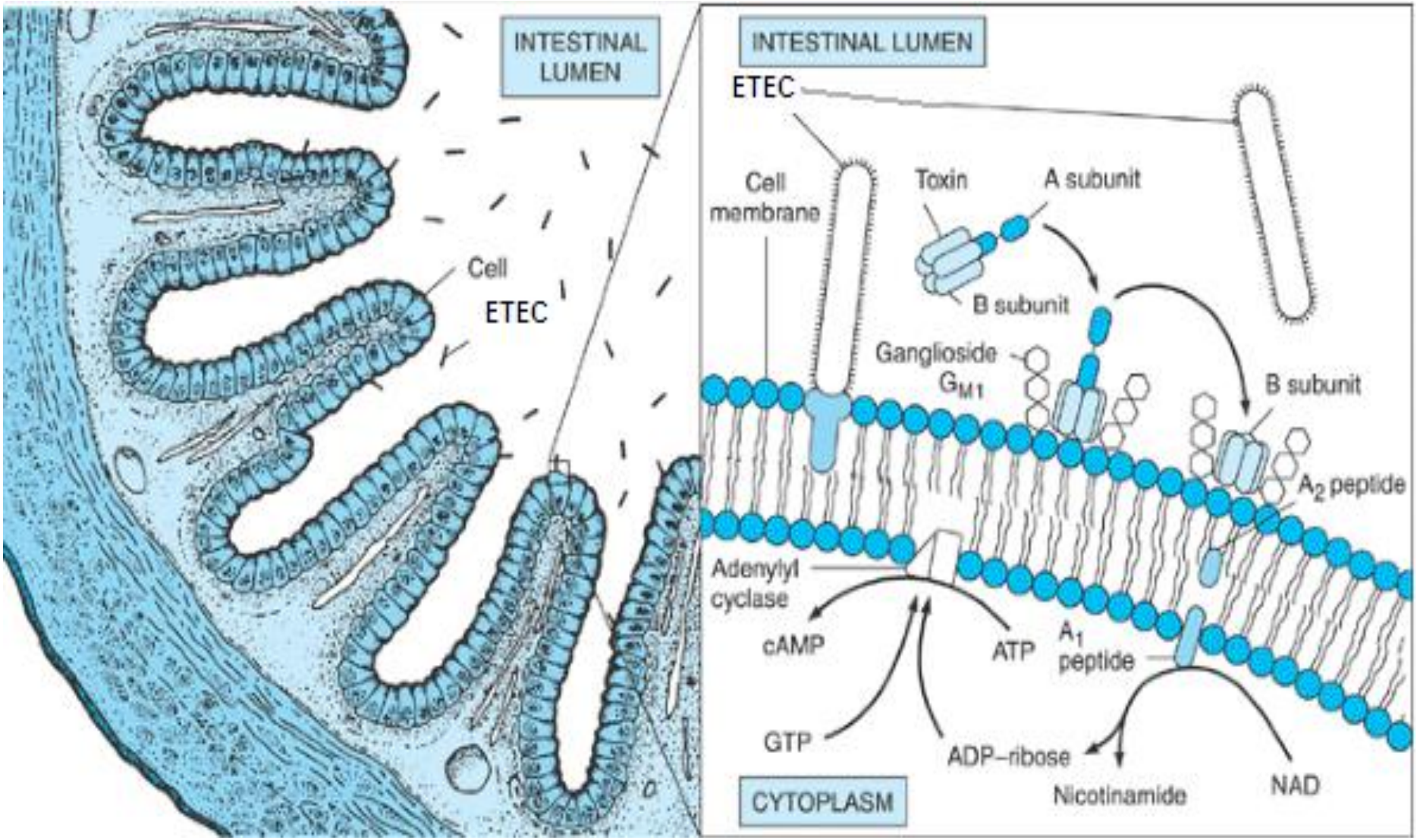
What is Colibacillosis ?

- is generally defined the infection with *Escherichia coli*
- cause of neonatal diarrhoea, post-weaning diarrhoea (PWD), oedema disease (ED), septicaemia, polyserositis, etc
- Two main pathotypes
- 1. enterotoxigenic E.coli (ETEC)***
- 2. enteropathogenic E.coli (EPEC)

What is Colibacillosis ?



What is Colibacillosis ?



Selvasingam, 2014

What is Colibacillosis ?

- Enterotoxigenic Escherichia coli (ETEC)
- Cause disease by attaching to epithelial cells in the small intestine
- using “fimbriae”
- There are many types of fimbriae
- Including K88 (F4), K99 (F5), 987P (F6) , F18 and F41
- Produce 2 important enterotoxin, i.e., heat-labile enterotoxin (LT) and heat-stable enterotoxin (Sta and STb)

What is Colibacillosis ?

Table 1: Characteristics of *E. coli* Strains that Cause Diarrhea in Young Pigs

Serogroup	Fimbriae	Toxins	Hemolysins	Age Affected
O8	K99±F41	STa	-	Neonate
O8	K88	LT, STb±STa	+	Neonate & Weaned
O9	K99±F41; 987P	STa	-	Neonate
O20	987P	STa	-	Neonate
O101	K99±F41	STa	-	Neonate
O138	F18ab;ac	STa,STb±Stx2e	+	Weaned
O139	F18ab	STa,STb±Stx2e	+	Weaned
O141	987P	STa	-	Neonate
O141	F18ac	STa,STb±Stx2e	+	Weaned
O149	K88	LT, STb±STa	+	Neonate & Weaned
O157	K88	LT, STb±STa	+	Neonate & Weaned

From: Francis, 2002. J. Swine Health and Prod. 10:171-175.

Mechanism of infection

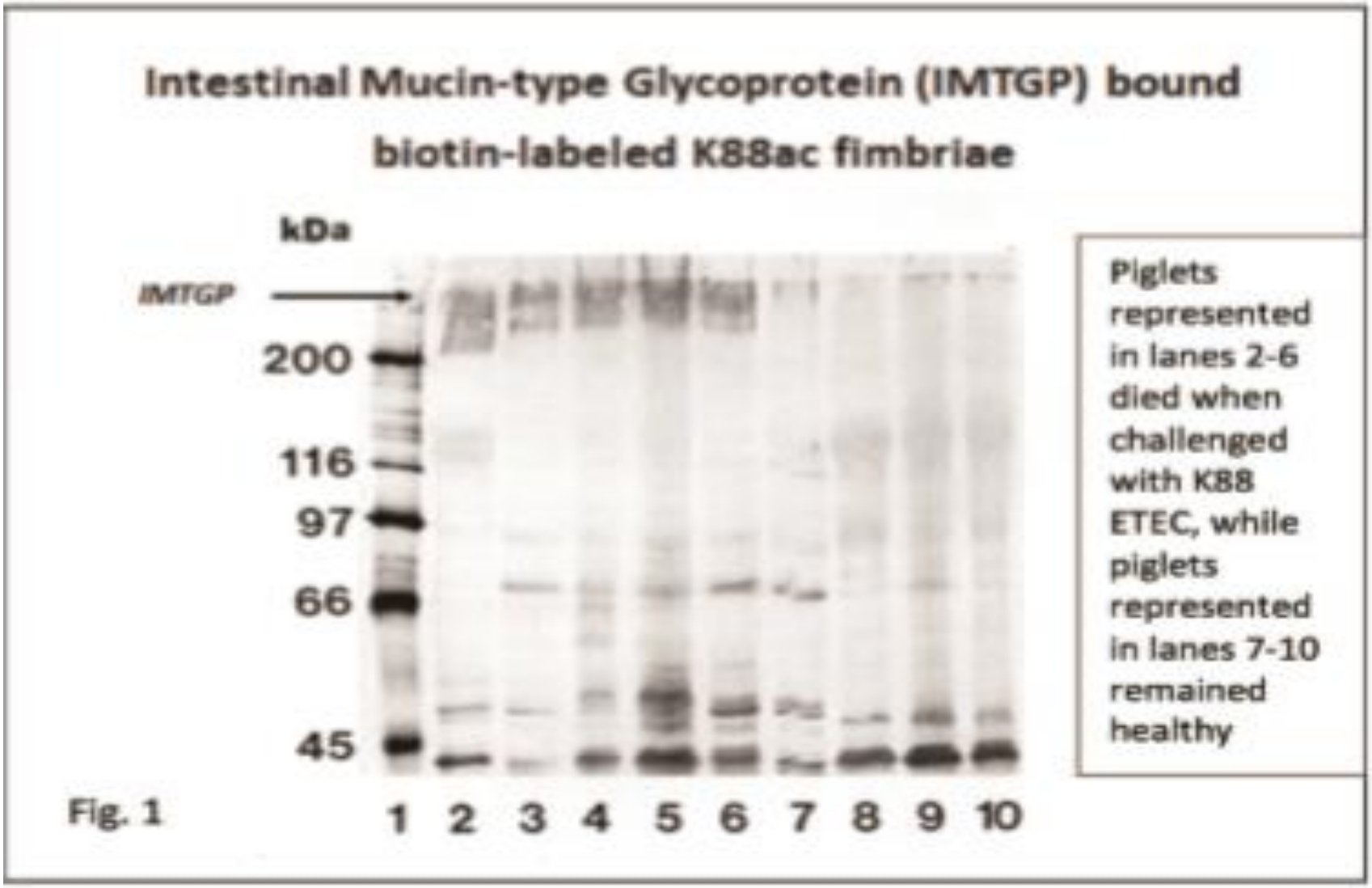
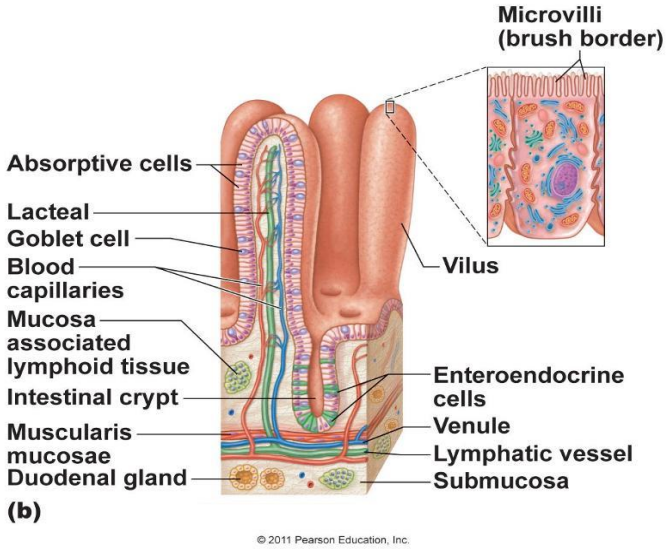
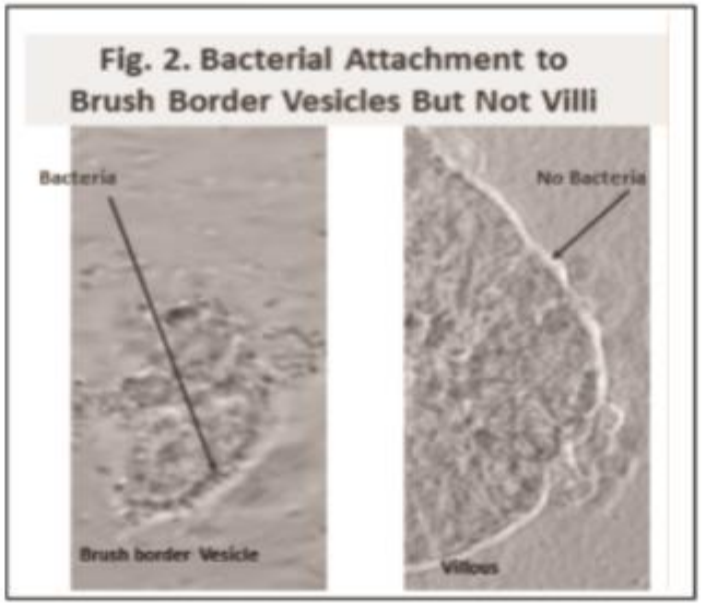
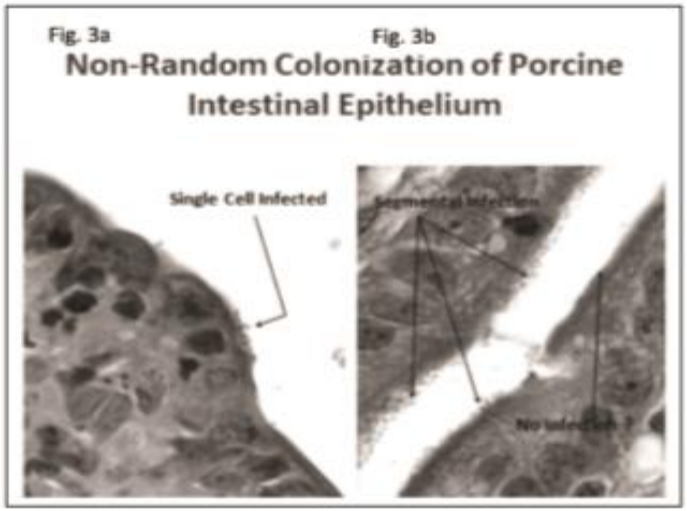


Fig. 1

Mechanism of infection







Mechanism of infection

- both adhesive fimbriae and enterotoxins is important for infection
- piglets show a modification of their intestinal receptor to ETEC fimbriae
- ex. intestinal mucin-type glycoprotein (IMTGP) is a biological receptor of K88 fimbriae
- Older pig resistant to ETEC infection than younger pig
- through thickening of the glycocalyx covering the epithelium

Diagnosis

Observation

- Neonatal diarrhea: 0-4 days of life
- PWD: 2-3 weeks after weaning

Diarrhea: yellowish, gray, slightly pink fluid

Gross lesion: dilated intestine, slightly edematous, sometime hyperaemia

Bacterial culture: hemolytic colonies (ETEC), non-hemolytic colonies (EPEC)

Diagnosis

Serotyping: agglutination test to determine O (cell wall LPS) ,F (fimbrial) antigens or H (Flagella)

Table 1: Important adhesins and serogroups of ETEC (modified from Fairbrother and Gyles, 2012).

ETEC Adhesins	O serogroups	Disease
F5, F6, F41	O8, O9, O20, O64, O101	Neonatal diarrhoea
F4	O8, O138, O141, O145, O147, O149, O157	
F4, AIDA	O8, O138, O139, O141, O147, O149, O157	PWD
F18, AIDA	O8, O138, O139, O141, O147, O149, O157	

Diagnosis

Genotyping:

- detection of genes encoding for virulence factors
- Mainly based on PCR
- ETEC: Sta, STb, LT, F4 , F5, etc
- EPEC: Eae
- STEC: Stx2e
- use of PCR for the identification of virulence factors directly in samples >> unreliable!!!

Table 2: Differential diagnosis of the main agents of neonatal diarrhoea (modified from Martelli et al., 2013).

Cause	Age	Diarrhoea	Gross Lesions	Mortality	Diagnosis
E.coli (ETEC, EPEC)	Most commonly from 0 to 4 days	Yellowish, gray or slightly pink alkaline pH	Distension, congestion of small intestine Stomach full of curdled milk	Can reach 70%	Culture/isolation Typing of isolates usually by PCR Histopathology
C.perfrigens type C	PA: 1 days A: 3 days SA: 7 days C: 10-14 days	PA: watery yellowish bloody A: brown bloody SA: watery grey/yellow C: yellow/grey	Jejunum and ileum mostly involved Haemorrhagic enteritis Bloody ascitis	100% in PA and A forms	Culture/isolation Typing/toxin identification Histopathology
C.perfrigens type A	Generally diarrhoea is observed within 48 hours of birth	Mucoid, pink without blood	Jejunum and ileum mostly involved Pasty content Presence of necrotic membrane	Generally low if not complicated	Culture/isolation Typing/toxin identification Histopathology
Clostridium difficile	In the first week of life	Pasty and yellow	Mesocolon oedema Typhlocolitis with focal erosions	Variable. Up to 50%	Culture/isolation Toxin identification
Coronavirus PEDV TGEV	All	Watery yellow/white/grey Watery yellow, white, grey, greenish; acid pH	Empty stomach Small intestine was thinned and congested	80-100% (1 week of age). Increasing the age decrease mortality	PCR Histopathology Viral isolation
Rotavirus	From 1 to 5 weeks	Watery, sometime pasty. Acid pH	Small intestine was thinned. Milk in the stomach	Low, < 20%	PCR Histopathology Viral isolation
Isospora suis	Not before 5 days More frequent around 14 days	Yellow and pasty. Alkaline pH	Small intestine. Enteritis with fibrino-necrotic membrane	Very low or not observed	Microscopic evaluation after flotation

PA: per-acute; A: acute; SA: sub-acute; C: chronic

Table 3: Differential diagnosis of the main agents of post-weaning diarrhoea (modified from Martelli et al., 2013).

Cause	Age	Diarrhoea	Gross Lesions	Mortality	Diagnosis
E.coli (ETEC, EPEC)	Most commonly from 0 to 4 days	Yellowish, gray or slightly pink alkaline pH	Distension, congestion of small intestine Stomach full of curdled milk	Can reach 70%	Culture/isolation Typing of isolates usually by PCR Histopathology
C.perfrigens type C	PA: 1 days A: 3 days SA: 7 days C: 10-14 days	PA: watery yellowish bloody A: brown bloody SA: watery grey/yellow C: yellow/grey	Jejunum and ileum mostly involved Haemorrhagic enteritis Bloody ascitis	100% in PA and A forms	Culture/isolation Typing/toxin identification Histopathology
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Isospora suis	Not before 5 days More frequent around 14 days	Yellow and pasty. Alkaline pH	Small intestine. Enteritis with fibrino-necrotic membrane	Very low or not observed	Microscopic evaluation after flotation

A: acute; C: chronic

Diagnosis

Can pooled pen floor samples be used for diagnosing Escherichia coli F18 positive diarrhoeic nursery pigs? (Nicolai Weber)

- pooled faecal pen floor samples (PFP) can be used for diagnosing ETEC in diarrhoeic nursery pigs ?
- Bacterial culture
- qPCR
- PFP vs Pig sample

Diagnosis

Method	Se	CL95%	Sp	CL95%
Culture	83.3	55.2-95.3	84.2	62.4-94-5
qPCR	83.3	55.2-95.3	68.4	46.0-84.6

Can pooled pen floor samples be used for diagnosing Escherichia coli F18 positive diarrhoeic nursery pigs? >> **YES** !!

Susceptibility test

- Disc diffusion >> low reliable
- Minimum inhibitory concentration = lowest concentration of a chemical that prevents visible growth of a bacteria
- MIC >> quantitative results (susceptible, intermediate, resistant)

dentification nformation	Card: GN		Lot Number: 241321840	Expires:	Oct 6, 2015 12:00 ICT
	Completed: Jun 24, 2015 15:59 ICT		Status: Final	Analysis Time:	6.00 hours
Selected Organism	95% Probability		Vibrio parahaemolyticus		
	Bionumber: 5025711340541262			Confidence:	Very good identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages: The following antibiotic(s) are not claimed: ESBL, Polymyxin B, Rifampicin,					
Contraindicating Typical Biopattern(s) Vibrio parahaemolyticus BGLU(16),URE(10),					

Card is for veterinary use only

Susceptibility Information	Card: AST-GN65		Lot Number: 585333910	Expires:	Feb 4, 2016 12:00 ICT
	Completed: Jun 24, 2015 17:29 ICT		Status: Final	Analysis Time:	7.50 hours
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL			Tobramycin	2	S
Ampicillin	>= 32	R	Imipenem	<= 1	S
Amoxicillin/Clavulanic Acid	4	S	Trimethoprim/Sulfamethoxazole	<= 20	S
Tiperacillin	>= 128	R	Chloramphenicol	<= 2	S
Cefalexin	>= 64	R	Enrofloxacin	<= 0.12	S
Cefovecin	>= 8	R	Marbofloxacin	<= 0.5	S
Cefpodoxime	>= 8	R	Polymyxin B		
Ceftiofur	>= 8	R	Tetracycline	<= 1	
Amikacin	<= 2	S	Nitrofurantoin	<= 16	S
Gentamicin	<= 1	S	Rifampicin		

= Deduced drug *= AES modified **= User modified

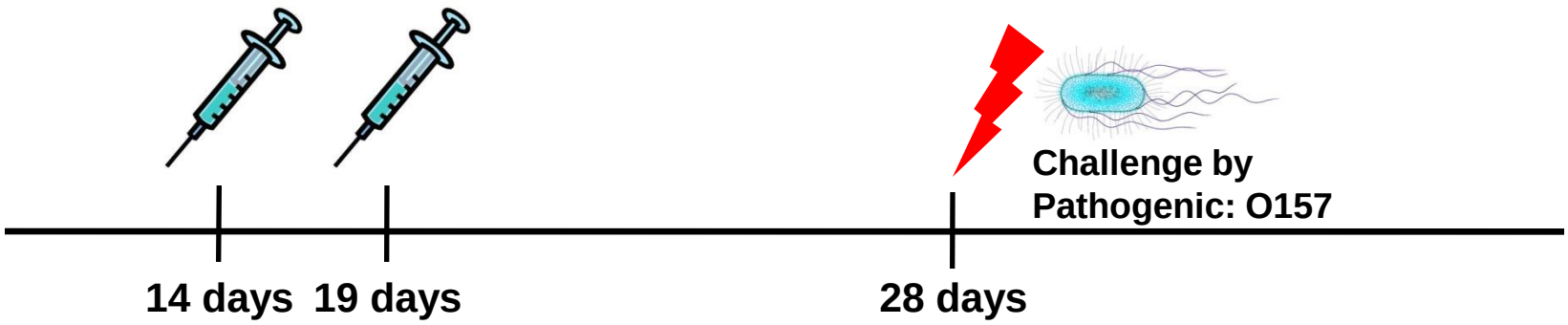
Immunization

- Vaccination: delivered to pregnant sows >> high efficiency
- But not weaned pig >> ???
- Concerning:
- 1. immunity immature in piglet
- 2. maternal immunity

Immunization

Experiment 1: live vaccine

- Piglets >> susceptible to K88 ETEC
- suckled for 10 days before separate from dam
- Vaccination (OS) 4 groups, i.e., i) live K88/Ltb ii) live K88, iii) live Ltb and iv) no antigen *E. coli*



Immunization

Experiment 1: live vaccine

Table 2: Clinical Outcomes following ETEC among Pigs Vaccinated with Constructed Vaccine Strain

Plasmid gene(s)	No. Pigs	Diarrhea	≥ 10% weight loss	Death*
K88/LTb	33	1(3)	0(0)	0(0)
K88	18	5(28)	2(11)	2(11)
LTb	14	14(100)	12(86)	10(71)
None	55	37(67)	23(42)	22(40)

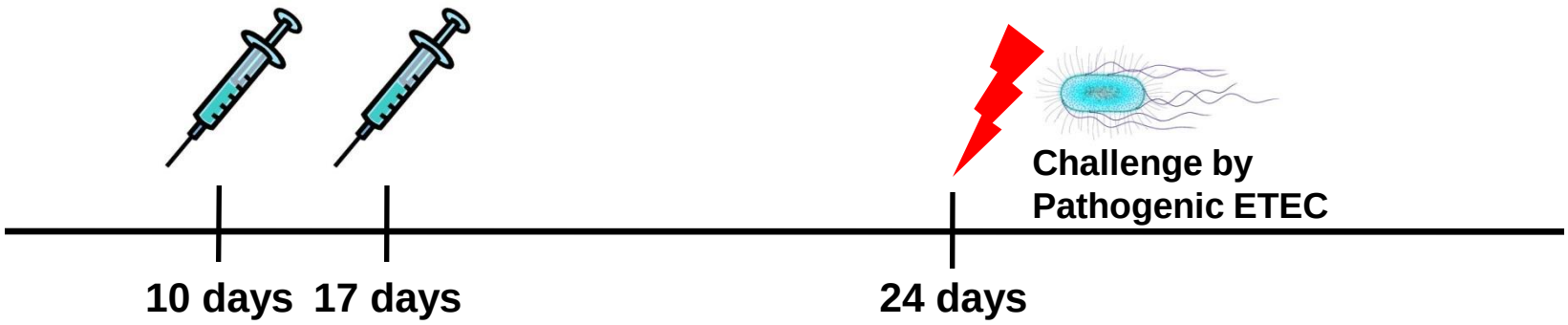
( )=%

*died or were euthanized when moribund

Immunization

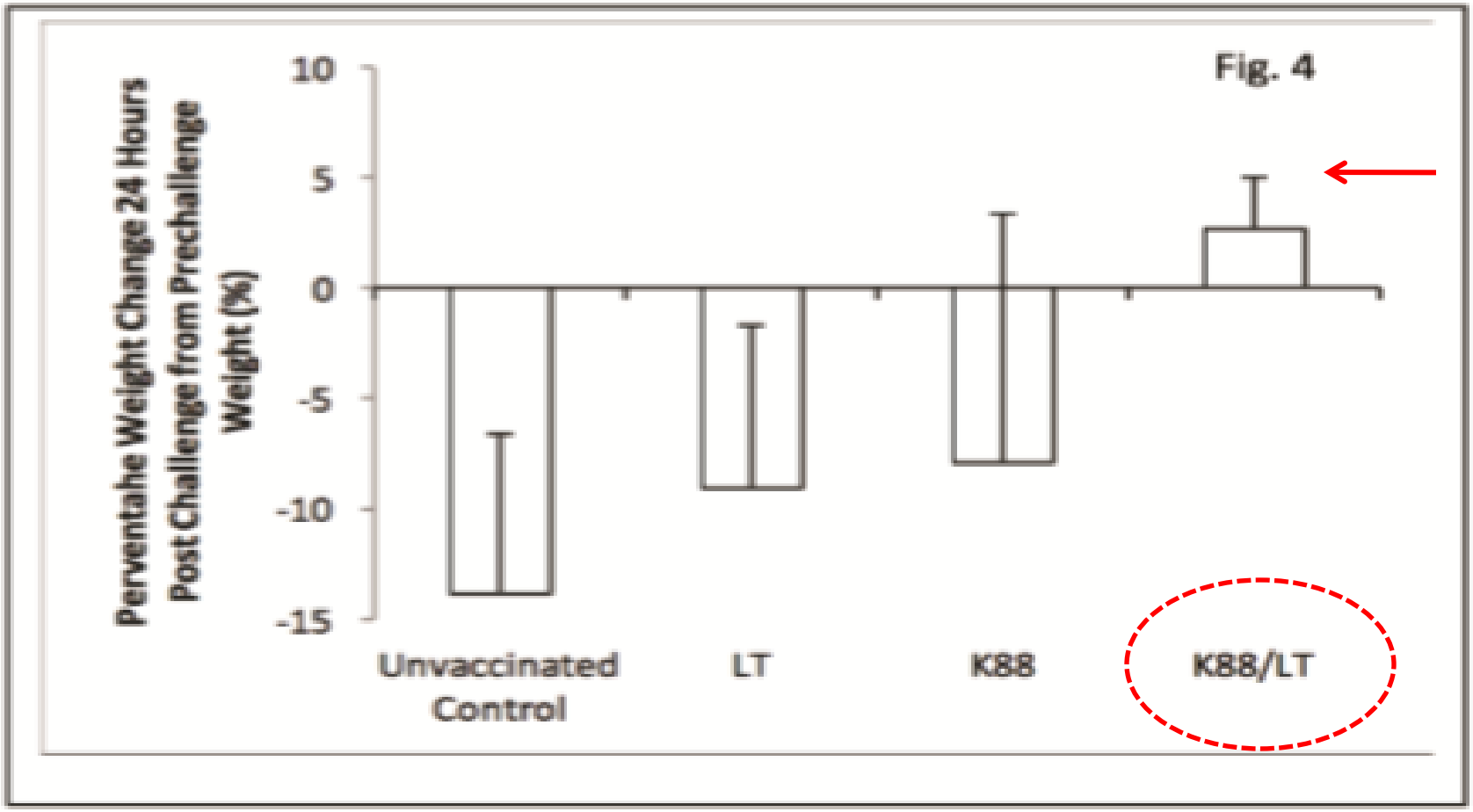
Experiment 2: subunit vaccine

- Piglets >> susceptible to K88 ETEC
- Vaccination (intranasally) 4 groups, i.e., i)purified K88/Lt ii) purified K88, iii) commercial Lt toxin and iv) diluent



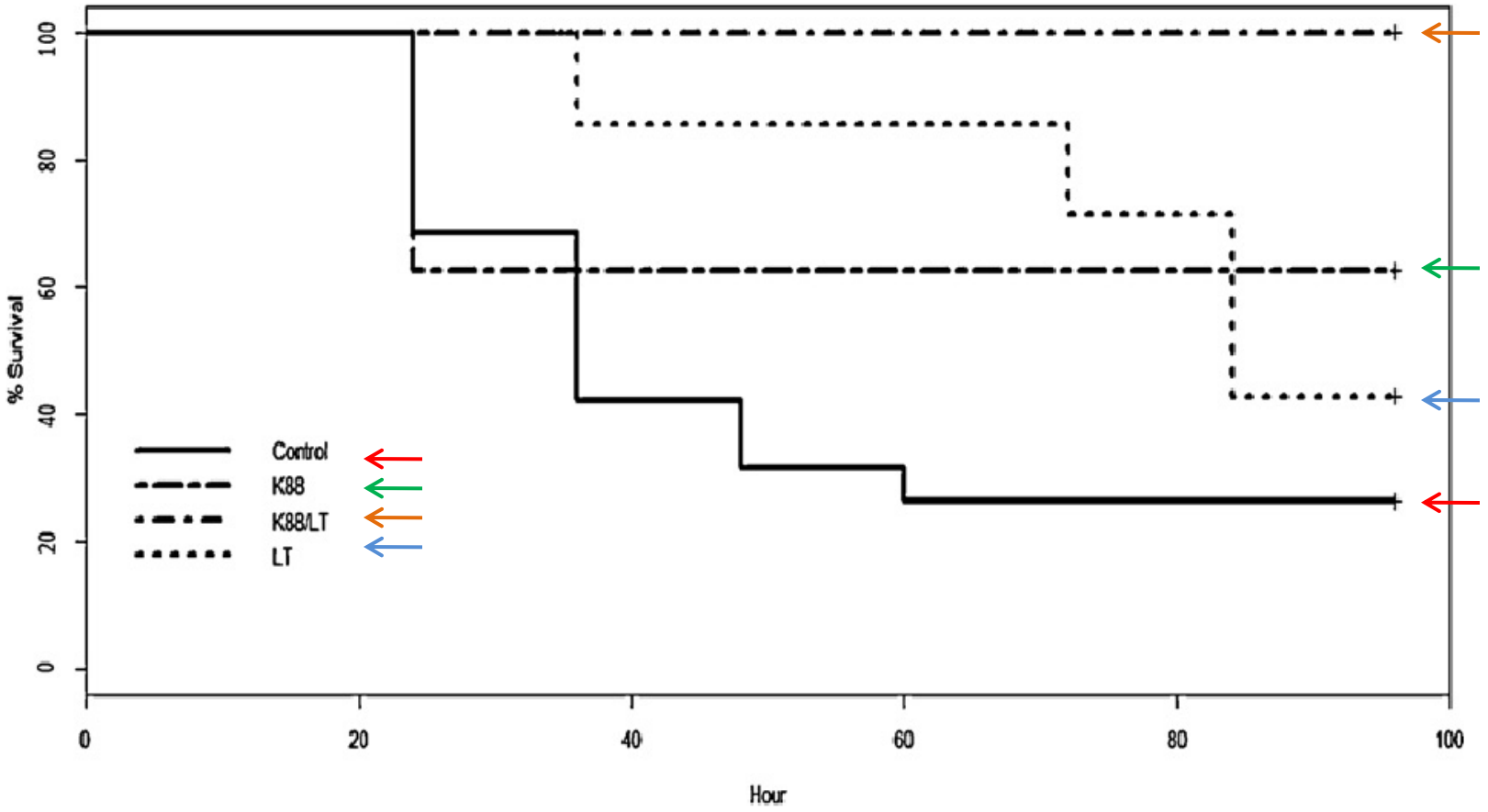
Immunization

Experiment 2: subunit vaccine



Immunization

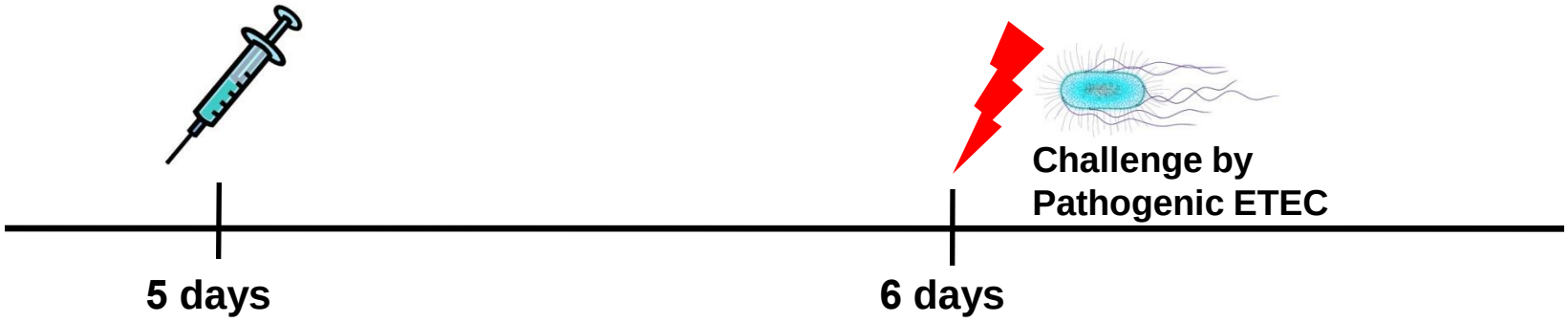
Experiment 2: subunit vaccine



Immunization

Experiment 3: live vaccine

- O8:H4 field isolate that expressed K88 fimbriae, but not LT or STb
- 4 groups (OS), i.e., i) mutate LT, ii) mutated Lt with STb, iii) O8:H4 with K88 and iv) no vaccine
- suckled for 12 hour before separate



Immunization

Experiment 3

Post-Challenge Observations Among K88-Receptor-Postivie Pigs Receiving Vaccine Strains 24h Before Challenge

Table 3

Vaccine Strain Gene(s)	No. Pigs	Diarrhea	Weight change	Change in Serum Total Protein
K88	8	5	-2.9±0.1	-5.5±2.9
K88/LT(R192G)	7	0	-2.1±0.1	-3.3±4.3
K88/STb-LT(R192G)	7	0	0.1±0.1	-5.5±3.8
Non-Vaccinates	9	9	-19.3±0.1	30.6±6.3

Immunization

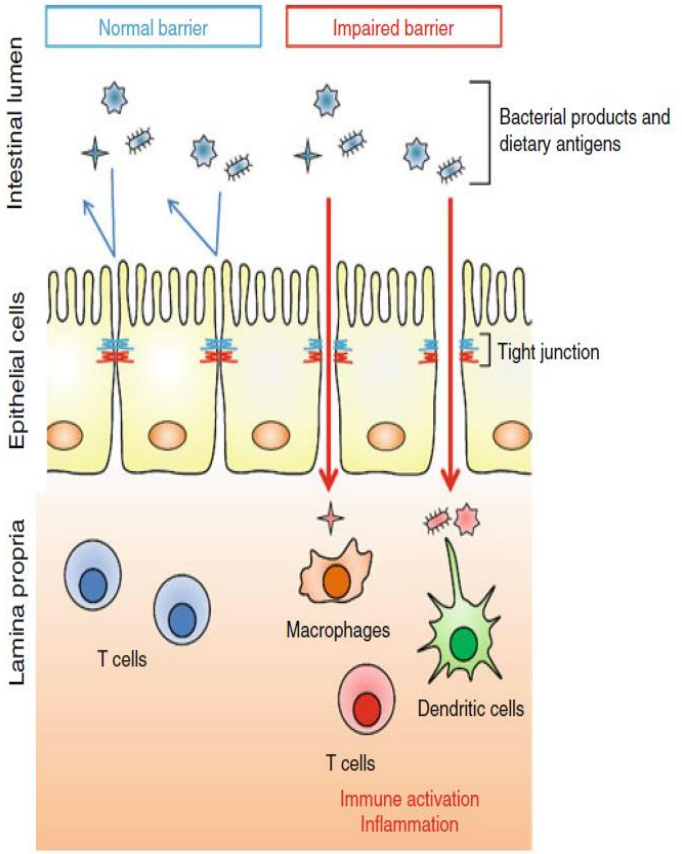
Conclusion

- efficacious K88 ETEC vaccines require “antigen” of both fimbriae and heat-labile enterotoxin
- mucosal route !!
- Live constructed vaccines >> oral route
- Subunit vaccines >> intranasally

Alternative method

Polyphenol

- positive regulatory effect on intestinal epithelium tight junctions (TJ)
- fruits, vegetables, tea, coffee
- Antioxidative
- anti-inflammatory
- anti-carcinogenic
- Decrease and ı permeability



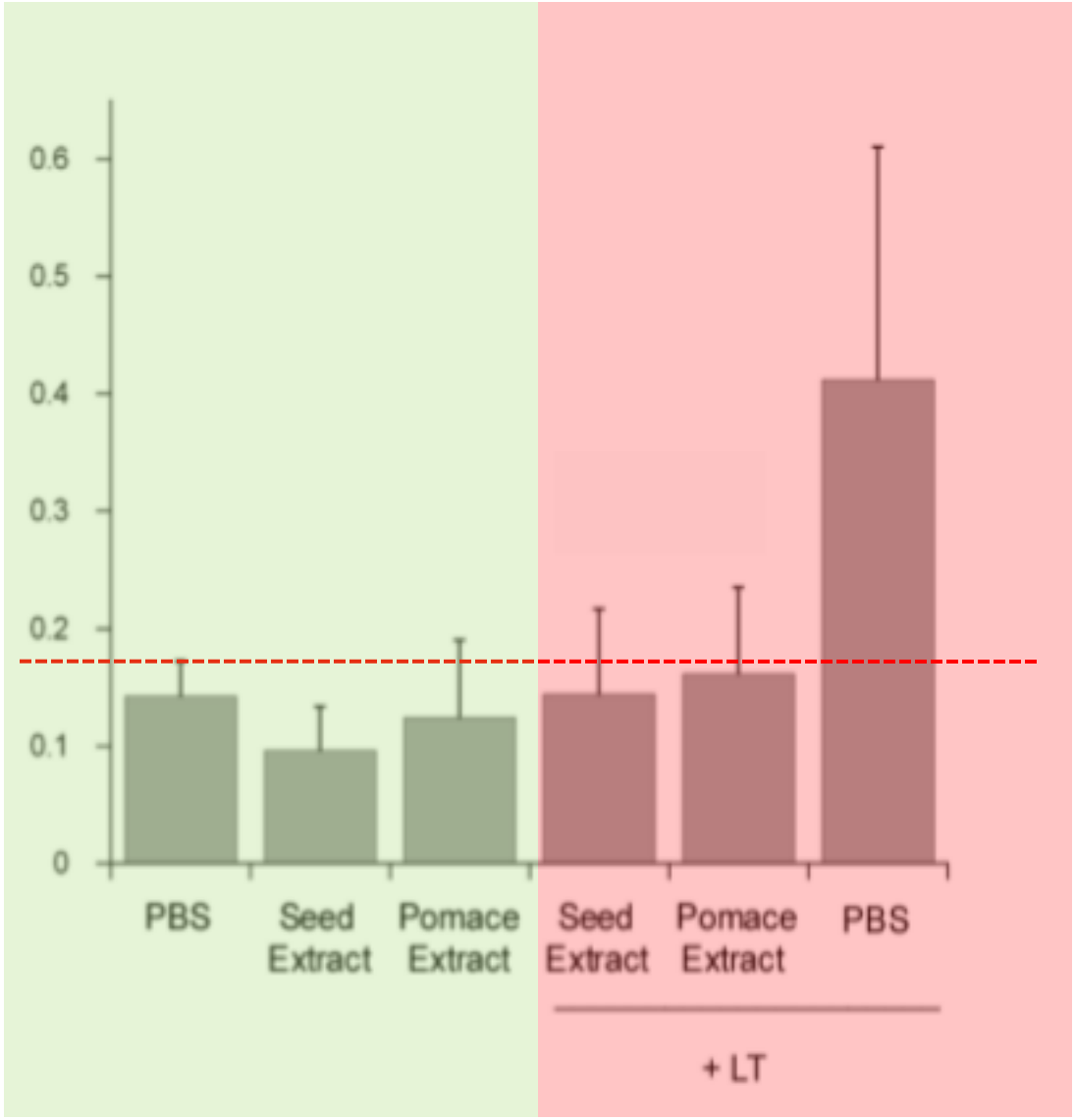
Suzuki, 2013

Table 4 Nutrients and food factors decrease and restore intestinal TJ permeability

Nutrients and food factors[a]	Permeability	Cell	Mechanism[b]
Amino acid			
Gln	Not determined	Caco-2	Claudin-1 ↔ [187]
Gln	Decrease	Caco-2	Neutralize acetaldehyde, restoration of occludin and ZO-1 distribution [189]
Trp	Decrease	Caco-2	Unknown [191]
Peptide			
Casein peptide	Decrease	Caco-2	Occludin ↑ [194]
Cheese peptide	Decrease	Caco-2	Unknown [192]
Fatty acid			
EPA, DHA, arachidonic acid, γ-LA, di-homo-γ-LA	Decrease	T84	Unknown [197]
EPA, DHA, arachidonic acid, di-homo-γ-LA	Decrease	T84	Neutralize IL-4 [197]
Acetic acid	Decrease	Caco-2, T84	Unknown [206]
Propionic acid	Decrease	Caco-2, T84	Unknown [206]
Butyric acid	Decrease	Caco-2	Promotion of occludin and ZO-1 assembly in Ca-induced TJ reassembly [205]
Vitamin			
Vitamin A	Decrease	Caco-2	Neutralize *Clostridium difficile* toxin A [213]
Vitamin D	Not determined	SW480	ZO1 ↑, claudin-1 ↑, claudin-2 ↑, E-cadherin ↑ [214]
	Decrease	Caco-2	Neutralize DSS [214]
Polyphenol			
Quercetin	Decrease	Caco-2	Claudin-4 ↑, ZO-2 ↔, claudin-1 ↔, occludin ↔ [217]
Kaempferol	Decrease	Caco-2	ZO-2 ↑, claudin-4 ↑ occluidn ↔, claudin-1 ↔, claudin-3 ↔ [218]
Myricetin	Decrease	Caco-2	Unknown [217]
Genistein	Decrease	Caco-2	Neutralize hydrogen peroxide, occludin ↔, ZO-1 ↔ [40]
	Decrease	Caco-2	Neutralize acetaldehyde, occludin ↔, ZO-1 ↔ [43]
Curcumin	Decrease	Caco-2	Neutralize TNF-α [106]
	Decrease	Caco-2	Neutralize IL-1β [116]
EGCG	Decrease	T84	Neutralize IFN-γ [221]
Probiotics			
EGN	Decrease	T84	Unknown [225]

Suzuki, 2013

B


Net Fluid Accumulation (g/cm)
0.6
0.5
0.4
0.3
0.2
0.1
0
PBS
Seed Extract
Pomace Extract
Seed Extract
Pomace Extract
PBS
+ LT

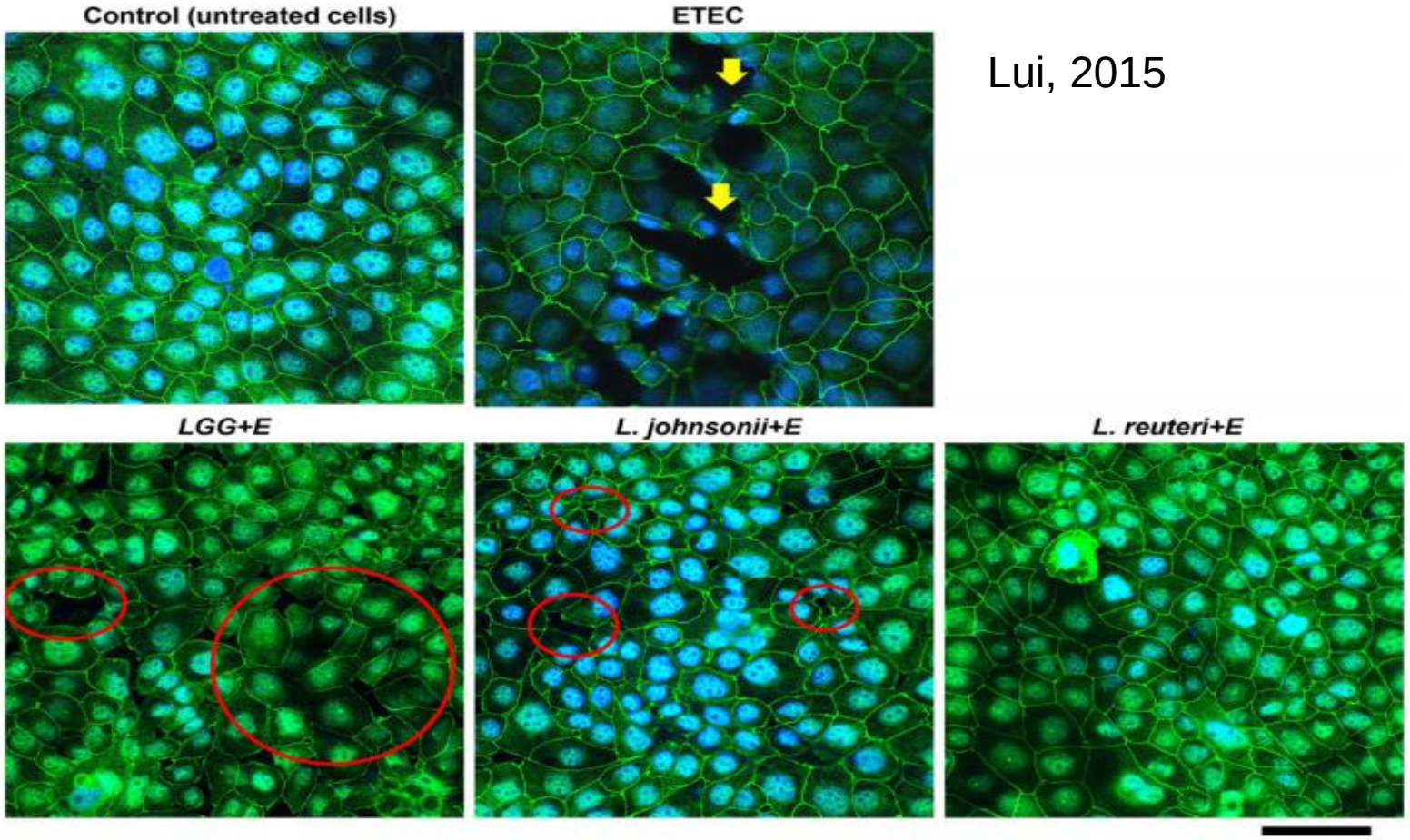
Alternative method

Probiotic and prebiotic

- Lactobacillus inhibit adherence of ETEC
- Saccharomyces stimulate intestinal immunity
- and inhibit binding of toxins to enterocyte receptors
- Prebiotic ,e.x., fermented ingredients
- stimulate the proliferation of potentially beneficial microorganism

Alternative method

Probiotic and prebiotic

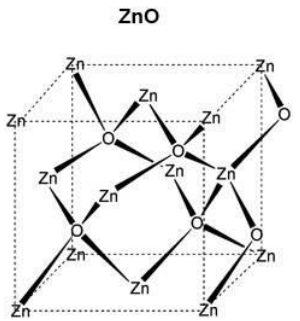


Lui, 2015

Alternative method

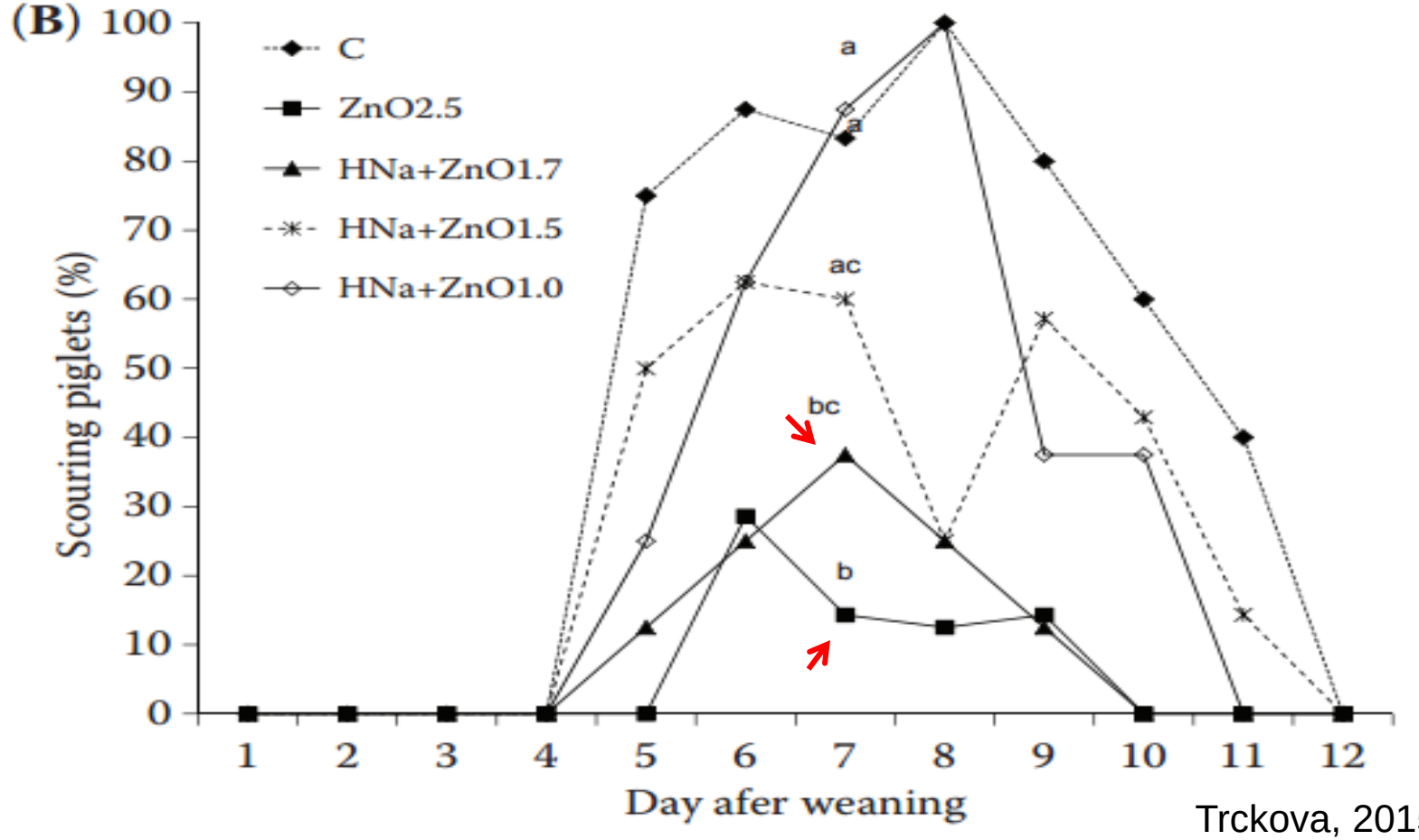
Zinc oxide

- 2,400 and 3,000 ppm of zinc can reduce diarrhea
- By reduce bacterial adherence of ETEC F4
- Can be an environmental pollution
- Resistant bacteria to Zn



Alternative method

Zinc oxide

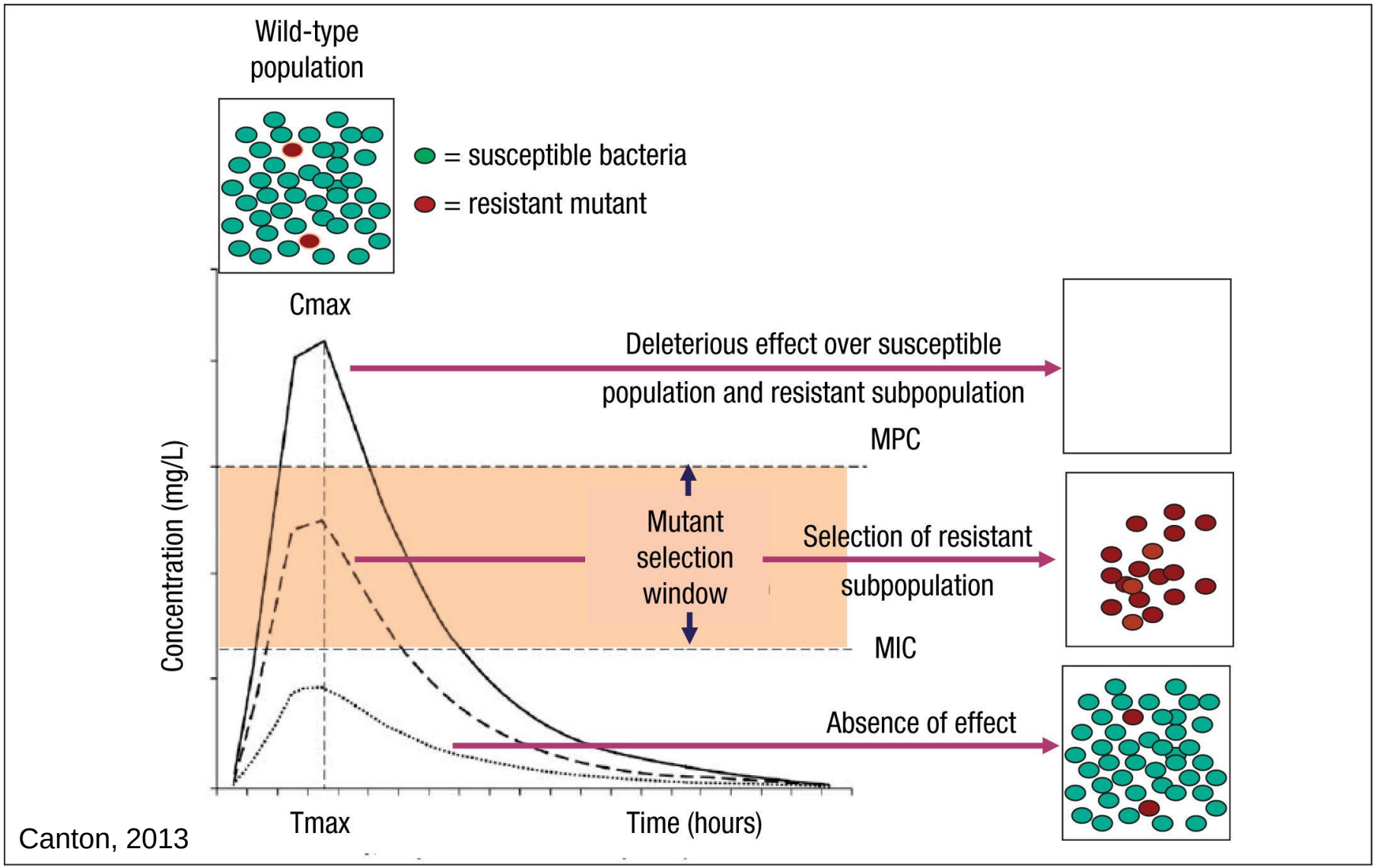


Trckova, 2015

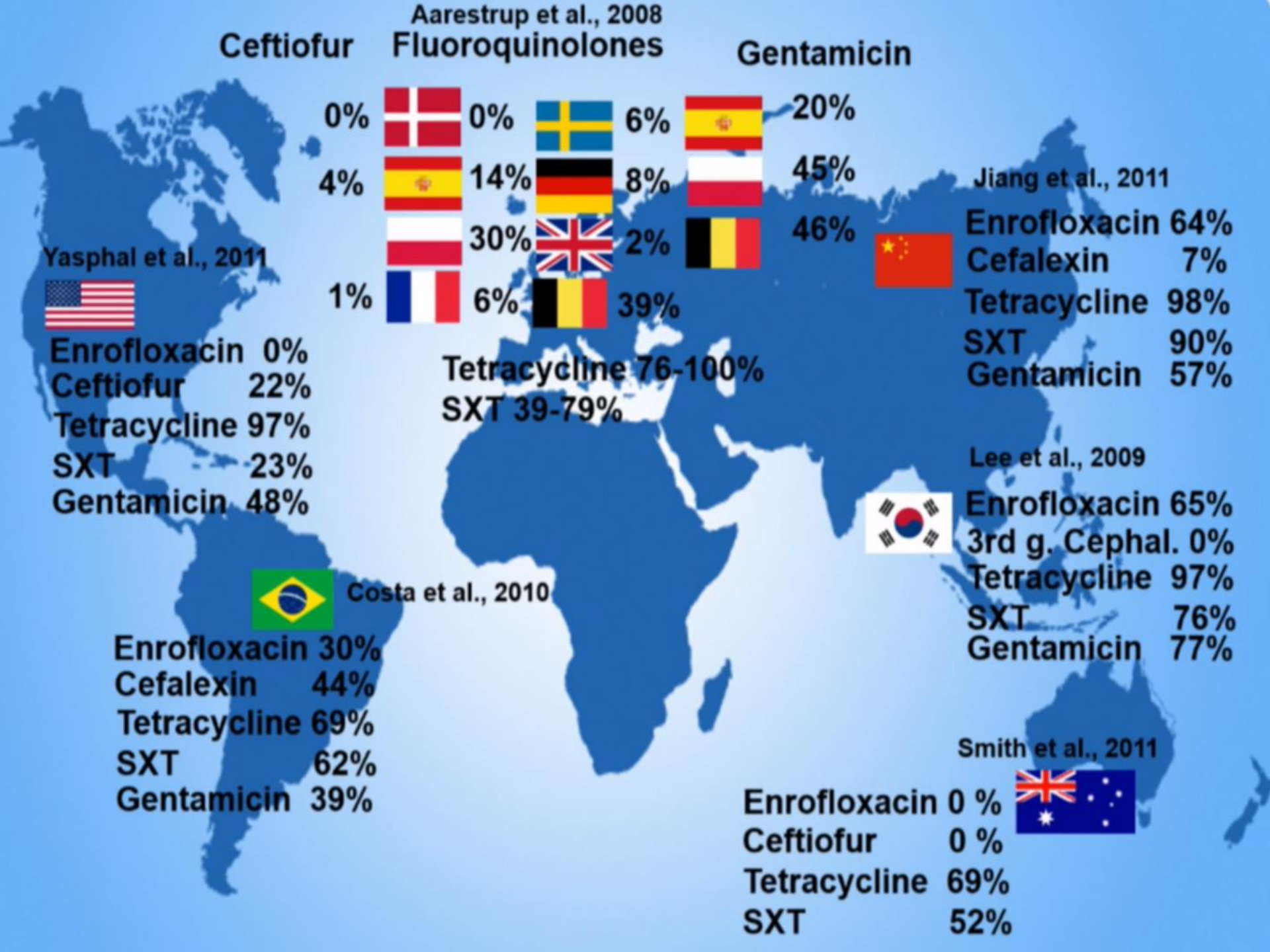
Resistant *E. coli*

- Under-dosing is frequent with oral administration in pigs
- Induce selection of resistant bacteria
- must be chosen for their ability to achieve therapeutic concentrations at intestinal level
- Multidrug-resistant pathogenic E. coli strains are often isolated from diarrhoeic pigs

Resistant *E. coli*



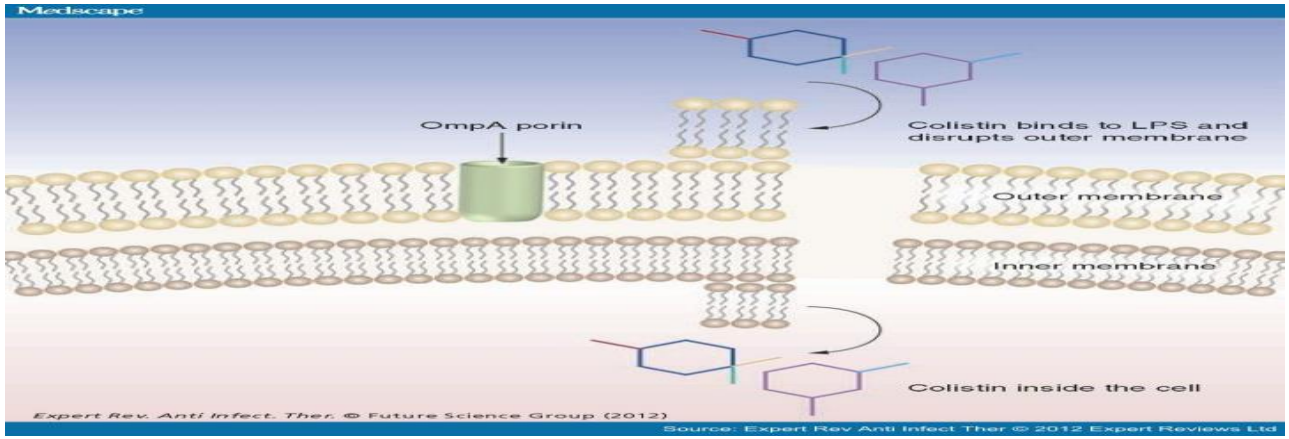
Canton, 2013


Aarestrup et al., 2008
Ceftiofur
Fluoroquinolones
Gentamicin
0%
0%
6%
20%
4%
14%
8%
45%
30%
2%
46%
1%
6%
39%
Yasphal et al., 2011
Enrofloxacin 0%
Ceftiofur 22%
Tetracycline 97%
SXT 23%
Gentamicin 48%
Tetracycline 76-100%
SXT 39-79%
Jiang et al., 2011
Enrofloxacin 64%
Cefalexin 7%
Tetracycline 98%
SXT 90%
Gentamicin 57%
Lee et al., 2009
Enrofloxacin 65%
3rd g. Cephal. 0%
Tetracycline 97%
SXT 76%
Gentamicin 77%
Costa et al., 2010
Enrofloxacin 30%
Cefalexin 44%
Tetracycline 69%
SXT 62%
Gentamicin 39%
Smith et al., 2011
Enrofloxacin 0 %
Ceftiofur 0 %
Tetracycline 69%
SXT 52%

Resistant *E. coli*

Colistin

- polymyxin group of polypeptide antibiotics
- activity against Gram-negative bacteria (GNB)
- lipopolysaccharide (LPS) and phospholipids in the outer cell membrane of GNB
- leading to disruption of the outer cell membrane



Resistant *E. coli*

Colistin resistant

Table 4: Percentage of colistin resistance in E.coli isolated from healthy and diseased pigs (modified from Kempf et al., 2013).

Country	Origin of the isolates	% of resistance/non-wild type strains	Reference
France	faeces, healthy pigs	0.5%	Belloc et al., 2008
Sweden	healthy pigs	0%	SVARM, 2011
Denmark	healthy pigs	0%	DANMAP, 2009
Belgium	pigs with diarrhoea	9.6%	Boyen et al., 2010
Croatia	pigs with diarrhoea	3%	Habrun B., 2011
Brazil	pigs with diarrhoea	6.3%	Morales et al., 2012
Brazil	pigs with diarrhoea	28.1%	Costa et al., 2010
UK	Slaughterhouse, healthy pigs	34,1%	Enne et al., 2008
China	pigs with diarrhoea	33.3%	Lu et al., 2010
Japan	pigs with diarrhoea	35.6%	Harada K., 2005
Italy	pigs with diarrhoea	42.2%	Data not published, 2015

Resistant *E. coli*

Colistin resistant

- resistant phenotype >> modification of LPS charge
- by modification of lipid A
- chromosomally mediated, ex., deletion of the *etk* gene (*E. coli*), pmrA (*S. Typhimurium*)
- plasmid-mediated colistin resistance mechanism >> **MCR-1 !!!**

Resistant *E. coli*

Colistin resistant

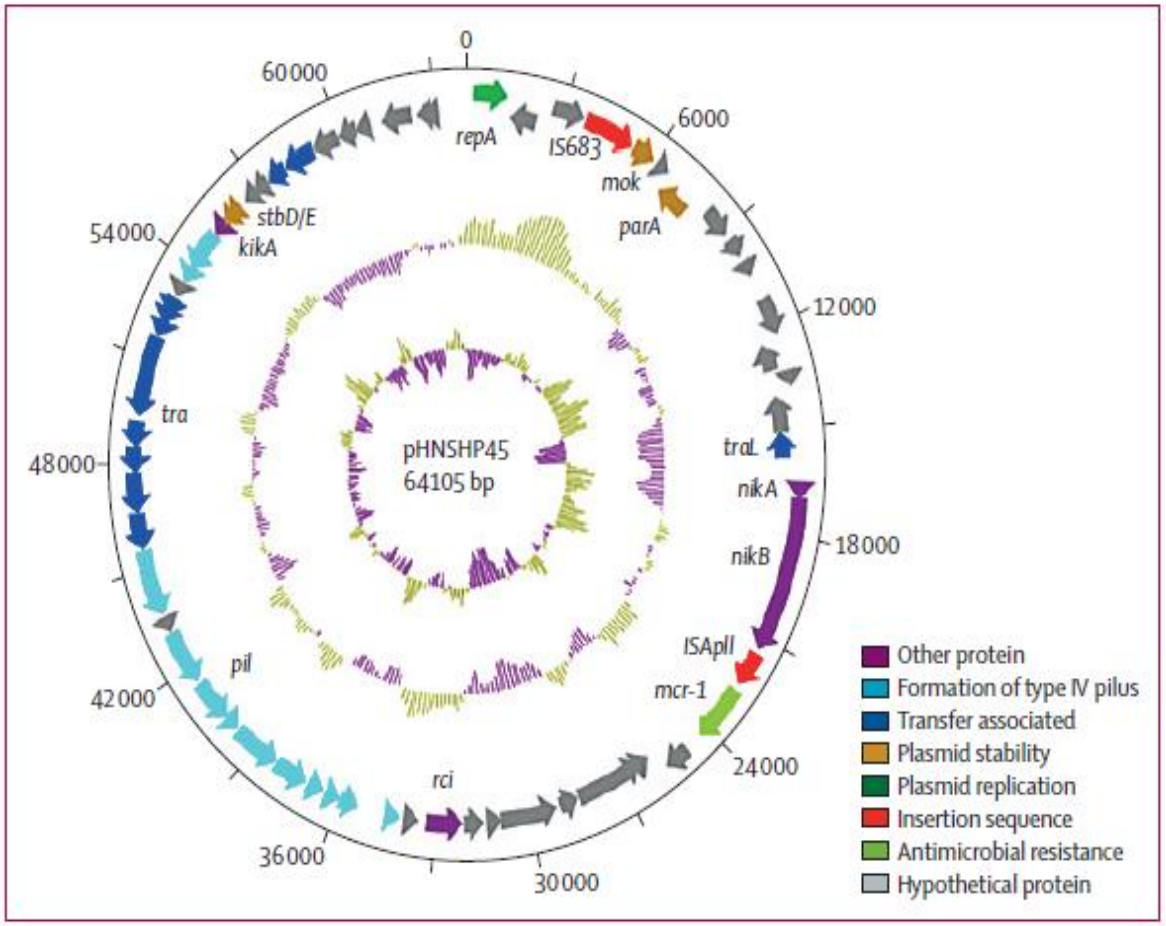
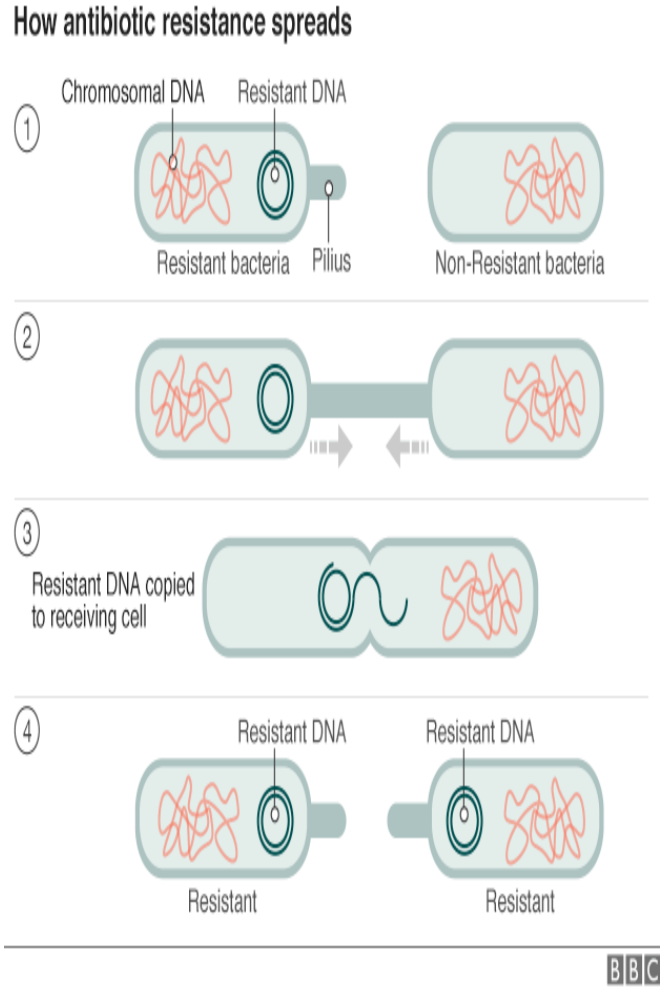


Figure 2: Structure of plasmid pHNSHP45 carrying *mcr-1* from *Escherichia coli* strain SHP45

Liu, 2016



Resistant *E. coli*

Colistin resistant

	Year	Positive isolates (%)/number of isolates
Escherichia coli		
Pigs at slaughter	All	166 (20·6%)/804
Pigs at slaughter	2012	31 (14·4%)/216
Pigs at slaughter	2013	68 (25·4%)/268
Pigs at slaughter	2014	67 (20·9%)/320
Retail meat	All	78 (14·9%)/523
Chicken	2011	10 (4·9%)/206
Pork	2011	3 (6·3%)/48
Chicken	2013	4 (25·0%)/16
Pork	2013	11 (22·9%)/48
Chicken	2014	21 (28·0%)/75
Pork	2014	29 (22·3%)/130
Inpatient	2014	13 (1·4%)/902
Klebsiella pneumoniae		
Inpatient	2014	3 (0·7%)/420

Table 2: **Prevalence of colistin resistance gene *mcr-1* by origin**

Liu, 2016

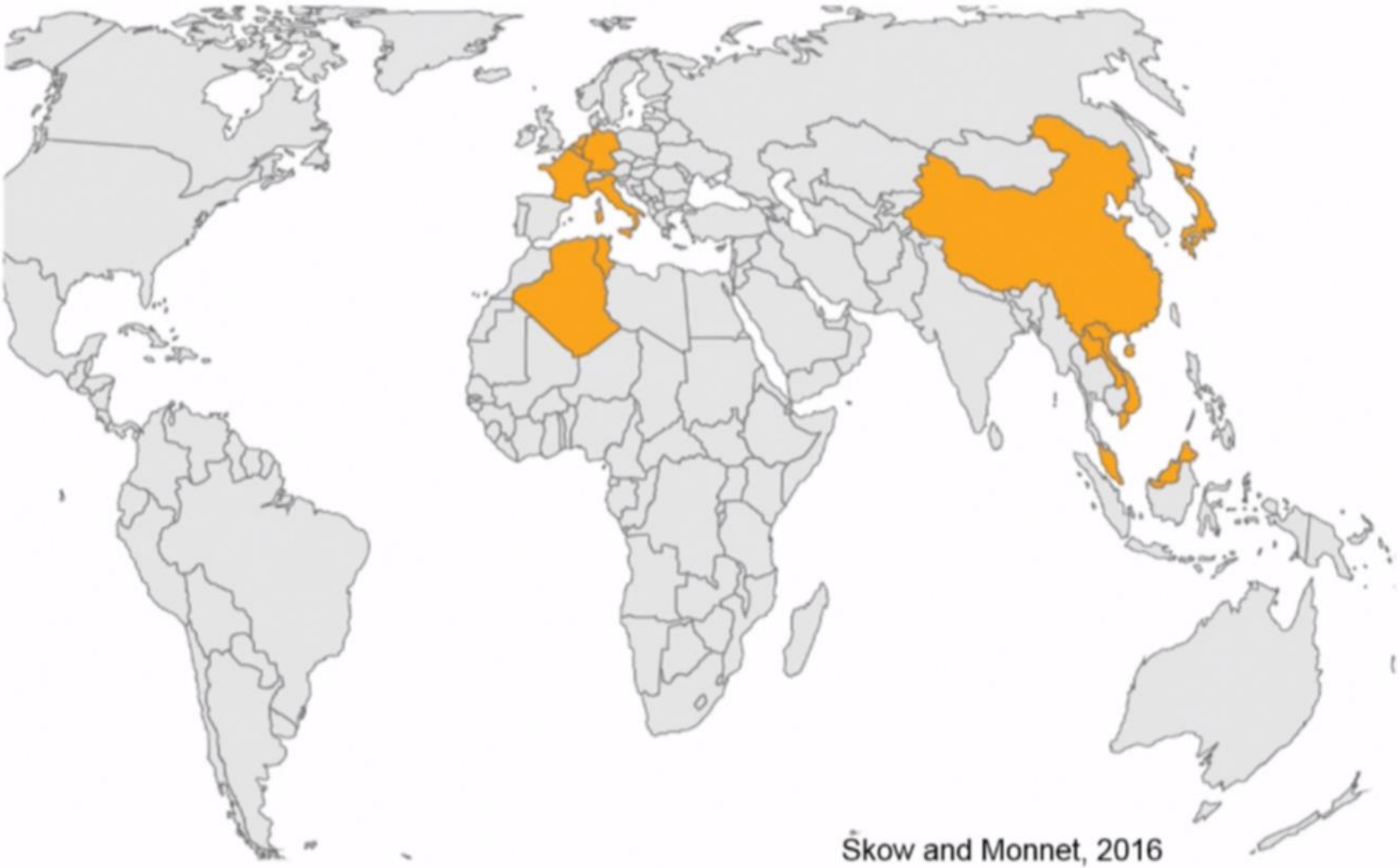
	Colistin minimum inhibitory concentration (mg/L)	Year	Source	Country	*mcr-1*
LH30	6	2012	Human	Laos	+
LH57	8	2012	Human	Laos	+
LH1	6	2012	Human	Laos	+
LH121	16	2012	Human	Laos	+
LH140	12	2012	Human	Laos	+
LH257	12	2012	Human	Laos	+
P10	6	2012	Pig	Laos	+
P6	6	2012	Pig	Laos	+
P17	4	2012	Pig	Laos	+
P7	4	2012	Pig	Laos	-
TH176	6	2012	Human	Thailand	-
TH214	6	2012	Human	Thailand	+
TH99	4	2012	Human	Thailand	+
FHM19*	12	2012	Human	France	-
FHA102†	12	2012	Human	France	-
FHA113‡	12	2012	Human	France	-
NH94§	12	2012	Human	Nigeria	-
235	4	2015	Chicken	Algeria	+
249	3	2015	Chicken	Algeria	-

Mutations in PmrB sensor kinase of the two-component system: *Pro7_Gln12del (deletion of 6 aminoacids); †Ala159Val; ‡Thr156Lys; and §Iso92 ins (insertion of isoleucine at position 92).

Table: **Colistin-resistant *Escherichia coli* with the associated *mcr-1* gene isolated from different sources**

Olaitan, 2016

Mcr-1 distribution in food animals (as of 1° March 2016)



Škow and Monnet, 2016

Advise for Colistin resistant

- colistin should be only used based on susceptibility testing
- duration of the treatment should be limited to the minimum time necessary for the treatment
- should not exceed 7 days
- use of the product deviating from the instructions given in the SPC may lead to treatment failures and increase the prevalence of bacteria resistant to colistin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Thank you !!
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