National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Whole genome sequence comparison of MDR *Salmonella* Infantis isolates from broilers and humans in the Netherlands

Angela van Hoek

17-09-2020

Introduction

- In recent years a shift is seen in the most prevalent *Salmonella* serotypes in broilers in the Netherlands
  - *Salmonella* Paratyphi B variant Java was the most prevalent serotype
  - Increase in the relative percentage of *Salmonella* Infantis.
- In 2018 *S*. Infantis was the most reported serotype in broilers as well as poultry meat in the Netherlands as well as in Europa
- *S*. Infantis ranked fourth among the serotypes isolated from human salmonellosis cases in the Netherlands and in Europe in the past few years

- *S*. Infantis increasingly associated with multi-drug resistance.
- In 2014, a multi-drug resistant *S*. Infantis strain was identified carrying a mega-plasmid of approximately 300 Kb called pESI (plasmid of emerging *S*. Infantis)

Introduction

- Yearly every EU member state has to report about prevalence and trends of zoonotic pathogens in animals, animal products and humans to ECDC and EFSA (zoonoses Directive (2003/99/EC)).
- Information regarding prevalence and risk factors of zoonotic pathogens in animals is required for adequate prevention measures.

The Netherlands: NVWA/RIVM project surveillance livestock

- NVWA (Netherlands Food and Consumer Product Safety Authority);
  - ’Monitoring pathogens in livestock’
- RIVM (National Institute of Public Health and the Environment);
  - ‘Monitoring of gastro-enteritis (GE) and GE-pathogens in humans’

Introduction

- Trends in prevalence of zoonotic pathogens in different livestock sectors:
  - Pigs (2013)
  - Layers (2015)
  - Beef cattle (2017)
  - Broilers (2018-2019)
  - Dairy cattle (2020)
  - Small ruminants; Dairy Goats/Sheep (2016)

- Research in livestock & farmers, family members and employees
- Faecal sampling and questionnaire

Broilers (2018-2019)

Zoonotic pathogens:

- Animals: *Campylobacter*, ESBL/AmpC-producing *E. coli*, *Listeria monocytogenes*, *Salmonella* and STEC.
- Humans: *Campylobacter*, ESBL/AmpC-producing *E. coli*, MRSA and *Salmonella*

  - The Netherlands: 625 broiler farms on 823 locations (CBS, 2018)
  - Randomly selected farms with >3000 broilers (n=230)
  - 200 put in a sampling scheme, 30 were backups

Response

- 198 farms visited; 379 flocks tested
- 81 farms (40.6%) participated in the human study; 132 farmers, family members and employees

Broilers (2018-2019)

Due to

- Commission Regulation (EU) No 200/2010 (reduction of the prevalence of *Salmonella* serotypes in adult breeding flocks of *Gallus gallus*) implementing Regulation (EC) No 2160/2003 (on the control of *Salmonella* and other specified food-borne zoonotic agents);

  The yearly maximum percentage of adult breeding flocks of *Gallus gallus* remaining positive for the relevant* *Salmonella* serotypes is to be 1% or less

- Wait for results of participating farms from the National Control Program *Salmonella* (AVINED)
- After the end of the complete sampling period *Salmonella* isolation was performed from the stored broiler faeces samples (1:1 glycerol, -20 °C)

* *S.* Enteritidis, *S.* Infantis, *S.* Hadar, *S.* Typhimurium, including monophasic S. Typhimurium with the antigenic formula 1,4,[5],12:i:- and S. Virchow

Methods *Salmonella* (1)

- Animals
  - 1 or 2 Broiler houses sampled
  - Three faecal samples (12 droppings each) collected (front, middle and back)
  - 10g Faeces/glycerol of each sample of was pooled to 30 g faeces/glycerol per broiler house
  - Incubated overnight together with 270 ml BPW at 37 °C

- Humans
  - 1 g Faeces was added to 9ml BPW and incubated overnight at 37 °C

Methods *Salmonella* (2)

- 100 µl Transferred to a MRSV agar plate supplemented with novobiocin and incubated at 41.5 °C for 24 and 28 h
- Suspected plates were streaked on BSA plates and incubated at 37 °C (18-22h)
- Biochemical confirmation
- Obtained isolates were analysed with a xMap *Salmonella* Serotyping kit (Biovet)

- *S*. Infantis isolates were analysed with short-read sequencing on a NovaSeq (Illumina) platforms.
- Core genome MLST (cgMLST) analysis was performed after *de novo* assembly.
- Isolates were screened for pESI plasmid linked genes as well as antibiotic resistance (AR) genes and *Salmonella* virulence factors.

Results *Salmonella*_broilers

	# Farms (houses)	Positive farms (houses)	Prevalence
RIVM	194 (371)	23 (35)	11.9% (9.4%)

Serotype	# Farms	Prevalence	# houses
S. Agona	1	4.3%	1
S. Infantis	10	43.5%	14
S. Paratyphi B variant Java	12	52.2%	20

- Farm-level prevalence of 5.1% and flock-level prevalence of 3.7% for *S.* Infantis

Results *Salmonella*_humans

- Response rate; 40.6%
  - 132 farmers, family members and employees
  - 81 farms

- Prevalence; 0.8%
  - 1 farmer positive with *S*. Infantis, no complaints
  - Flocks were *Salmonella* negative at time of sampling

Whole Genome Sequencing

- Broiler isolates (14)
- Human isolate; farmer (1)

- Routine *Salmonella* surveillance at RIVM (Centre of Diagnostics and Laboratory Surveillance (IDS))
  - >100 *S.* Infantis isolates (July 2018 – May 2019)
  - Focus on MDR isolates (phenotypic data)

- Human isolates (13), Pig (2), Broiler (1), Broiler meat product (1)

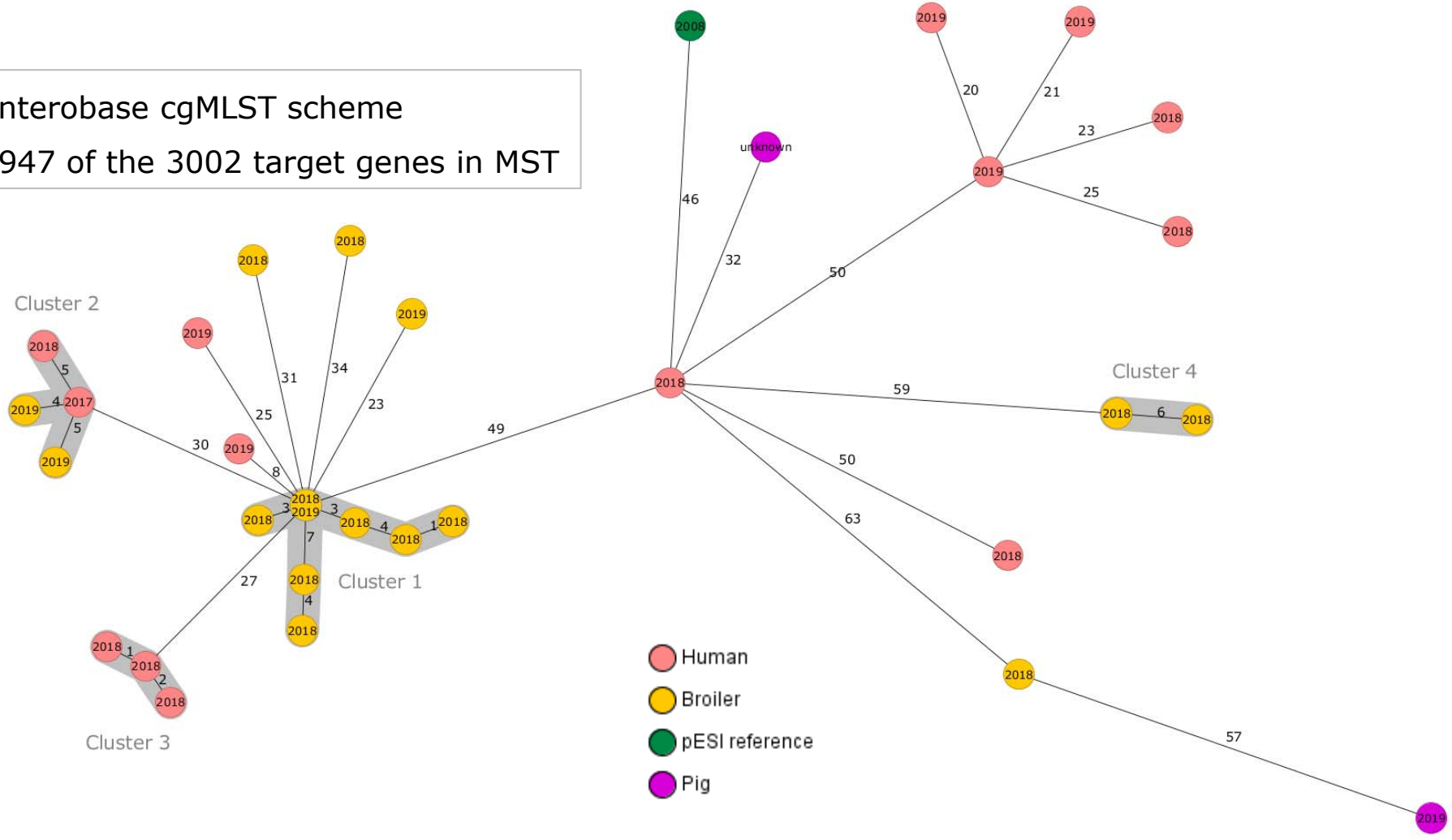
(cg)MLST analysis

pESI Reference: *Salmonella enterica* subsp. *enterica* serovar Infantis strain 119944 (CP047881)

- All 32 *S.* Infantis isolates belonged to ST32

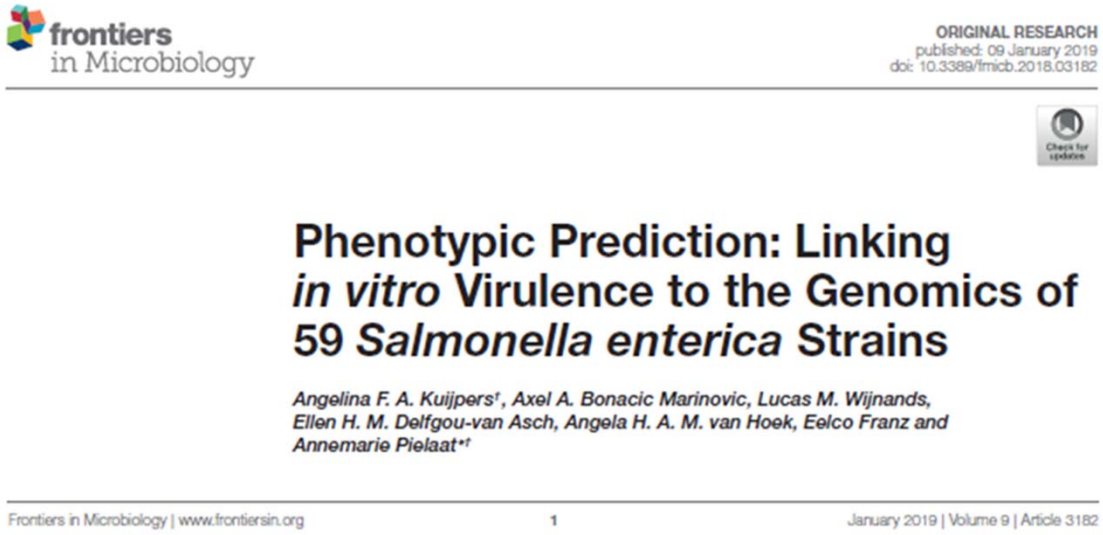
Enterobase cgMLST scheme

2947 of the 3002 target genes in MST



Virulence Factors (VFs)

- 233 *Salmonella* VFs from public databases and literature
- Listed in



frontiers in Microbiology

ORIGINAL RESEARCH
published: 09 January 2019
doi: 10.3389/fmicb.2018.03182

Phenotypic Prediction: Linking *in vitro* Virulence to the Genomics of 59 *Salmonella enterica* Strains

Angelina F. A. Kuijpers†, Axel A. Bonacic Marinovic, Lucas M. Wijnands, Ellen H. M. Delfgou-van Asch, Angela H. A. M. van Hoek, Eelco Franz and Annemarie Pielaat†*

Frontiers in Microbiology | www.frontiersin.org 1 January 2019 | Volume 9 | Article 3182

- Implemented in one target scheme (SeqSphere^{+})

VFs

- 156 VFs present in all *S*. Infantis isolates investigated
- Most of these VF gene sequences were identical (83.3%; 130/156)
- Ten genes contained a synonymous SNP which does not result in an amino acid substitution

VFs

Gene	Function	SNP	AA substitution	Source (#)
fimD	Outer membrane usher protein	C1855A	R619S	Pig (1)
fimZ	Fimbria biosynthesis transcriptional regulator	C136T	R46C	Pig (1)
fimF	Type I fimbriae adaptor protein	T62C	V21A	Broiler (1)
lpfC	Long polar fimbrial usher protein	2451 INS 1nt	PMSC	Human (3)
stfA	Fimbrial subunit	G192A	M64I	Broiler (2)
tcfC	Fimbrial outer membrane usher	G1201A	M67I	Broiler (2)
mgtB	Magnesium-translocating P-type ATPase	T1662A	D554E	Human (1)
ratB	Outer membrane protein	T3898C	Y1300H	Broiler (2)
sinI	Outer membrane protein	T589G	E197PMSC	Broiler (13), Human (6), Farmer (1)
hilC	Transcriptional regulator	T310C	Y104H	Human (1)
ssrA	Sensor kinase	C1894T	H632Y	Broiler (1)
sscB	Secretion system chaperone	T238A	F80I	Broiler (13), Human (6), Farmer (1)
ssaU	Type III secretion system protein	G353A	S118N	Pig (1)
slrP	E3 ubiquitin-protein ligase	C178A	A60I	Broiler (2)
sopA	Type III secretion system effector	T629A	I210K	Human (1)
sifB	Type III secretion system effector	G766A	V256I	Broiler (2)
sspH2	Type III secretion system effector	G1224T	M408I	Broiler (1)

PMSC = premature stop codon

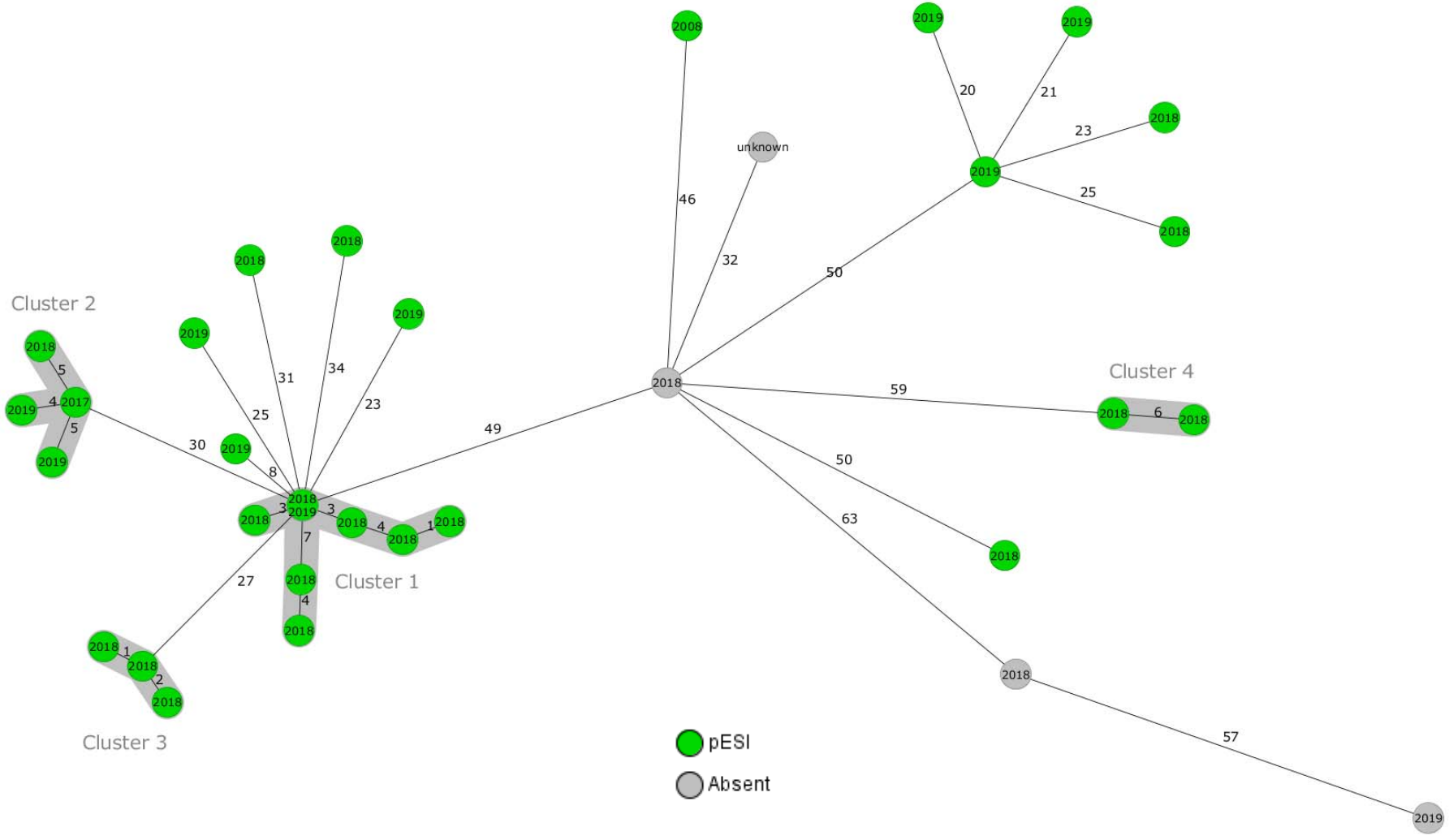
In silico analysis of AMR genes and point mutations in *gyrA* and *parC*

Source	#	*aadA*	*aph*(3')-I	*aph*(4)-I	*str*	*bla*	*sul*	*tet*	*dfr*	GyrA p83*	GyrA p87*
Farm P	2	*aadA1*					*sul1*	*tet*(A)	*dfrA14*	WT	**G**
Farm Q, R, S, T, U,V	8	*aadA1*	*aph*(3')-Ic				*sul1*	*tet*(A)	*dfrA14*	WT	**G**
Farm W	2	*aadA1*					*sul1*	*tet*(A)		**Y**	WT
Farm X	1	*aadA1*					*sul1*	*tet*(A)		WT	**G**
Farm Y	1									WT	WT
Farm Z, farmer	1	*aadA1*					*sul1*	*tet*(A)	*dfrA14*	WT	**G**
Broiler	1	*aadA1*	*aph*(3')-Ic				*sul1*	*tet*(A)	*dfrA14*	WT	**G**
Broiler meat	1	*aadA1*	*aph*(3')-Ic		*strAB*	TEM-1b	*sul1*, *sul2*	*tet*(A)	*dfrA8*	WT	**G**
Pig	1				*strAB*		*sul1*, *sul2*	*tet*(A)	*dfrA23*	WT	WT
	1									WT	WT
Human	3	*aadA1*	*aph*(3')-Ic			CTX-M-65	*sul1*	*tet*(A)	*dfrA14*	WT	**Y**
	3	*aadA1*					*sul1*	*tet*(A)	*dfrA14*	WT	**G**
	1	*aadA1*	*aph*(3')-Ic			TEM-1b	*sul1*	*tet*(A)	*dfrA14*	WT	**G**
	1	*aadA1*	*aph*(3')-Ic				*sul1*	*tet*(A)		WT	**G**
	1	*aadA1*	*aph*(3')-Ic	*aph*(4)-Ia		CTX-M-65	*sul1*	*tet*(A)	*dfrA14*	WT	**Y**
	1	*aadA1*				CTX-M-65	*sul1*	*tet*(A)	*dfrA14*	WT	**Y**
	1	*aadA1*					*sul1*	*tet*(A)		WT	**G**
	1		*aph*(3')-Ia		*strAB*		*sul2*	*tet*(A)	*dfrA32*	**Y**	WT
	1									WT	WT

* p = position; WT = wildtype; In all isolates ParC p57: **S**

pESI

- Prevalence 87.5% (28/32), absent in 1 broiler, 1 human and 2 pig isolates

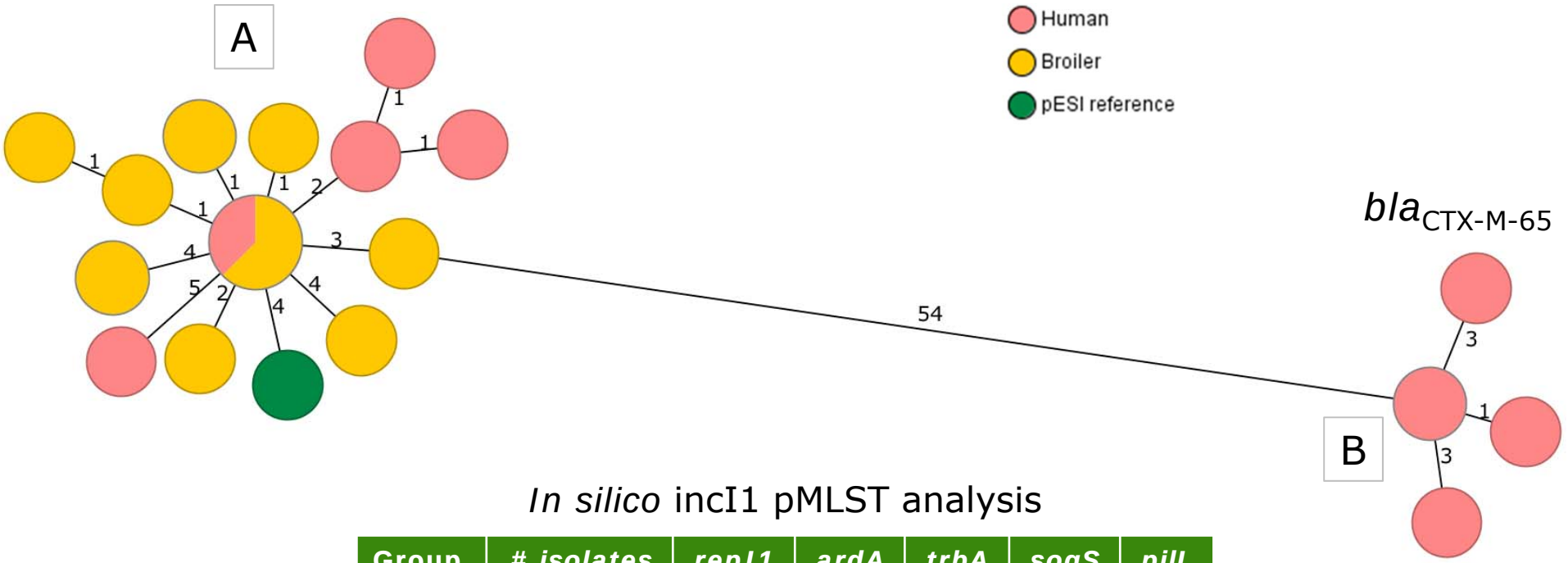


Whole plasmid MLST (wp(MLST))

- Reference; *Salmonella enterica* subsp. *enterica* serovar Infantis strain 119944 plasmid pESI, complete sequence (CP047882 (285,081 bp))

- A wpMLST scheme was constructed containing 282 gene targets (SeqSphere^{+})
  - IncI1-type genes (*traE*, *traF*…*traX*, *trbA*…*trbC*)
  - IncP replicase
  - Plasmid SOS inhibitors (*psiA*, *psiB*)
  - Fimbrial operon (*faeA*, *faeD*, *faeH*)
  - Type IV pilus operon (*pilL*..*pilR*, *pilX*)
  - Class 1 integron (*intI1*), class 2 integrons (*intI2*)
  - AMR genes (*aadA1*, *dfrA14*, *sul1*, *tet*(A))
  - Mercury resistance operon (*merCBDEPTR*)
  - Yersiniabactin operon (*fyuA*, *ybtETU*, *irp1*, *irp2*, *ybtAAPQXS*)

pESI wpMLST



In silico incI1 pMLST analysis

Group	# isolates	repI1	ardA	trbA	sogS	pilL
A	22	NA	2	21	9	3
	1	NA	2	46	9	3
B	5	NA	11	8	14	3

Conclusions (1)

- Surveillance farm animals (broilers)
  - 198 farms visited; 379 flocks tested
  - Participation in the human study; 40.6% (81 farms, 132 persons)

  - *Salmonella* prevalence on the farms; 11.9%, the flocks; 9.4%
  - *S*. Infantis prevalence farm-level; 5.1%, flock-level; 3.7%

  - *Salmonella* prevalence in the human study; 0.8%
  - Human isolate; *S*. Infantis

Conclusions (2)

- WGS analysis
  - All belonged to ST32
  - cgMLST analysis revealed considerable variation among the isolates included in this study. Even isolates from different flocks of the same farm were never 100% identical, although they did cluster together
  - 156 VFs present in all *S*. Infantis isolates investigated
  - Most of these VF gene sequences were identical (83.3%)
  - Sixteen genes contained a non-synonymous SNP which resulted in an amino acid substitution or PMSC

Conclusions (3)

- WGS analysis
  - *In silico* screening of the assembled genomes showed a high prevalence of 5 antibiotic resistance genes (*aadA1*, *aph*(3')-Ic, *drfA14*, *sul1*, *tet*(A))
  - Five human Infantis isolates were ESBL producers ($bla_{\text{CTX-M-65}}$)
  - (Fluoro)quinolone resistance was prevalent among the Infantis isolates due to mutations in *gyrA*, which resulted in amino acid substitutions
  - pESI prevalence was 87.5% (28/32)
  - Based on (w)pMLST two subtypes of pESI were shown

Acknowledgements

NVWA

Ben Wit

Coen van der Weijden

Olaf Stenvers

RIVM

Tryntsje Cuperus

Cecile Dam-Deisz

Wendy van Overbeek

Lapo Mughini Gras

Cindy Dierikx

Joke van der Giessen

Kim van der Zwaluw

Maaike van den Beld

Eelco Franz

Marieke Opsteegh

THANK YOU FOR YOUR ATTENTION