“Situation of STEC infections in Argentina”

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EU Reference Laboratory for *E.coli*
Department of Food Safety, Nutrition and Veterinary Public Health
Unit of Food Microbiology and Foodborne Diseases
Istituto Superiore di Sanità

HUS in Argentina

- High Incidence Rate
  - 8-10/100000 children < 5 years old
  - ~ 400 cases /year

- In children…
  - First cause of acute renal failure
  - Second cause of chronic renal failure
  - Responsible of 9 % renal tranplant

- STEC associated to 70% of HUS cases
  - O157:H7 / O145:NM predominant
  - *Shigella dysenteriae* type 1 NOT isolated

National Reference Laboratory for HUS & Diarrheas associated to STEC and Diarrheagenic *E. coli*

Pathogens

- **STEC O157 y non-O157**
- **Diarrheagenic *E. coli* (ETEC, EPEC, EIEC, EAEC, DAEC)**

➢ Standardized protocols for the diagnosis and subtyping of strains of human, animal and food origin applied to:

- ➢ Epidemiological surveillance
- ➢ Outbreak investigation

- **Mandatory notification - Ministry of Health Resolution No. 346/00. National Health Surveillance System (SNVS)**
- **National Laboratory Network for the surveillance of Diarrheas and pathogens associated to foodborne diseases**
- **HUS Centinel Units (25 laboratories)**

National Reference Laboratory for Hemolytic Uremic Syndrome and Diarrheas associated with STEC

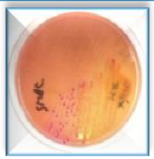
1. Isolation and characterization of the microorganism
   - Typing: virulence factors, biotype, serotype, antimicrobial resistance
   - Subtyping: PFGE and genotyping of Stx1 / Stx2 variants

2. Detection of free Shiga toxin in stool samples

   Cytotoxicity on Vero cells

3. Detection of anti-LPS antibodies
   - Recombinant glycoproteins based iELISA for detection of a-O157, a-O145, a-O121, a-O103

1- ENRICHMENT & CULTURE



SMAC Agar

2- SCREENING

PCR - Confluent zone and pools

screening mPCR-1
eae / *lt* / *sth* / *stp*

mPCR-1 +

Screening mPCR-2
IpaH / *aggR*

mPCR-2 +

Screening mPCR-STEC
stx_1 / stx_2 / rfb_{O157}

mPCR-STEC +

3- CONFIRMATION

PCR - individual colonies

eae (+) EPEC isolate

lt / st_h / st_p (+) ETEC isolate

IpaH (+) EIEC isolate

aggR (+) EAEC isolate

stx_1 / stx_2 / rfb_{O157} (+) STEC isolate

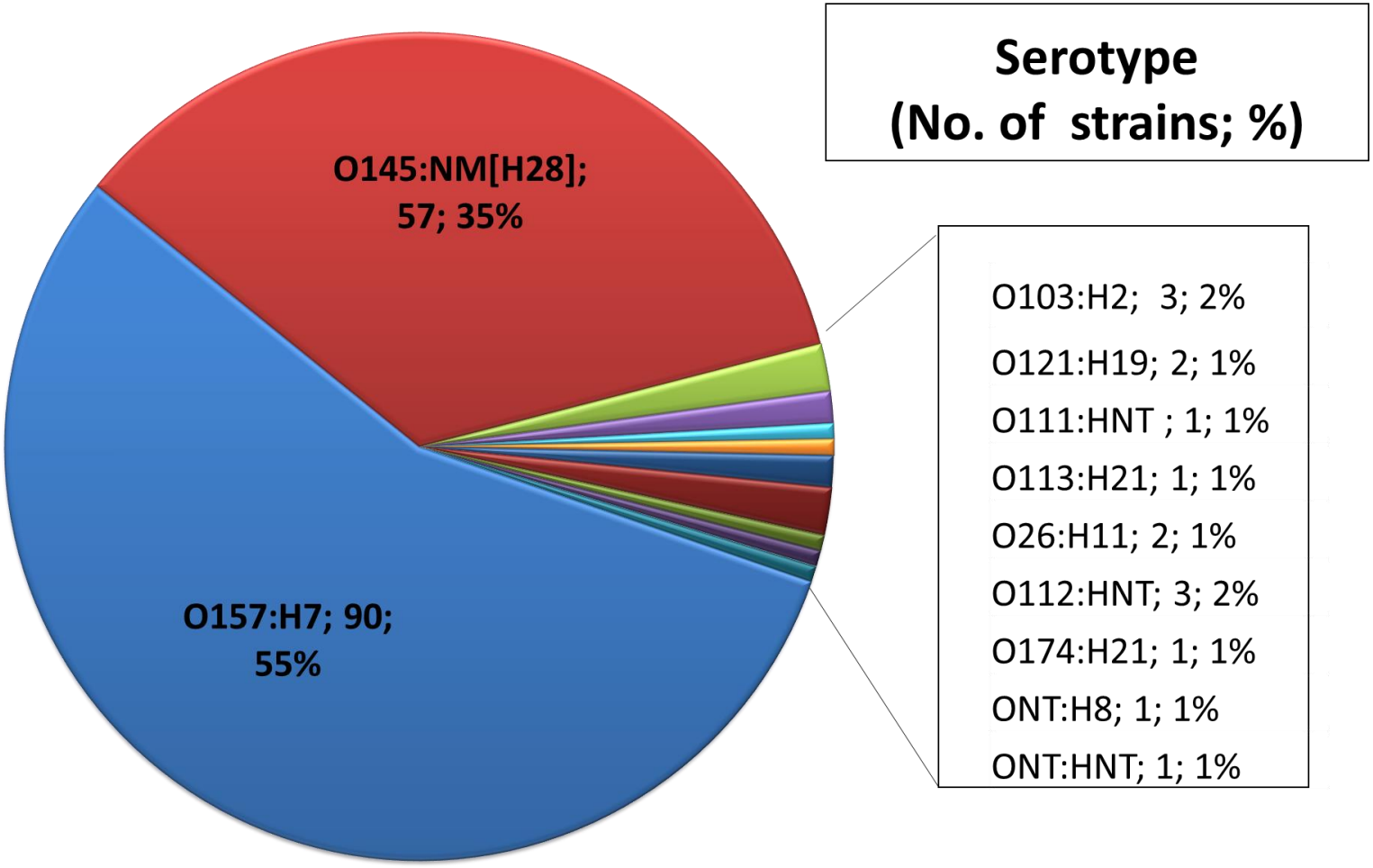
If stx_2 (+) *eae* (-) EAEC-*stx* isolate

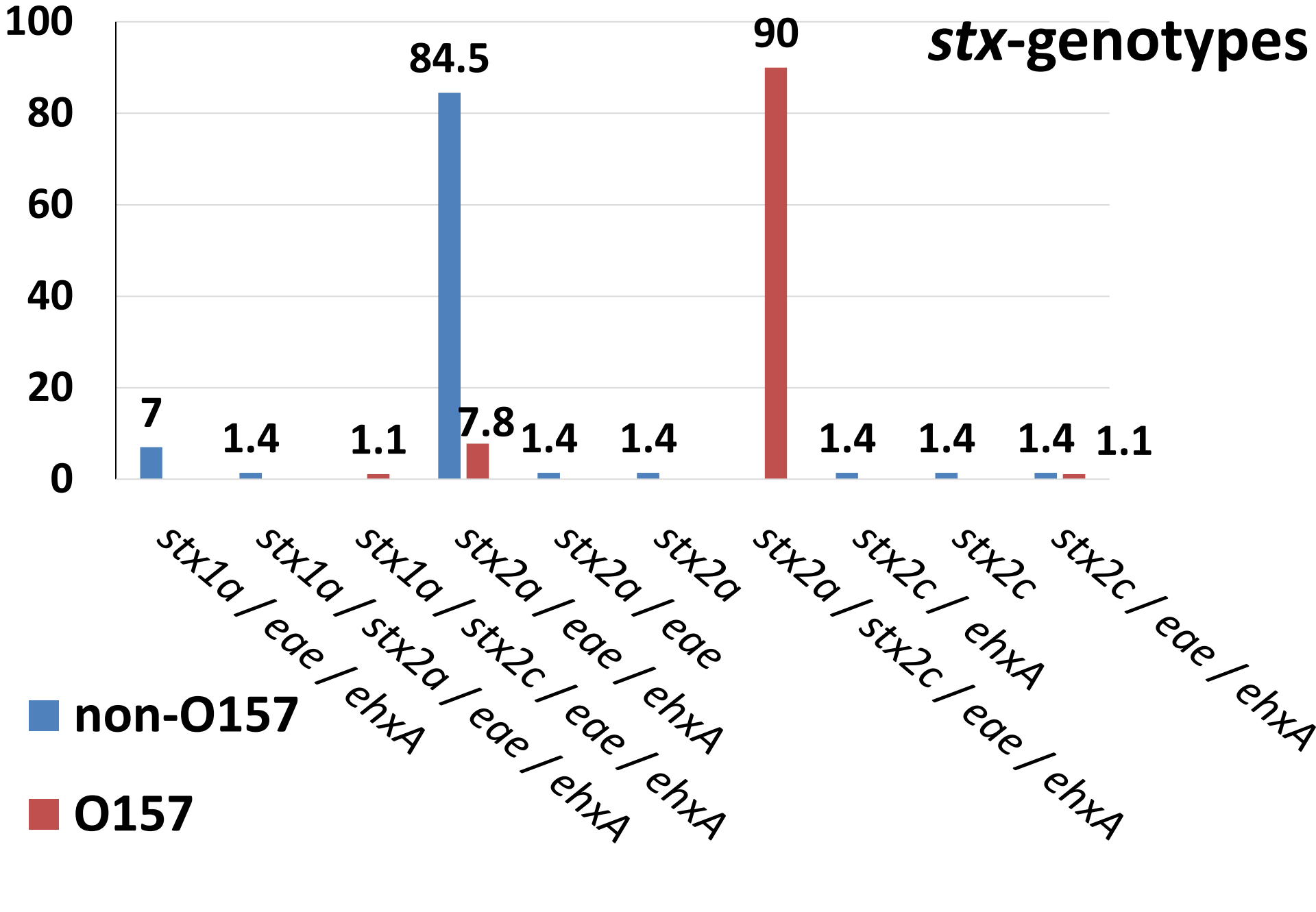
If stx_2 (+) *eae (-) aggR* (+) EAEC-*stx* isolate

Biochemical identification and Serotyping

4- Report to SNVS 2.0-SISA and deliver the isolates to NRL

Serotypes of STEC strains




stx-genotypes
100
80
60
40
20
0
7
1.4
1.1
84.5
7.8
1.4
1.4
90
1.4
1.4
1.4
1.1
stx1a / eae / ehxA
stx1a / stx2a / eae / ehxA
stx1a / stx2c / eae / ehxA
stx2a / eae / ehxA
stx2a / eae
stx2a / stx2c / eae / ehxA
stx2c / ehxA
stx2c
stx2c / eae / ehxA
non-O157
O157

Clinical Diagnostic	No. Cases Received NRL	STEC O157 / STEC no-O157	Glyco-iElisa O157 / O145 / O121 ONLY = 221	Stx-MF ONLY=143	Total
HUS	339	**79 (24%)** 54 O157 25 no-O157	**138 (40%)** O157 (85/44%) O145 (49/19%) O121 (4/1%)	**8 (2%)**	**225 (66%)**
BD	133	**19 (14%)** 13 O157 6 no-O157	**6 (4,5%)** O157 (4/2.7%) O145 (2/1%)		**25 (19%)**
D	135	**36 (27%)** 21 O157 15 no-O157			**36 (27%)**
Total	**607**	**134 (22%)**	**144 (23,7%)**	**8 (2%)**	**286 (47%)**

STEC infections

66%

SUH / DS / D cases - Total STEC diagnosed = 286/607 (47,1%)

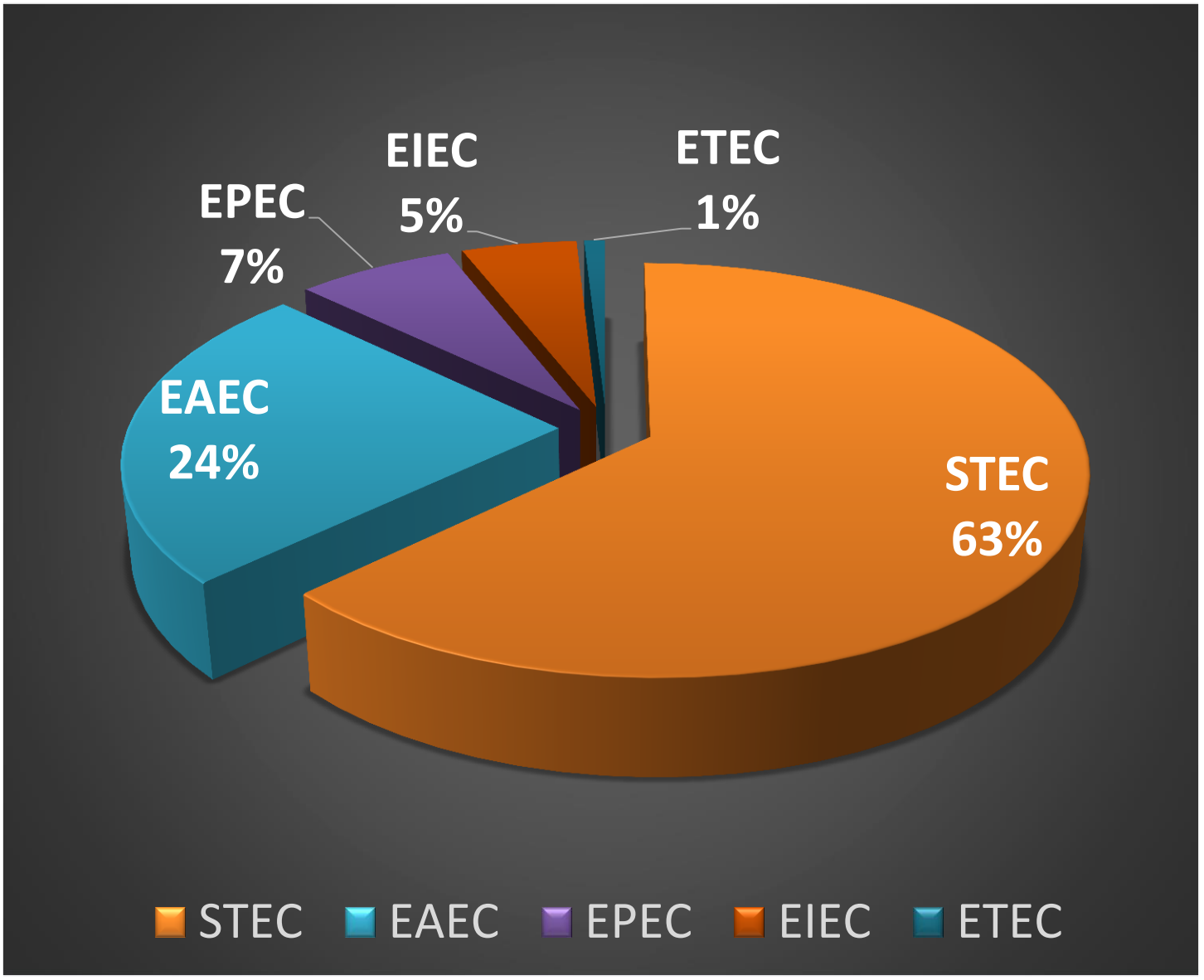
STEC bacteriological confirmed = 134 (22%)

a-LPS STEC O157/O145/O121= 144 (23,7%)

OUTBREAKS

Outbreak	STEC-positive cases (No.)	Characteristics of the STEC strains		
		Serotype	Genotypic profile	*Xba*I-PFGE
1	BD / Healthy contact	O157:H7	*stx*$_{2a}$ / *stx*$_{2c}$ / *eae* / *ehxA*	AREXHX01.0011
2	HUS / HUS	O157:H7	*stx*$_{2a}$ / *stx*$_{2c}$ / *eae* / *ehx*A	AREXHX01.0650
3	BD / BD	O157:H7	*stx*$_{2a}$ / *stx*$_{2c}$ / *eae* / *ehx*A	AREXHX01.0650
4	BD / Healthy contact	O157:H7	*stx*$_{2a}$ / *stx*$_{2c}$ / *eae* / *ehx*A	AREXHX01.1058
5	BD / NBD / Healthy contact	O157:H7	*stx*$_{2a}$ / *stx*$_{2c}$ / *eae* / *ehx*A	AREXHX01.1309
6	HUS / Healthy contact	O157:H7	*stx*$_{2a}$ / *stx*$_{2c}$ / *eae* / *ehx*A	AREXHX01.1356
7	HUS / Healthy contact (2)	O145:NM[H28]	*stx*$_{2a}$ / *eae* / *ehxA*	ARENMX01.0316
8	BD / Healthy contact (5)	O145:NM[H28] O157:HNT	*stx*$_{2a}$ / *eae* / *ehxA* *stx* (-)	ARENMX01.0319 AREXHX01.1415
9	HUS / Healthy contact	O145:NM[H28]	*stx*$_{2a}$ / *eae* / *ehxA*	ARENMX01.0335
10	HUS / Healthy contact	O145:NM[H28]	*stx*$_{2a}$ / *eae* / *ehxA*	ARENMX01.0340
11	HUS / Healthy contact (2)	O112:HNT	*stx*$_{2c}$ / *ehxA*	ARE12X01.0002

Diarrheagenic *E. coli*



DEC vs Clinical diagnosis

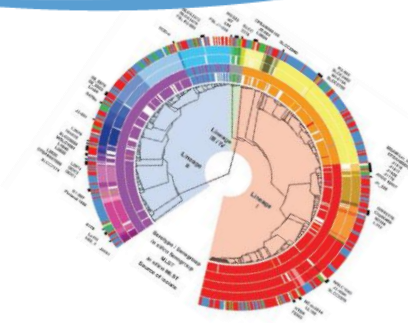
	HUS (n=339)	BD (n=133)	D (135)	Total
STEC	79	19	36	134 (63%)
EAEC	15	8	30	53 (24%)
EPEC	6	3	6	15 (7%)
EIEC	2	7	2	11 (5%)
ETEC	0	2	0	2 (1%)
Total	102	39	74	215

O-antibodies HUS-DEC+

	HUS (n=339)	Glyco iELISA	
STEC	79	42/47	30 a-O157 12 a-O145
EAEC	15	6/11	4 a-O157 2 a-O145
EPEC	6	4/5	3 a-O157 1 a-O145
EIEC	2	1/2	1 a-O145
ETEC	0	0	
	102	53/65	

Cases	Coinfection / strains
NBD	• STEC O157:H7 • EAEC
Healthy contact	• STEC O157:H7 • STEC O112:HNT
HUS	• STEC O157:H7 • EAEC
HUS	• STEC O145:NM[H28] • EAEC-*stx*+ O59:H19
BD	• O145:NM[H28] • O157:HNT
HUS	• O145:NM[H28] • O157:H16
BD	• O145:NM[H28] • O157:HNM

PROJECTS



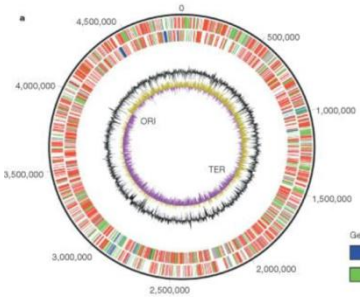
- **Global surveillance and monitoring of the foodborne pathogens/WGS implementation in Argentina**

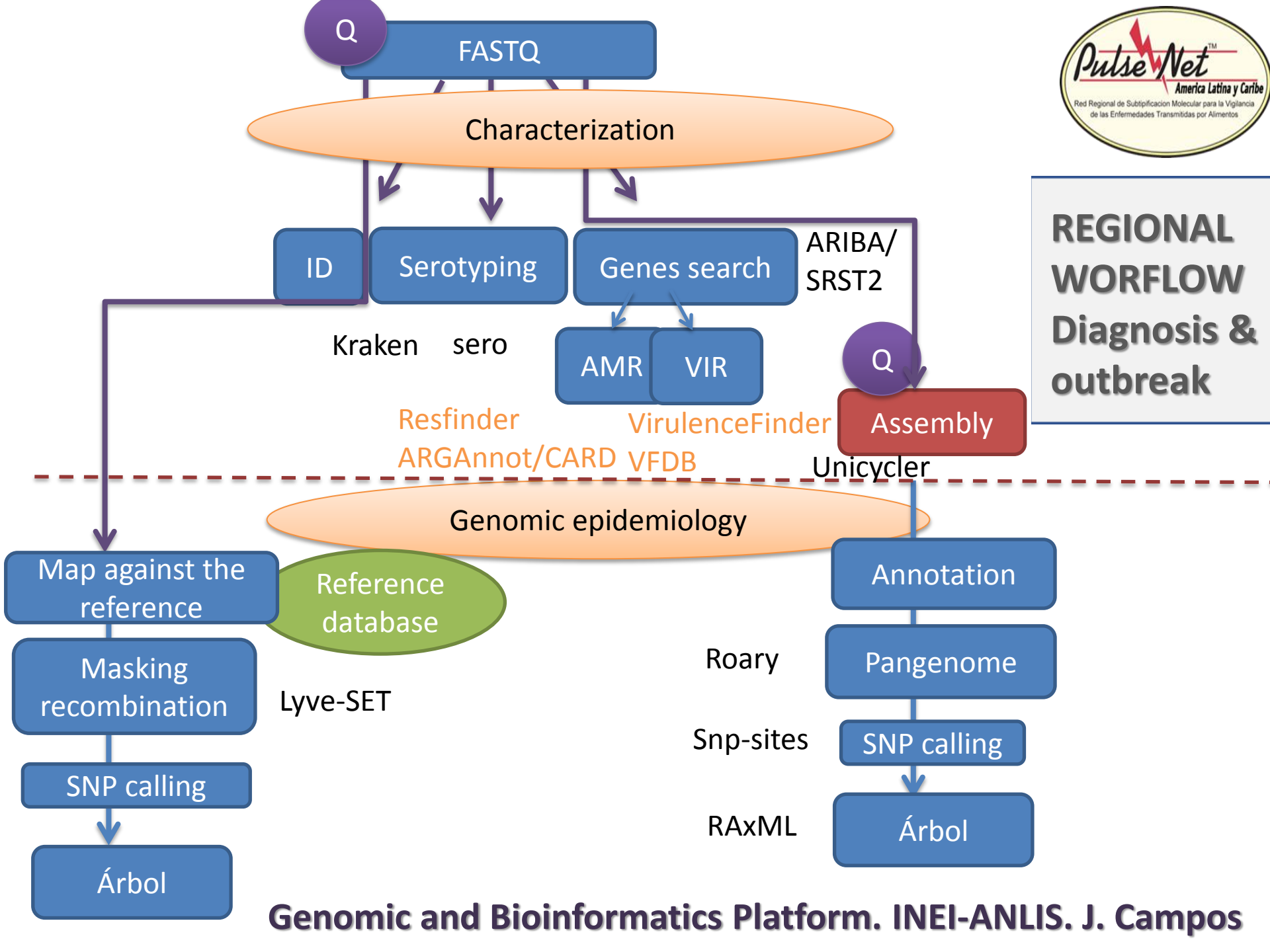
  FDA – Genome TRAKR Project

  WHO-FDA Pilot Project

- **wg/cgMLST Strategy for the foodborne surveillance- PulseNet Latinamerica and The Caribbeean**

  CDC-PAHO



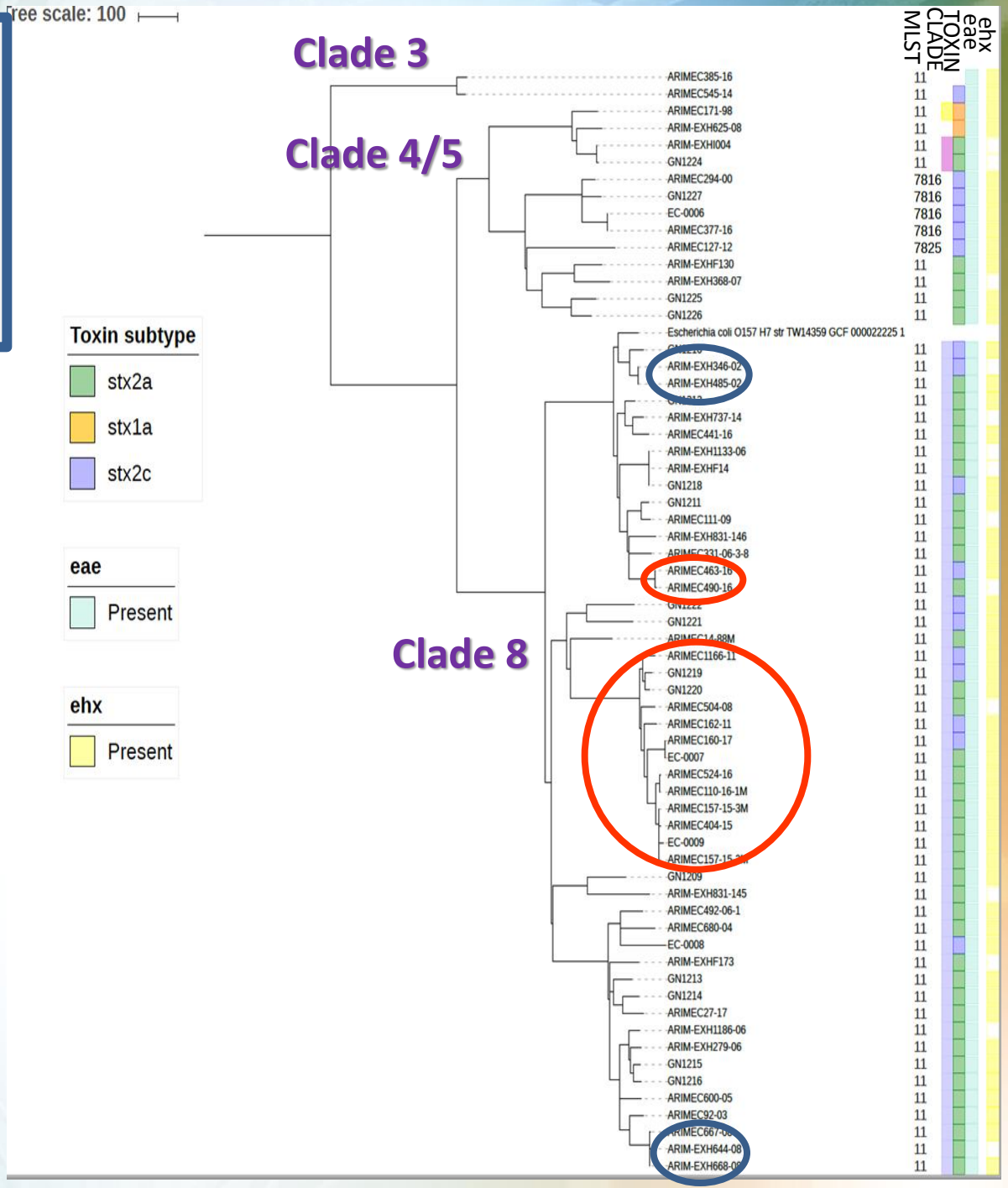

Q
FASTQ
Characterization
ID
Serotyping
Genes search
ARIBA/
SRST2
Kraken
sero
AMR
VIR
Resfinder
ARGAnnot/CARD
VirulenceFinder
VFDB
Q
Assembly
Unicycler
REGIONAL
WORFLOW
Diagnosis &
outbreak
Genomic epidemiology
Map against the
reference
Reference
database
Masking
recombination
Lyve-SET
SNP calling
Árbol
Annotation
Roary
Pangenome
Snp-sites
SNP calling
RAxML
Árbol
Genomic and Bioinformatics Platform. INEI-ANLIS. J. Campos

WGS for STEC surveillance – Argentina

Genome TraKr – Pilot Project WHO/FDA

SNPs Tree O157:H7

- Outbreaks
- Case/Food



Instituto Nacional de Enfermedades Infecciosas INEI
A.N.L.I.S. "Dr. Carlos G. Malbrán" - Argentina

ANLIS
Administración Nacional de Laboratorios e Institutos de Salud

"Enteroaggregative *Escherichia coli*: its importance in human diarrheic disease in Argentina, characterization and virulence factors study"

1- Retrospective analysis of EAEC strains (Period 2002 - 2017)

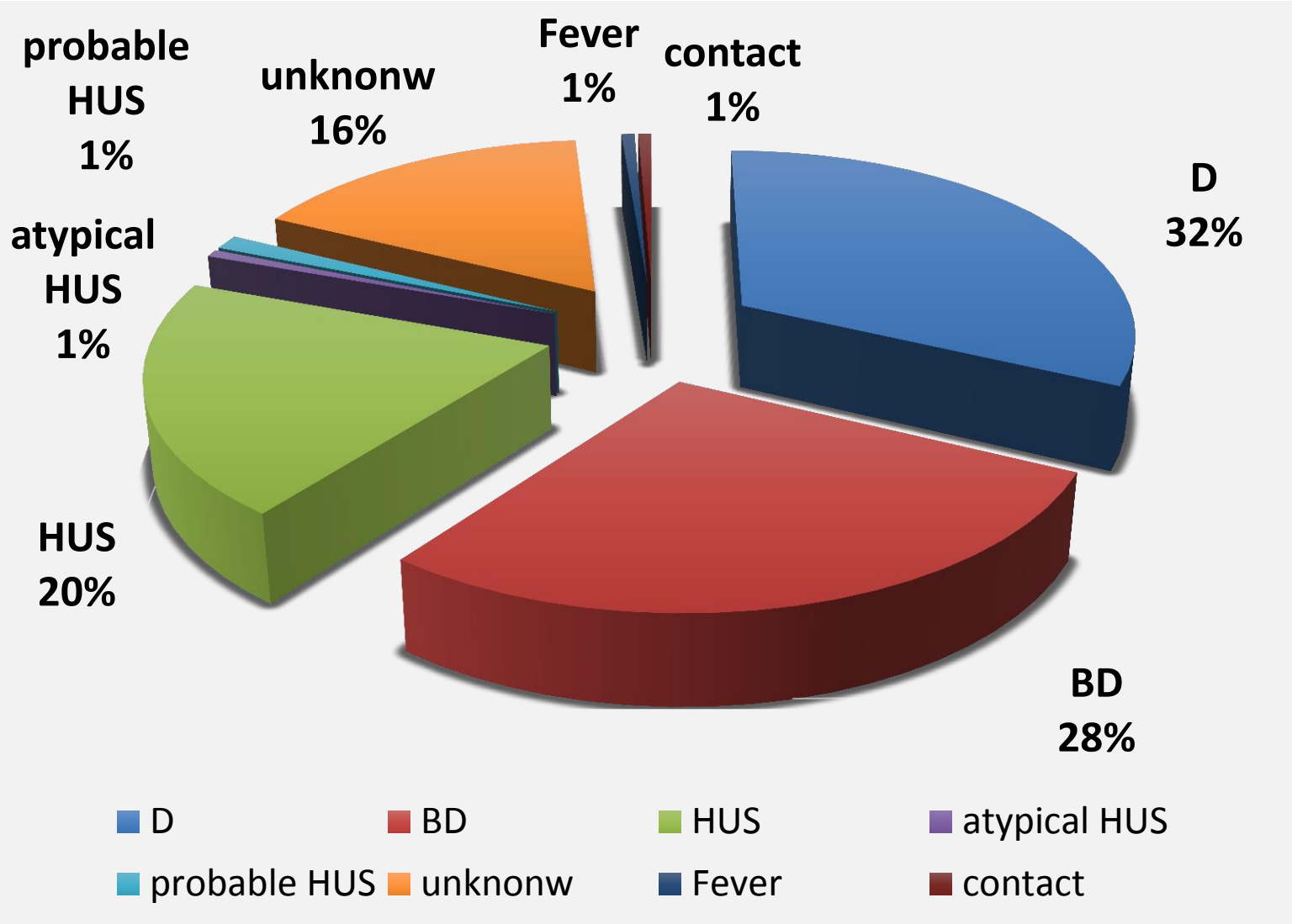
2- Prospective study of EAEC (Period 2018)

- General aim: characterize and subtype the strains EAEC and EAEC-stx isolated in Argentina, and to determine the frequency of disease associated with this pathogen from the implementation of a diagnostic protocol.

- Specific objectives:
  - To create the EAEC Database (PFGE patterns, characteristics of the strains and epidemiological data of the patient).
  - To implement the Whole Genome Sequencing (WGS) for the surveillance and identification of new tools associated with epidemic strains.
  - To determine characteristics and phylogeny of EAEC - Stx strains by SGC.
  - To determine ability of causing serious disease.
  - To develop and implement a protocol using a specific real-time PCR and based on the results obtained in the retrospective study, to improve the detection and diagnosis of EAEC strains in the surveillance framework.

Carolina Carbonari . Servicio Fisiopatogenia. INEI-ANLIS.

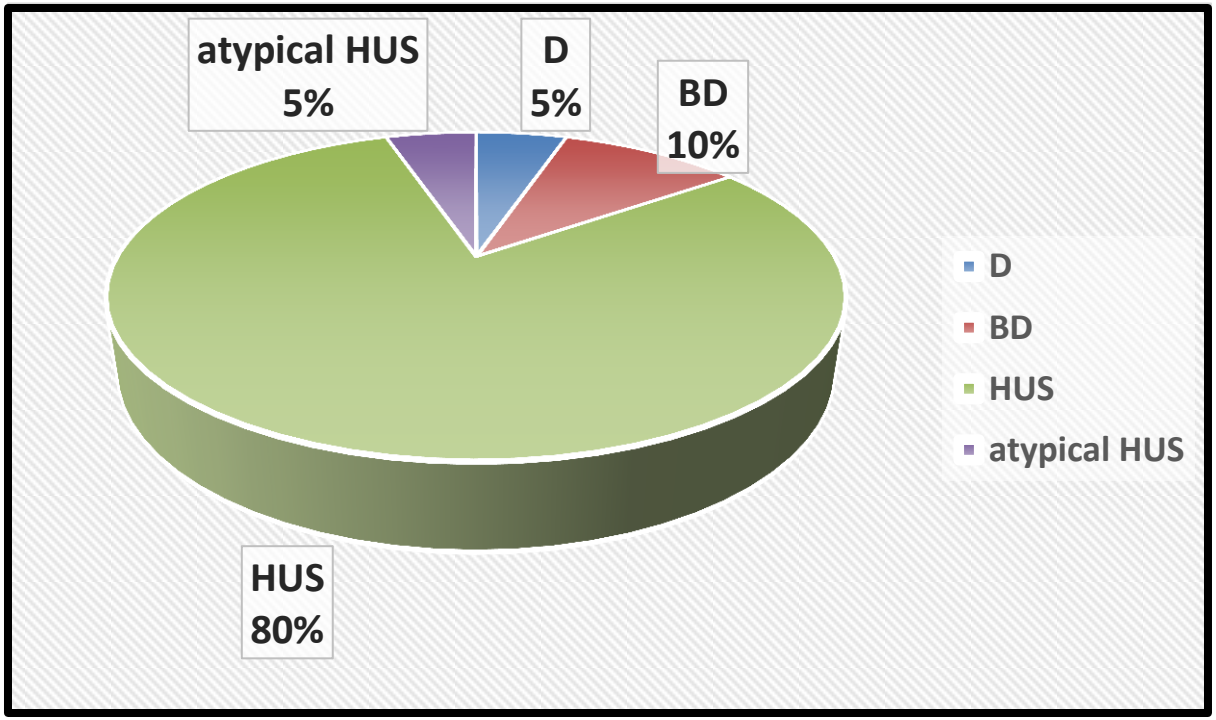
Cases associated with EAEC



170 strains/159 cases associated with EAEC

EAEC-stx

- 13.5% of total ⟶ EAEC-stx
- 23 strains /21 cases



EAEC-stx

23 strains /21 cases

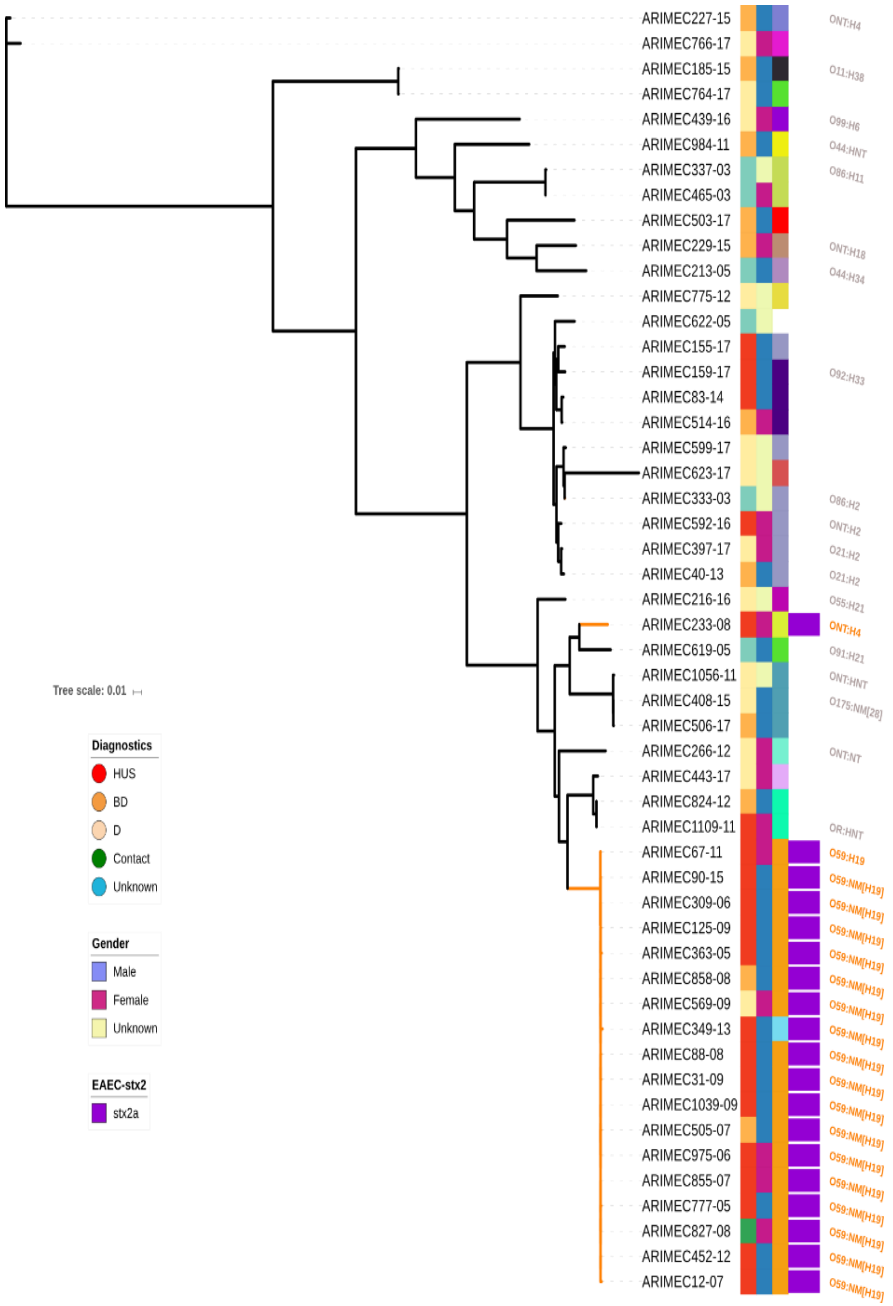
22 O59:H19 *stx*2a *agg*R S-TMS

1 ONT:H4 *stx*2a *agg*R/*aai*C

HUS and bloody diarrhea cases						Characteristics of EAEC/STEC strains analyzed in the study																
ID No.	Age	Sex	Location	Year	Diagnosis	Serotype	AAF type ✹	Genes frequently associated with STEC ♦						Genes frequently associated with EAEC ♦							Antibiotic resistance ■	Vero cells DC50/90 µl §
								stx genotype	*iha*	*lpf* $_{O26}$	*lpf* $_{O113}$	*terA*	*saa*	*aggR*	*aatA*	*aap*	*pet*	*astA*	*aaiC*	*pic*		
363/05	16 m	M	Buenos Aires	2005	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	< 100
777/05	12 m	M	Buenos Aires	2005	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	400
309/06	15 y	M	Córdoba	2005	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TETRA-TMS	400
975/06 ϑ	92 m	F	Entre Ríos	2006	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	800
12/07	47 m	M	Buenos Aires	2006	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	800
855/07	13 y †	F	Buenos Aires City	2007	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	200
505/07	18 y	M	Buenos Aires	2007	BD	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	400
452/12	76 m	M	Santa Fe	2012	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	400
90/15 #	8 y	M	Chubut	2015	HUS	O59:NM[*fliC* $_{H19}$]	IV	*stx* $_{2a}$	+	+	+	-	-	+	+	+	-	-	-	-	S-TMS	600

EAEC + EAEC-stx

Diagnostic
Gender
MLST
Stx
Serotype



Partnership / International Project

- Wellcome Trust Sanger Institute (WTSI)
- Food and Drug Administration (FDA)
- Center for Diseases Control and Prevention (CDC)
- PulseNet International
- Istituto di Sanitá
- National Public Health Canada (NPHC)
- Pasteur Institute
- Public Health Ontario (PHO)
- Panamerican Health Organization (PAHO)
- World Health Organization (WHO)

WGS for Public Health in LA

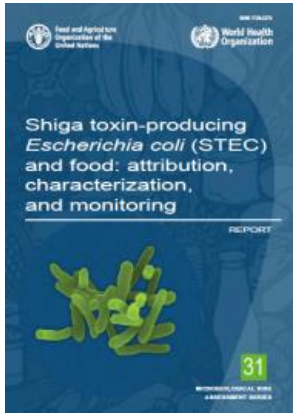
CONCLUSIONES

- **In Argentina, it is installed the capacity for the surveillance of STEC O157 / no-O157, DEC and new pathotypes in human samples**

- **Implementation of new technology (RT-PCR, iELISA, lateral flow, WGS and MALDI-TOF)**

- **Reinforcing the capacity for the integrated surveillance (Human-Food-Animal). Ministry of health, Food Agency (INAL) and Animal Agency (SENASA)**

  - **IHR (WHO/PAHO) autoevaluation**
  - **Manual of Standards for HUS - Update**



Servicio Fisiopatogenia