4th Annual Workshop of the National Reference Laboratories for *E. coli* in the EU

Epidemiology and Molecular Surveillance of VTEC Infections in South America

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MINISTERIO de SALUD
PRESIDENCIA de la NACION

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INEI
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HUS IN SOUTH AMERICA
INCIDENCE/100,000 < 5 YEARS OLD

STEC IN BRAZIL

- A nationwide surveillance system for HUS has been recently established but there is still a paucity of data
- Non-O157 strains have been circulating as agents of infantile diarrhea since the late 1970s
- O157 and non-O157 STEC strains have been recovered from:
  - Bloody diarrhea
  - Hemolytic anemia
  - HUS
  - Food
  - Animals
- Prevalence in acute diarrhea ranged from 0 (Western Amazon) to 6% (Southern Region)

STEC IN CHILE
LABORATORY BASED SURVEILLANCE SYSTEM. 2007-2008

- National Surveillance System for STEC was established in 1999

- 96 strains

O157[H7,H-]	66
O26[H11,21,H-]	24
O111[H-]	1
ONT	6

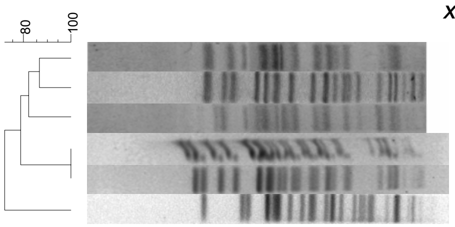
- *stx* genotypes
  - *stx*2 (72.9%)
  - *stx*1 (27.1%)

- 54 *Xba*I-PFGE patterns → 3 prevalent patterns (96% similarity)

STEC IN PARAGUAY

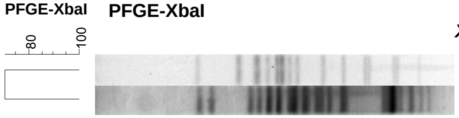
- HUS is mandatory since 2005
- 2006-2008: 15 HUS cases reported by the Laboratory-based Surveillance System
- Estimated annual rate: 0.6 cases/100,000 children < 5y
- 3 Sentinel Sites have been established
- National Databases have been created for molecular surveillance purposes with strains isolated from HUS and diarrhea cases

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]
PFGE-XbaI PFGE-XbaI



*Xba*I PFGE pattern	Serotype	Genotype	Year	Diagnostic	Origin
PYEXHX01.0001	E. coli O157:H7	eae / ehxA / stx2 / stx2c (vh-a)	2002	BD	Human
PYEXHX01.0003	E. coli O157:H7	eae / ehxA / stx2c (vh-a)	2004	HUS	Human
PYEXHX01.0002	E. coli O157:H7	eae / ehxA / stx2 / stx2c (vh-a)	2002	D	Human
PYEXHX01.0005	E. coli O157:H7	eae / ehxA / stx2 / stx2c(vh-a)	2009	D	Human
PYEXHX01.0005	E. coli O157:H7	eae / ehxA / stx2 / stx2c(vh-a)	2009	D	Human
PYEXHX01.0004	E. coli O157:H16	eae	2004	HUS	Human

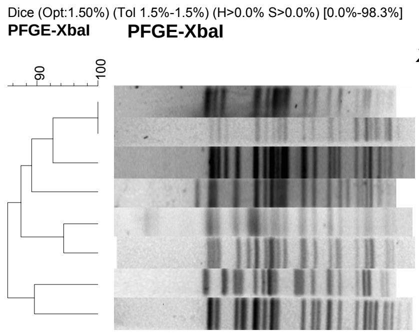
Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]
PFGE-XbaI PFGE-XbaI



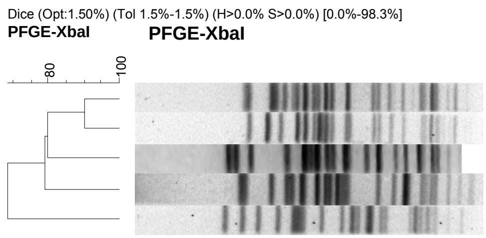
*Xba*I PFGE pattern	Serotype	Genotype	Year	Diagnostic	Origin
PYEXSX01.0002	E. coli ONT:H7	ehxA / stx1 / stx2	2006	BD	Human
PYEXSX01.0001	E. coli ONT:HNT	eae / ehxA / stx1	2005	D	Human

STEC IN URUGUAY

- Reporting of HUS is not mandatory
- O157 and non-O157 STEC strains are detected in humans, food and animals
- 2003-2005: 42% (173/412) of grazing cattle and sheep, shedding non-O157 strains, *stx*2 was prevalent
- Some serogroups detected (O26 and O111) were previously associated with HUS and diarrhea cases



***Xba*I PFGE pattern**	**Serotype**	Genotype	PT	Year	Diagnostic	Origin
UYEXHX01.0002	E. coli O157:H7	eae / ehxA / stx1	UT	2002	ground beef	Food
UYEXHX01.0002	E. coli O157:H7	eae / ehxA / stx1 / stx2c (vh-a)	32	2005	ground beef	Food
UYEXHX01.0004	E. coli O157:H7	eae / ehxA / stx1 / stx2c (vh-a)	39	2004	unknown	Human
UYEXHX01.0003	E. coli O157:H7	eae / ehxA / stx1 / stx2c (vh-a)	45	2002	ground beef	Food
UYEXHX01.0005	E. coli O157:H7	eae / ehxA / stx2c (vh-a)	39	2005	Food	Food
UYEXHX01.0007	E. coli O157:H7	eae / ehxA / stx1 / stx2c (vh-a)	14	2004	Food	Food
UYEXHX01.0001	E. coli O157:H7	eae / ehxA / stx2 / stx2c (vh-a)	2	2002	HUS	Human
UYEXHX01.0006	E. coli O157:H7	eae / ehxA / stx2c (vh-a)	32	2006	Food	Food



***Xba*I PFGE pattern**	**Serotype**	Genotype	Year	Diagnostic	Origin
UYEXSX01.0002	E. coli O26:HNT	eae / ehxA / stx1	2002	HUS	Human
UYEXSX01.0004	E. coli O26:H11	eae / ehxA / stx1	2002	HUS	Human
UYEXSX01.0001	E. coli O111:NM	eae / ehxA / stx1 / stx2	2002	BD	Human
UYEXSX01.0005	E. coli O111:HNT	eae / stx1 / stx2	2006	unknown	Animal
UYEXSX01.0003	E. coli O145:HNT	eae / ehxA / stx2	2002	HUS	Human

ARGENTINA - BACKGROUND

- Highest rate of HUS in the world
  - 17.0 cases/100,000 children <5 years old
- In children
  - Leading cause of acute renal failure
  - 2nd leading cause of chronic renal failure
  - Cause of 20% of kidney transplants
- Evidence of STEC infection in 60% of HUS
  - O157:H7 predominant serotype
  - *Shigella dysenteriae* type 1 NOT isolated

ARGENTINA
EPIDEMIOLOGICAL SURVEILLANCE STRATEGIES

- Reporting of HUS cases to the National Health Surveillance System (SNVS) is mandatory (Resolution No. 346/00), and must be immediate and individualized
- Sentinel Surveillance System: 25 HUS Sentinel Units
- Laboratory-based Surveillance (SIVILA): National Diarrheal and Foodborne Pathogens Network
- Molecular Surveillance: PulseNet Latin America and Caribbean

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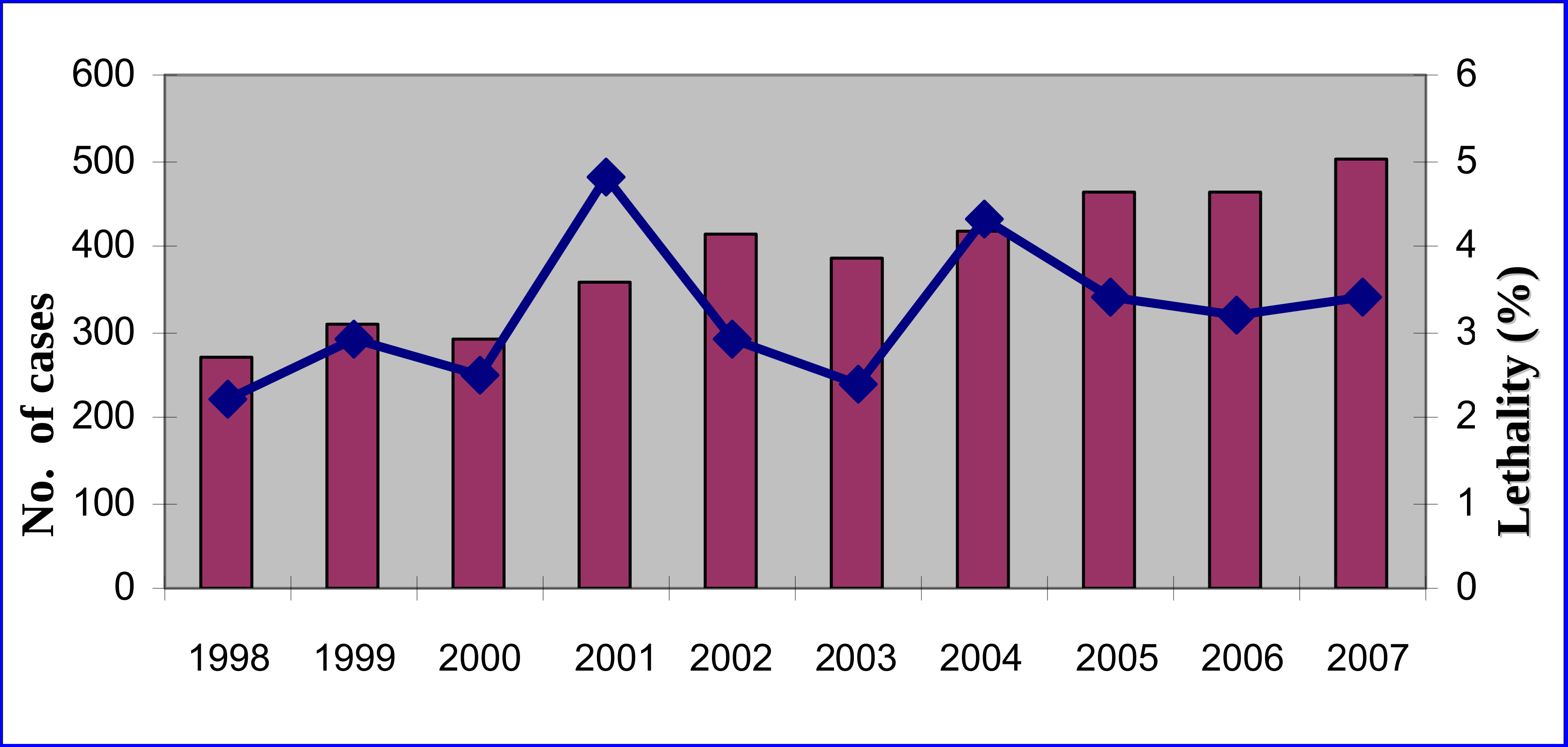
HUS – ARGENTINA. 1998-2007



Year	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Incidence	8.2	7.8	8.4	10.4	12.2	11.5	12.5	13.9	13.9	15.0

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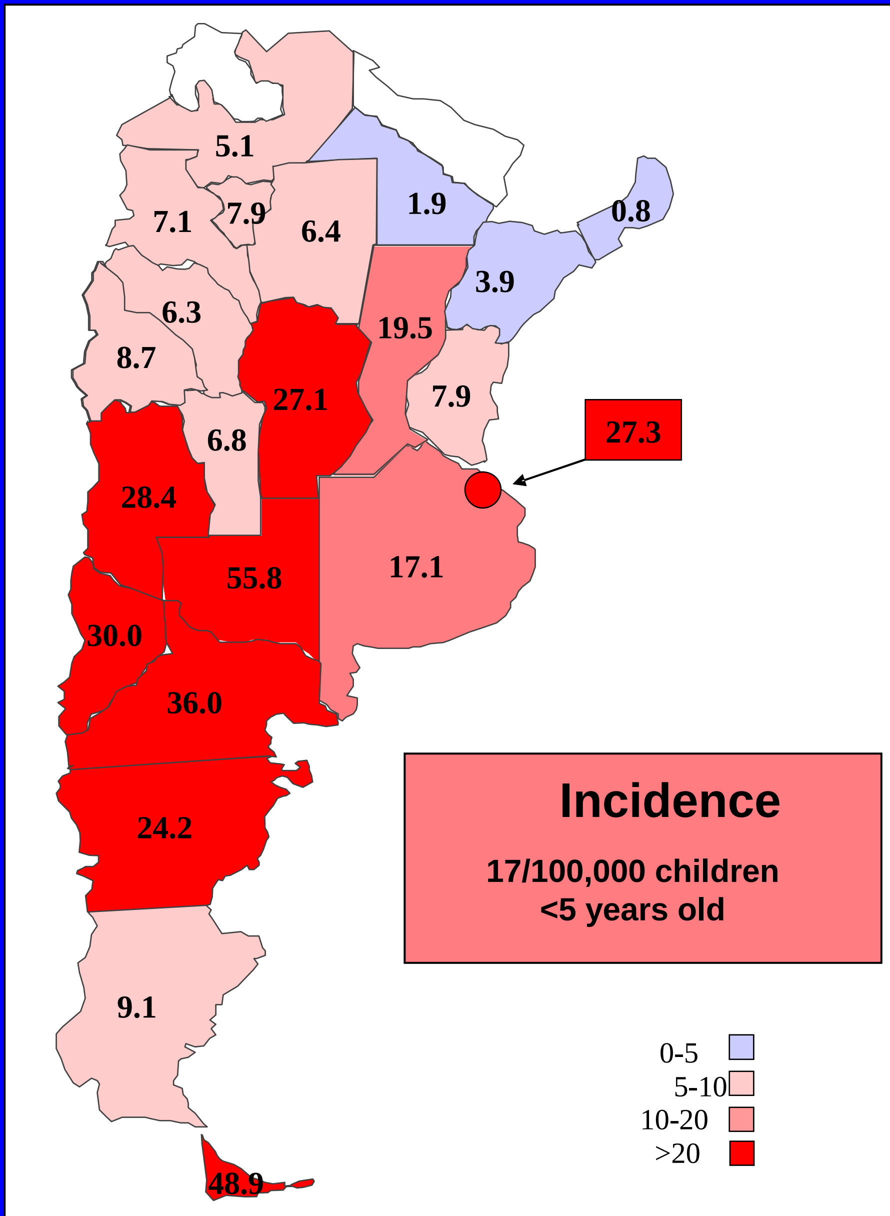
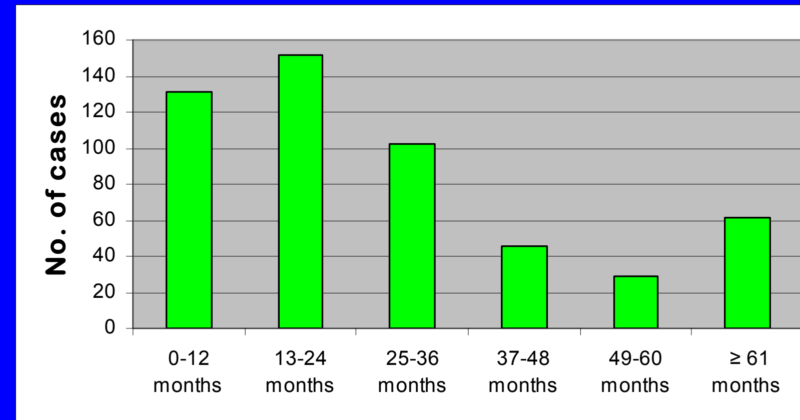
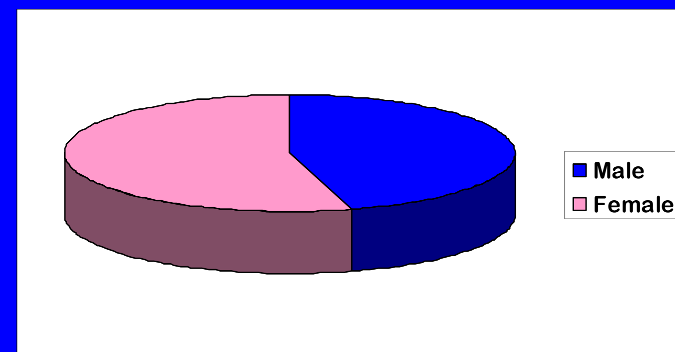
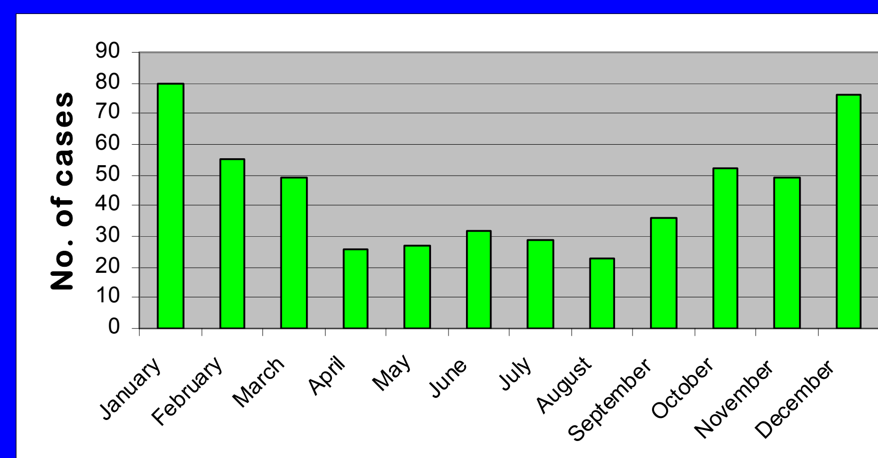
HUS - ARGENTINA 2008









NRL - DIAGNOSTIC CRITERIA

1. Isolation, pheno-genotypic characterization and typing of STEC strains

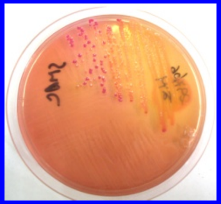
2. Detection of specifically neutralizable free fecal Shiga toxin

3. Serological tests to detect Stx and LPSO157 antibodies

ISOLATION AND CHARACTERIZATION

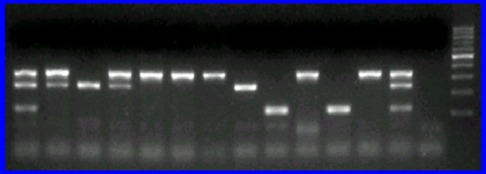
IMS ← PCR (-) ← Fecal Specimen

↓



Culture on SMAC (directly and after enrichment)

↓



Screening by Multiplex PCR
*stx*1/*stx*2/*rfb*O157

↓

Phenotypic and Genotypic Characterization
Typing

↓

RESULT

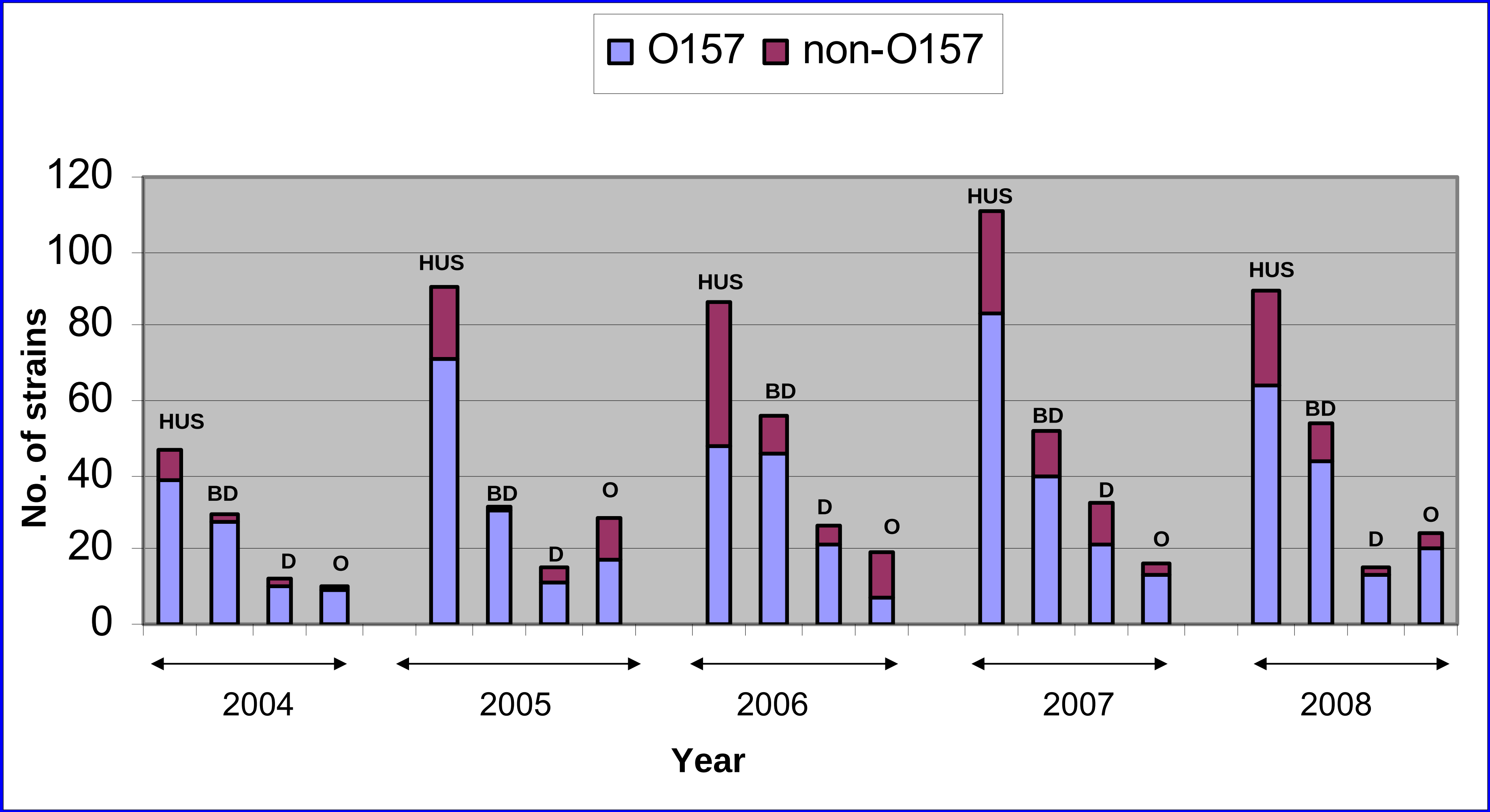
PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

- Biotyping
- Serotyping
- Antimicrobial susceptibility
- Enterohemolysis production
- Cytotoxicity assays on Vero cells
- Accessory virulence markers: *eae*, *exh*A, putative adhesins

TYPING TECHNIQUES

- Genotyping of *stx*1 and *stx*2 variants by PCR-RFLP (Zhang et al., 2002; Tyler et al., 1991; Pierard et al., 1998; Jelacic et al., 2003)
- Typing of *eae* by PCR-RFLP (Ramachandran et al., 2003)
- Phage typing by Ahmed et al. (1987), and Khakria et al. (1990) methods
- *Xba*I-PFGE using the 24-h CDC protocol, with minor modifications

DISTRIBUTION OF O157 AND NON-O157 STEC STRAINS ISOLATED FROM HUMAN SPECIMENS. 2004 - 2008



O157 AND NON-O157 STEC STRAINS OF CLINICAL ORIGIN. 2004-2008

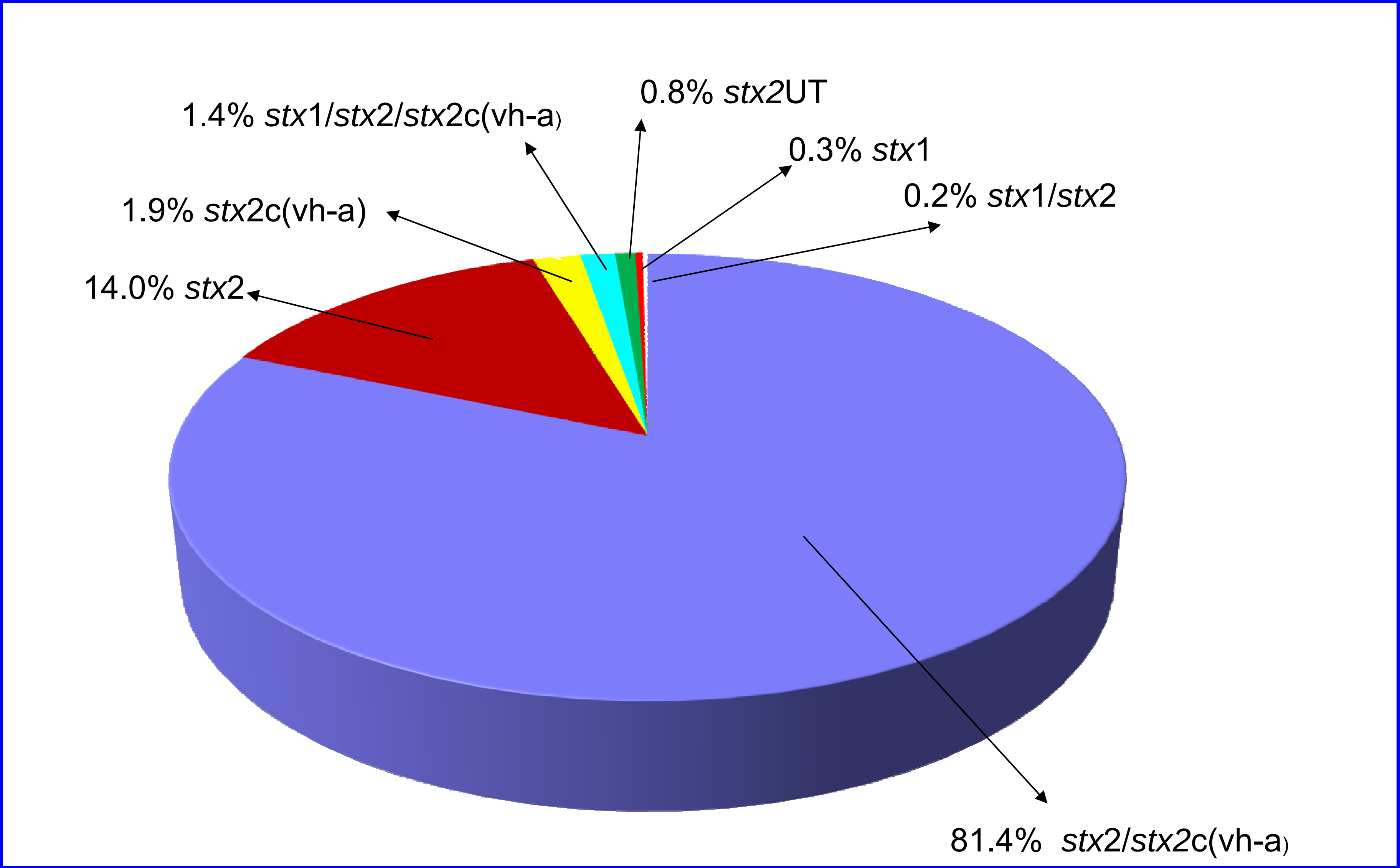
STEC STRAINS		
ORIGIN	**O157 No. (%)**	**Non-O157 No. (%)**
HUS	305 (48.0)	121 (56.8)
BD	188 (29.6)	35 (16.4)
NBD	76 (12.0)	26 (12.2)
OTHERS	66 (10.4)	31(14.6)
TOTAL	635	213

STEC SEROTYPES

- No. of strains = 848

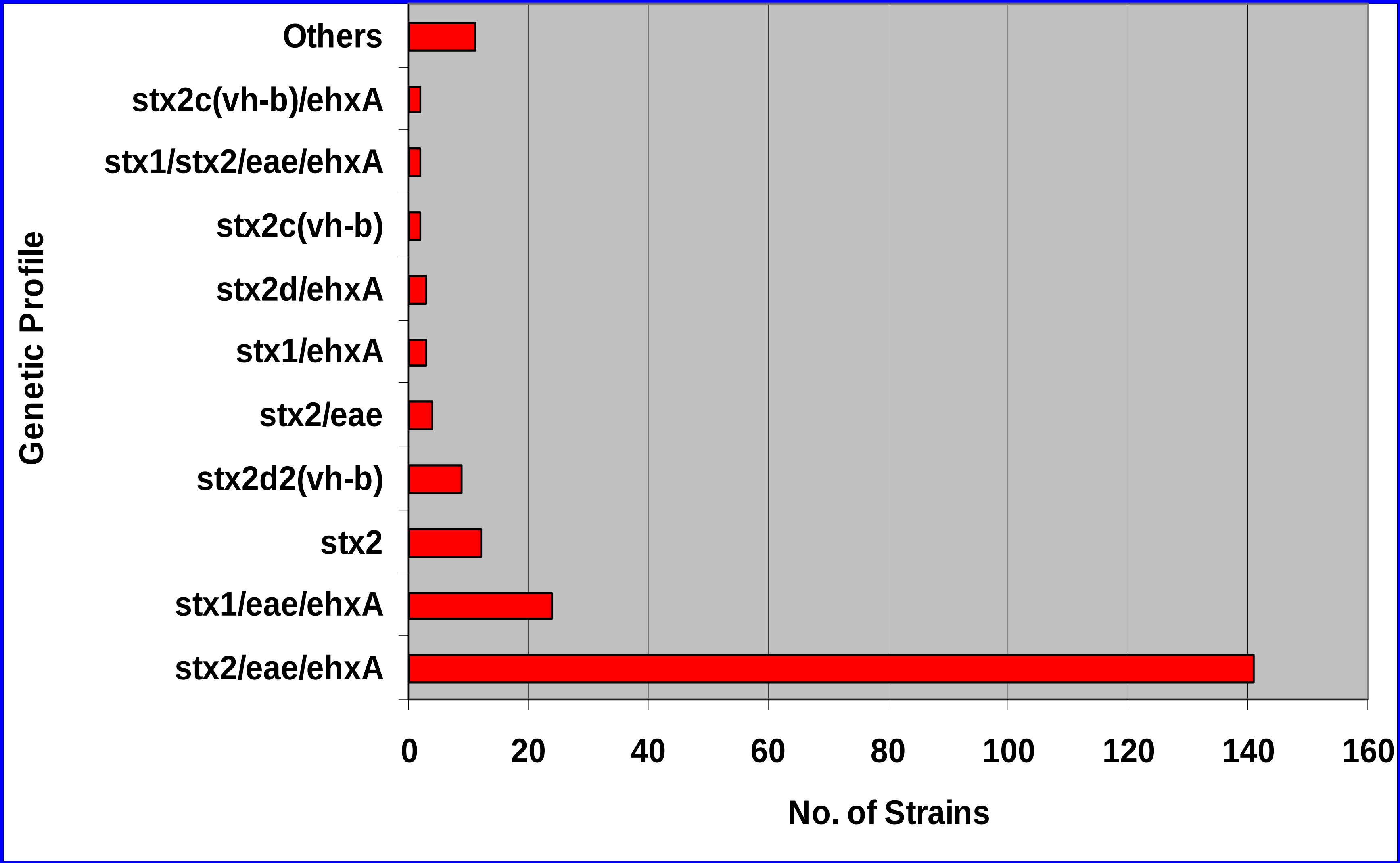
O157[H7]	74.9%	O113[H4,19,21]	0.3%
O145[H-,NT]	13.7%	O59[H19]	0.3%
O121[H19]	2.2%	O171[H2,NT]	0.2%
O26[H2,11,NT]	1.8%	O5[H-]	0.1%
O174[H8,21,28,H-]	0.8%	O20[H19]	0.1%
O111[H-]	0.6%	O22[HNT]	0.1%
O8[H16,19]	0.5%	O130[H11]	0.1%
O103[H2,NT]	0.3%	ONT[H7,11,12,49,NT]	3.1%
		OR[H-,NT]	0.6%

GENETIC PROFILE OF STEC O157 STRAINS



Antimicrobial susceptibility: 97.6%

GENETIC PROFILE OF NON-O157 STEC STRAINS



Antimicrobial susceptibility: 81.2%

MOLECULAR SURVEILLANCE IN REAL TIME

- **Genetic diversity and clonal relatedness**
  - 24-h CDC Protocol of PulseNet
  - Enzymes : *Xba*I and *Avr*II/*Bln*I
  - Reference strain: S. Braenderup CDC#H9812
  - Software: BioNumerics Ver. 4.01 (Applied Maths) 1998-2004

Databases 1988-2008

STEC O157

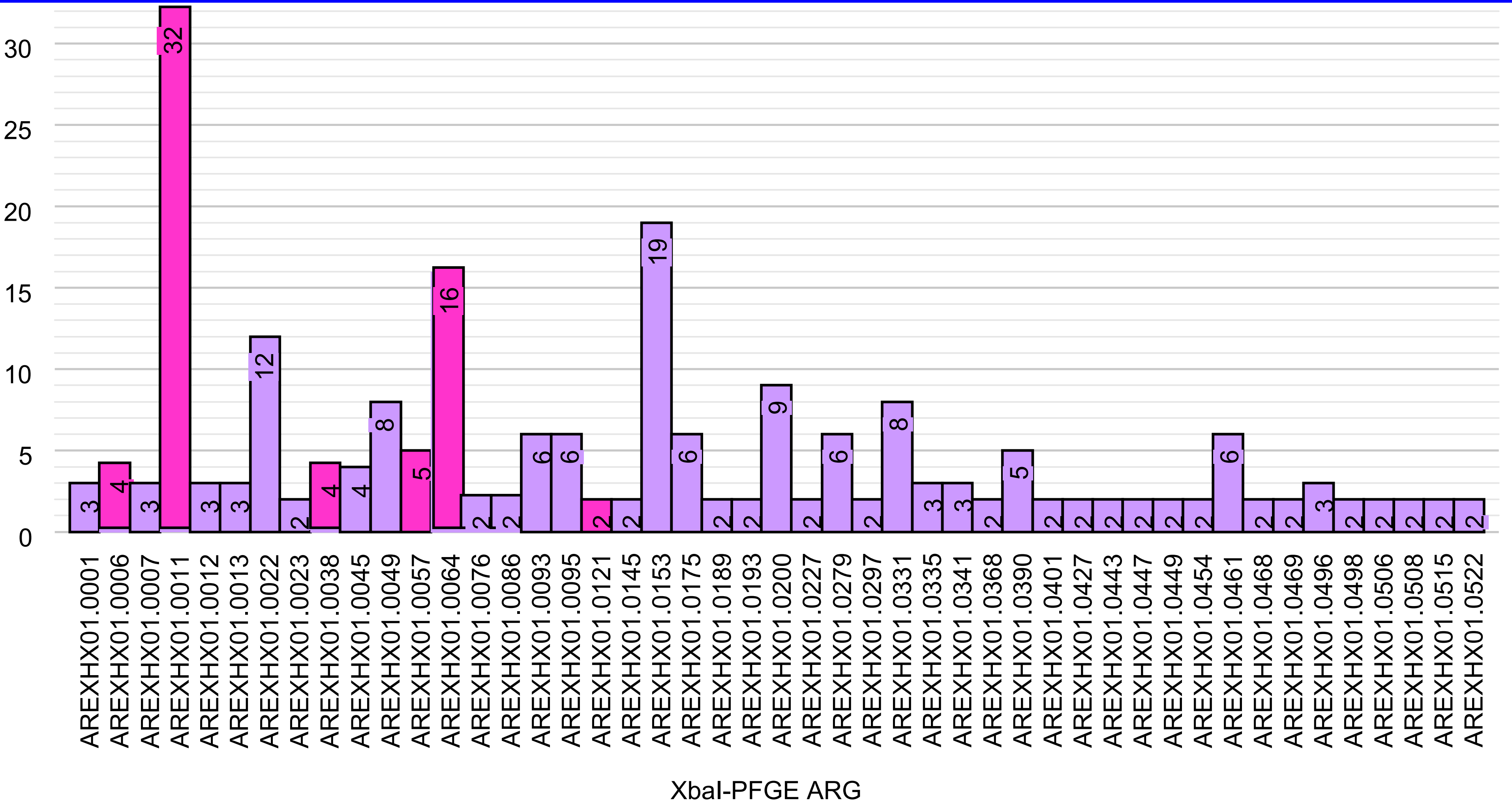
No. of strains: 1389
No. of *Xba*I-PFGE patterns: 584
Prevalent *Xba*I-PFGE patterns (17%)
AREXHX01.0011 (147 strains)
AREXHX01.0022 (91 strains)

Non-O157 STEC

No. of strains: 463
No. of *Xba*I-PFGE patterns: 322
Prevalent *Xba*I-PFGE patterns (7%)
AREXSX01.006 (17 strains)
AREXSX01.0124 (15 strains)

Outbreak detection
Clusters detection → Diffuse outbreaks
Source identification

ARGENTINE *E. coli* DATABASE. 2007-2008



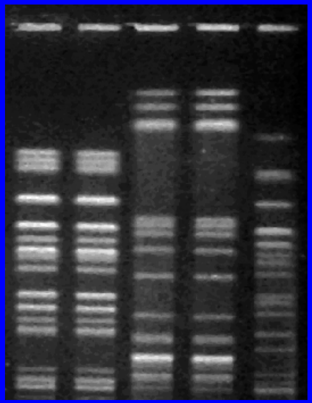
No. of strains: 363

No. of *Xba*I-PFGE: 189

No. of *Xba*I-PFGE > 2 strains: 47

Home-prepared Hamburger and Sporadic HUS

- Case: 2-year-old girl, attended in "Hospital Garrahan" on April 26, 2002 with bloody diarrhea, developed HUS three days later
- Food Consumption: home-prepared hamburger 48 h before diarrhea onset
- STEC isolated from stool and food:
  - *Escherichia coli* O157:H7
  - *eae*/*stx*2+*stx2c* (vh-a)/*ehxA*, PT4
  - identical pattern by *Xba*I-PFGE and *Bln*I-PFGE



Rivas et al. Emerg. Infect. Dis. 9: 1184 -1186, 2003.

COMMUNITY OUTBREAK ASSOCIATED WITH STEC O157 . NEUQUÉN. 2007

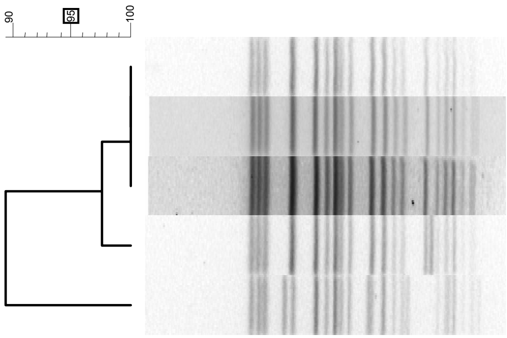
Cases: 3 BD / 1 HUS

Period: 02/24/07 - 03/03/07

Place: San Martín de los Andes, Neuquén

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]

PFGE-XbaI PFGE-XbaI



No. *Xba*I PFGE	Genotype	Origin	age (y)	Gender	City	date
AREXHX01.0200	*stx*2	BD	2.2	F	San Martín de los Andes	03/07
AREXHX01.0200	*stx*2	BD	3.9	M	San Martín de los Andes	03/07
AREXHX01.0200	*stx*2	HUS	3.5	M	San Martín de los Andes	03/07
AREXHX01.0440	*stx*2	BD	1.2	M	San Martín de los Andes	03/07
AREXHX01.0086	*stx*2 /*stx*2c	HUS	1.8	M	Neuquén City	03/07

STEC INFECTION IN HOUSEHOLD CONTACTS OF HUS CASE

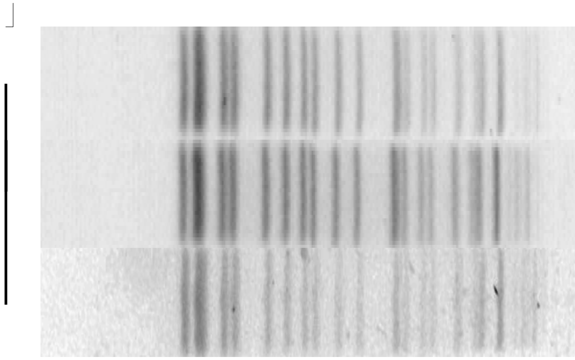
HUS case: 20 months old - girl
Sister: 13 years old
} negative diagnostic criteria

Father: 38 years old
Sister: 15 years old
Sister : 4 months old
} *E. coli* O145:NM Stx2, Int-γ, EHEC-Hly

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]

PFGE-XbaI

100



No. *Xba*I-PFGE	No. Cluster/Outbreak	Diagnostic	Age (y)	Gender	City
AREXSX01.0006	06MLEXS-11C	contact	15	FEMALE	Neuquén
AREXSX01.0006	06MLEXS-11C	contact	0-4	FEMALE	Neuquén
AREXSX01.0006	06MLEXS-11C	contact	38	MALE	Neuquén

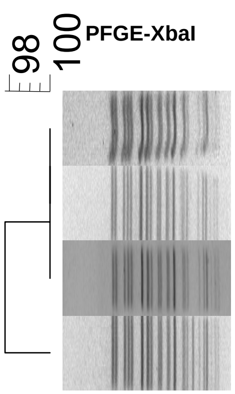
CLONAL RELATEDNESS OF STEC O157:H7 ISOLATED FROM HUS, ASYMPTOMATIC CONTACT AND FOOD

HUS case: boy, 1 year old
Household contact: brother, 11 year old
Food: ground beef and sausage
Period: July-August 2008
Place: Alpachiri, La Pampa

Isolates: *Escherichia coli* O157:H7 *eae* / *ehx*A / *stx*2 / *stx*2c (vh-a) / PT49

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]

PFGE-XbaI



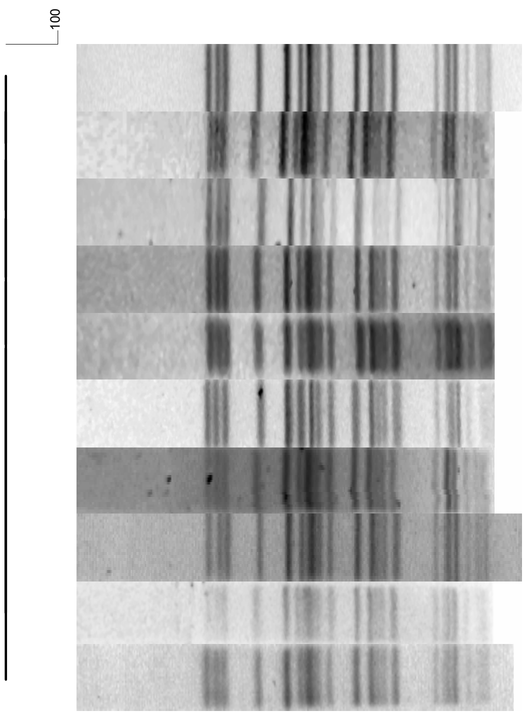
***Xba*I-PFGE**	**Date**	**Origin**	**Age**	**Gender**	**Cluster**	**Procedence**
AREXHX01.0153	08/05/08	Contact	11	M	08LPEXH-10C	Hospital Dr. L. Molas
AREXHX01.0153	08/11/08	Sausage			08LPEXH-10C	Food Laboratory
AREXHX01.0153	08/11/08	Ground beef			08LPEXH-10C	Food Laboratory
AREXHX01.0344	07/25/08	HUS	1	M	08LPEXH-10C	Hospital Dr. L. Molas

SPINACH OUTBREAK IN THE US
CDC CONSULTATION - 2006

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]

PFGE-XbaI

PFGE-XbaI8



Strain No.	No. *Xba*I-PFGE	Origin	Year	*stx* - Genotype	City
06BC005123					
25/02	AREXHX01-0057	BD	2002	stx2/stx2vh-a	BA
279/00	AREXHX01-0057	HUS	2000	stx2+stx2vh-a	BA
467/03	AREXHX01-0057	Beef	2003	stx2+stx2vh-a	Viedma
535/01	AREXHX01-0057	BD	2001	stx2	BA
58/01	AREXHX01-0057	BD	2001	stx2/stx2vh-a	BA
667/02	AREXHX01-0057	BD	2002	stx2	Me
698/04	AREXHX01-0057	D	2004	stx2+stx2vh-a	BA
73/02	AREXHX01-0057	Ground Beef	2002	stx2only	BA
88/02	AREXHX01-0057	HUS	2002	stx2	Tucumán

OUTBREAKS IN NURSERIES AND KINDERGARTENS

Year	No. Patients BD/HUS	No. Asymptomatic	Serotype	*stx*-Genotype	Source
2002	1/0	1	O157:H7	*stx*2/*stx*2c	unknown
2003	13/1	0	O26:H11 O103:H2	*stx*1 *stx*1	person-to-person
2004	3/1	2	O157:H7	*stx*2/*stx*2c	Swimming pool
2005	27/4	1	O145:NM ONT:HNT	*stx2* *stx2*d	Swimming pool

Miliwebesky et al., Rev. Arg. Microbiol. 39: 90-92, 2007

STEC IN FOOD

FOOD	STEC		TECHNIQUE	REFERENCE
	O157	non-O157		
GROUND BEEF	3.8%	-	IMS	Chinen et al., 2001
FRESH SAUSAGE	4.8%	-	IMS	Chinen et al., 2001
DRY SAUSAGE	3.3%	-	IMS	Chinen et al., 2001
HAMBURGER	ND	8.4%	PCR	Gómez et al., 2002
SOFT CHEESE	ND	0.9%	PCR	Gómez et.al., 2002
PRECOOKED				
SAUSAGE (MORCILLA)	2.0%	1.0%	PCR	Oteiza et al., 2006
RAW BEEF BURGER	11.2%	-	IMS	Chinen et al., 2009
COOKED BEEF BURGER	3.3%	-	IMS	Chinen et al., 2009
RAW CHICKEN BURGER	7.6%	-	IMS	Chinen et al., 2009
COOKED CHICKEN BURGER	4.0%	-	IMS	Chinen et al., 2009

STEC RESERVOIRS

RESERVOIR	O157	NON-O157	TECHNIQUE	REFERENCE
SHEEP	2.5%	-	PCR	Marguet et al., 1999
CATTLE	ND	26.6%	PCR	Parma et al., 2000
STEERS	ND	5.0%	PCR	Gioffre et al. 2002
CALVES	0.5%	-	PCR	Chinen et al., 2003
CALVES	0.5%	35.0%	PCR	Meitchtri et al., 2004
DOGS	1.4%	2.0%	PCR	Gallego et al., 2006
CATS	ND	4.2%	PCR	Gallego et al., 2006
RUMINANTS	ND	50.8%	PCR	Leotta et al., 2006
CATTLE (FS)	3.8%	73.3%	SIM/PCR	Tanaro et al., 2009
CATTLE (FS)	38.4%	45.2%	SIM/PCR	López et al., 2009
CATTLE (FS)	3.6%	35.0%	SIM/PCR	Masana et al., 2009
CATTLE (C)	2.5%	17.0%	SIM/PCR	Masana et al., 2009

PREVALENCE OF STEC O157:H7/NM AND NON-O157 IN COMMERCIAL BEEF CATTLE IN PROCESSING PLANTS

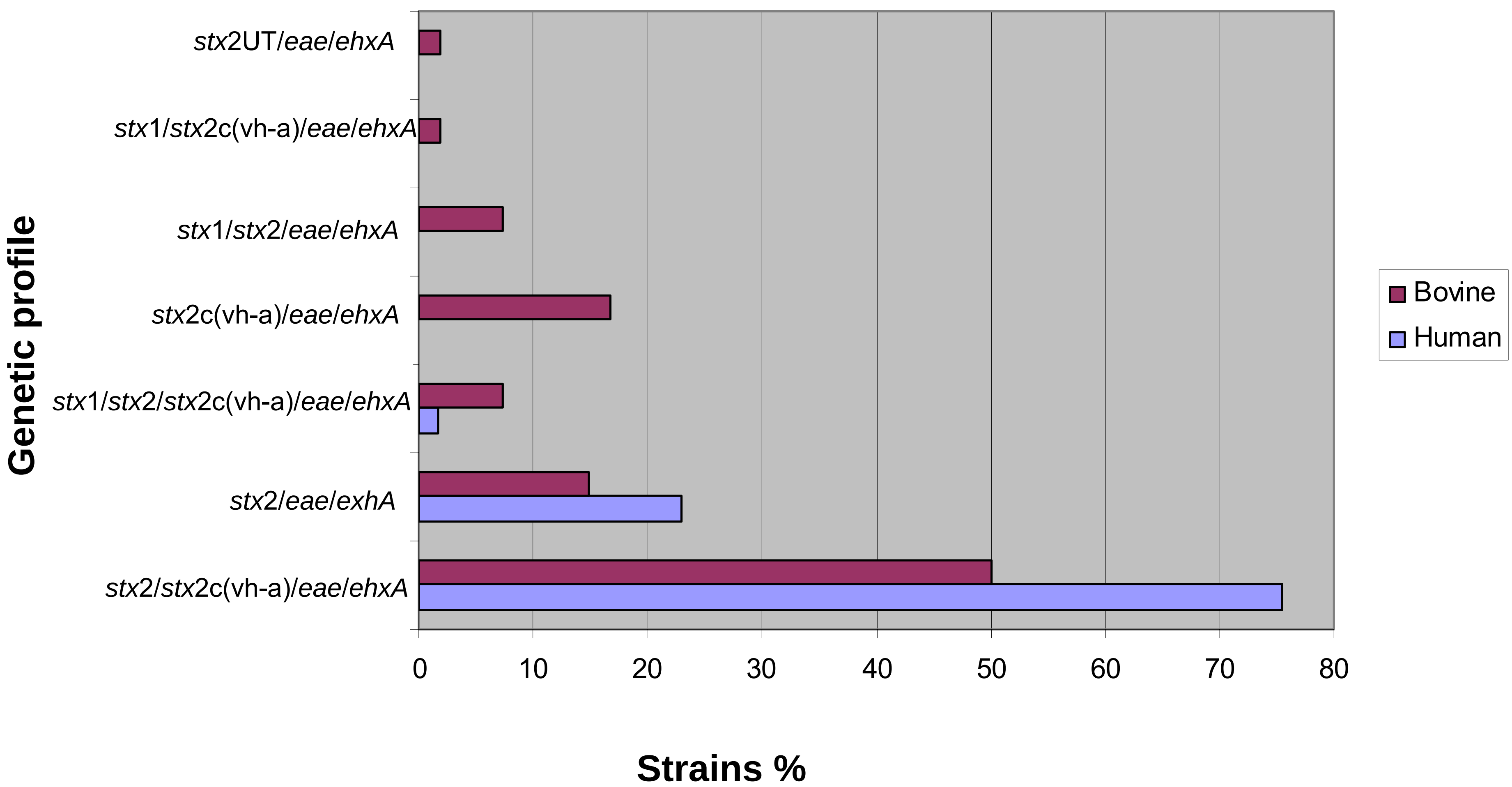
- Period of study: November 2006 – April 2008
- Place: 9 abattoirs (20% of annual slaughter)
- No. of animals = 811
- No. of samples = 1622
- 6 animals from each lot of 30-60 animals (single-source)
- Fecal samples: collected from the descendent colon near the rectum
- Carcass samples: obtained by swabbing the surface with a humidified sponge (6500.2/2005, FSIS/USDA)
- Animal types: young steers, steers, heifers, cows, calves
- Methodology: O157 by IMS and non-O157 by MK-PCR-screening after enrichment steps

RESULTS

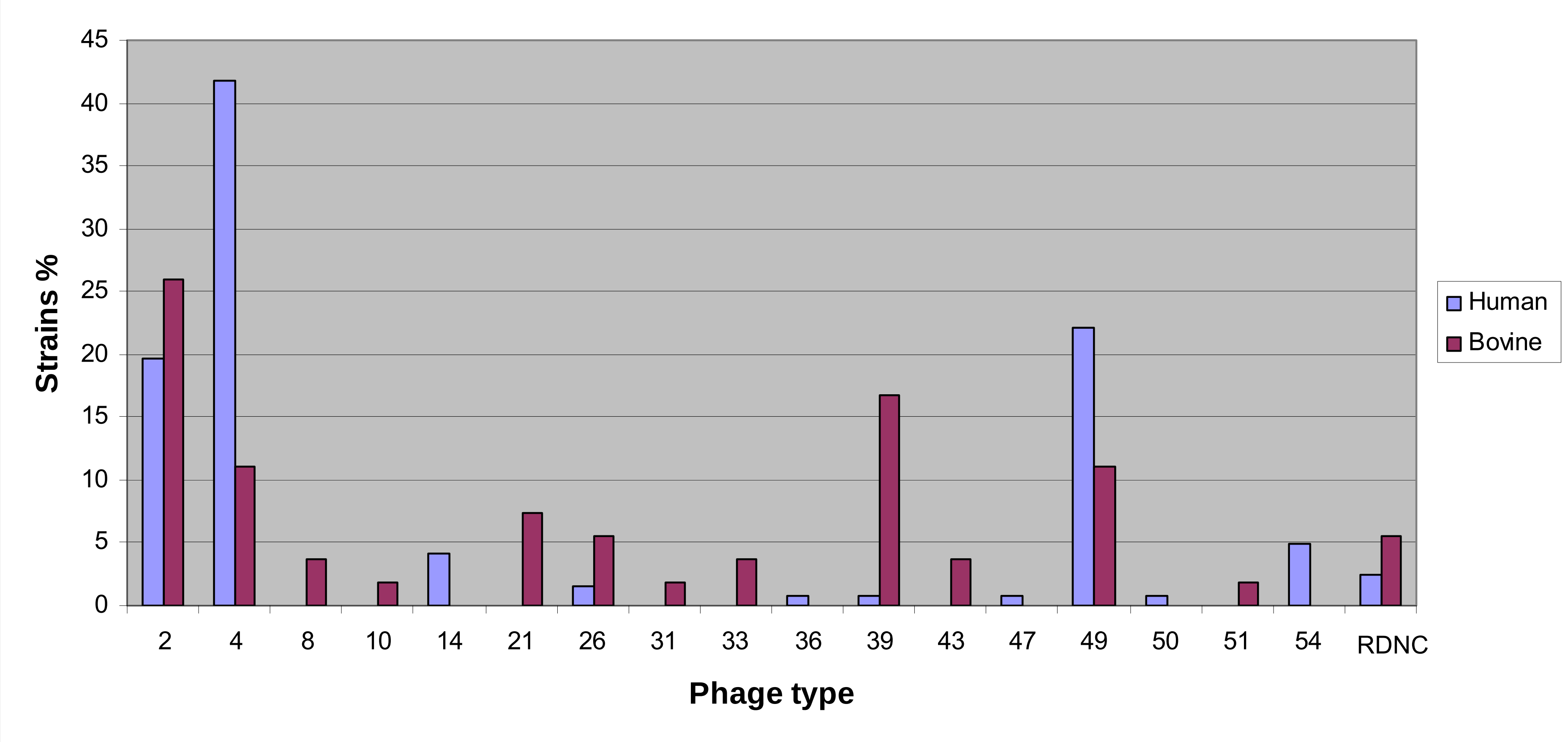
	O157:H7/NM	NON-O157
FECAL SAMPLE	3.6%	35%
CARCASSES	2.5%	17%
stx-GENOTYPE	*stx*2 / *stx*2c (vh-a)	*stx*2

- 26.7% of non-O157 serotypes detected were associated with human disease, previously
- No differences in the seasonal distribution
- Higher prevalence in calves and young steers
- Differences among abattoirs

COMPARISON OF GENETIC PROFILE OF STEC O157 STRAINS ISOLATED FROM HUS CASES AND CATTLE. NOVEMBER 2006 - APRIL 2008



COMPARISON OF PHAGE TYPES OF STEC O157 STRAINS ISOLATED FROM HUS CASES AND CATTLE. NOVEMBER 2006 - APRIL 2008



CONCLUSION I

- STEC infections are widespread in South America
- STEC strains are recovered from human, food and animal sources
- Different serotypes and *stx*-genotypes are detected
- STEC strains showed a high diversity because different restriction patterns are recognized in each country

A REGIONALSURVEILLANCE SYSTEM AND AN ENHANCED LABORATORY–BASED SURVEILLANCE SYSTEM ARE NEEDED TO ASSESS THE REAL BURDEN OF DISEASE DUE TO STEC IN OUR REGION

Conclusion II

- In Argentina, HUS is endemic
- *E. coli* O157:H7 is the main STEC serotype detected (>50%), other serotypes detected are: O26:H11, O91:NM, O111:NM, O113:H21, O145:NM
- *stx*2/*stx*2c (vh-a) is the prevalent genotype among O157 strains, and *stx*2 among non-O157 strains
- PT4 and PT49 are predominant
- Different *Xba*I-PFGE patterns identified – HIGH diversity
- Identical patterns are recognized in different regions, at different times
- High correlation between PT and *Xba*I-PFGE results

THANK YOU FOR YOUR ATTENTION