Prevalence of Non-O157 Shiga Toxin-Producing *Escherichia coli* in Live Animals and at Various Steps During Harvest

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Outline

-Introduction

-Detection/Isolation of non-O157 STEC

-Non-O157 STEC prevalence in cattle

-Non-O157 STEC prevalence on beef carcasses

-Conclusion

Shiga toxin-producing *Escherichia coli* (STEC)

- -Foodborne pathogens
- -Cause serious disease and death in humans
- -Cattle serve as reservoir

Nomenclature

- Shiga toxin initially discovered as a product of *Shigella dysenteriae*

- Antibodies against Shiga toxin shown to inhibit cytotoxicity of an *E. coli* strain
- *E. coli* strain discovered to be toxic to vero cells

Shiga-like toxin (SLTEC)

Vero toxin (VTEC)

Shiga toxin (STEC)

Cause disease in humans

Enterohemorrhagic *E. coli* (EHEC)

Non-O157 STEC

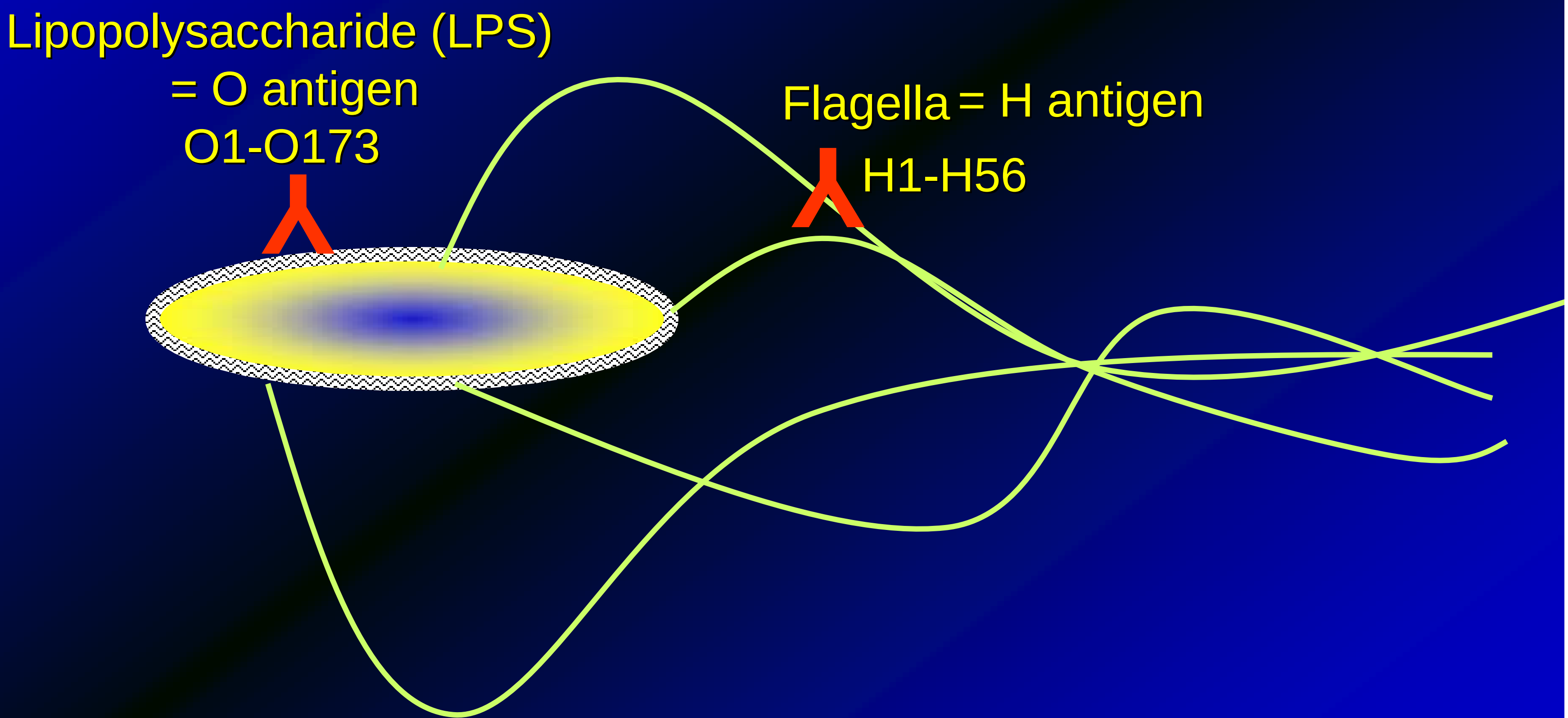
Estimated to cause one-third of the clinical EHEC cases, however, this is probably an underestimation.

Over 200 STEC serotypes have been isolated from cattle.

The proportion of non-O157 STEC able to cause disease in humans is unknown.

E. coli serotyping

O157:H7
O111:H8
O26:H11



Non-O157 disease outbreaks in U.S.

Approximately 35 *E. coli* O157 outbreaks occur each year, but only 3 non-O157 outbreaks ever reported in the U.S.

Montana	O104:H21	11 persons	milk?	1994
Connecticut	O121:H19	11 persons	swimming in lake	1999
Texas	O111:H8	58 persons	ice?	1999

Non-O157 STEC disease in US

O2.H7	HUS	O83.H1	HUS	O121.H19	HUS/D
O4.H-	HC	O85.H-	D	O125.H-	BD/HUS
O5.H-	BD/HUS	O88.H-	D	O126.H27	HUS
O14.H-	HUS	O103.H2	BD/HUS	O137.H41	HUS
O22.H5	HUS	O103.H2	HUS	O145.H-	HUS/BD
O26.H11	BD/HUS	O103.H25	D/BD	O153.H2	BD/HUS
O26.H2	D	O103.H6	D	O165.H25	HUS/BD
O38.H21	D	O104.H-	BD/HUS	O172.H-	HUS/D
O45.H2	BD/HUS	O104.H2	BD	O?.H11	BD/HUS
O45.H2	HC	O111.H-	HUS	OR.H-	HUS
O48.H21	BD	O111.H8	BD/HUS	OR.H9	BD/HUS
O50.H7	BD/HUS	O113.H21	BD/HUS	OR:H2	D
O68.H-	BD/HUS	O118.H16	BD	OX3.H21	HUS
O79.H7	HUS	O119.H-	D		

HUS – hemolytic uremic syndrome
HC – hemorrhagic colitis
BD – bloody diarrhea
D – diarrhea

STEC virulence factors

Shiga toxins – two types: *stx*1 and *stx*2
- ribosome inactivating proteins

Intimin (*eae)* – attachment to epithelial cells

EHEC-hemolysin – iron acquisition?

E. coli

Family: Enterobacteriaceae
Gram negative
Rod-shaped
Motile
Ferment: several sugars
including sorbitol
β-glucuronidase positive

E. coli O157

Family: Enterobacteriaceae
Gram negative
Rod-shaped
Motile: flagella
Ferment: several sugars
not sorbitol
β-glucuronidase negative

Produce Shiga toxins
Infectious dose <50 organisms

Non-O157 STEC

Family: Enterobacteriaceae
Gram negative
Rod-shaped
Motile: flagella
Ferment: several sugars
including sorbitol
β-glucuronidase positive

Produce Shiga toxins
Infectious dose ~10 organisms

Non-O157 STEC Detection

-Sampling is the same as for O157
-Enrichment is the same as for O157
-No universal immunomagnetic separation method available
-Cannot use sorbitol-based detection
-Cannot use β-glucuronidase-based detection

Detection has focused on identification of strains carrying the Shiga toxin genes or expressing the toxin proteins

PCR	EIA
Colony hybridization	Vero cell assay

Vero Cell Assay (VCA)

A cultured cell line, vero cells, is grown with the addition of culture supernatant in microtiter plates.

After incubation for 48 to 72 hours, the microtiter plates are examined microscopically.

Killing of the Vero cells is considered presumptive evidence of the presence of Shiga toxins in the broth culture supernatants.

Other organisms may produce toxins unrelated to Shiga toxins but toxic to Vero cells. To avoid false positive samples, antibodies which neutralize both Stx1 and Stx2 are used.

All positive samples are streaked for isolated colonies, which are then tested by the same assay or by PCR to isolate STEC strains.

Enzyme-Immunoassay (EIA)

Enzyme-immunoassays use anti-Shiga toxin antibodies to capture and detect the toxins in culture supernatants.

96-well format

Commercially available

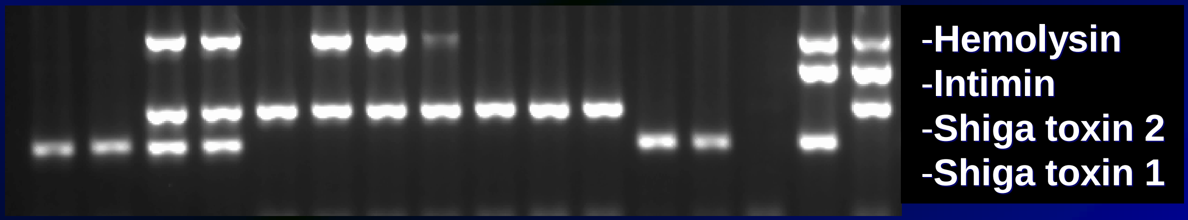
Test samples - then individual isolates

PCR

DNA primers for specific virulence factor genes

Can identify multiple genes in one reaction

Detects viable and nonviable cells



Colony hybridization

Grow colonies from sample enrichments on agar media

Transfer colonies to nylon membranes

Lyse cells and fix DNA to the membrane

Hybridize with DNA probe

Detect bound probe

Identify target colonies

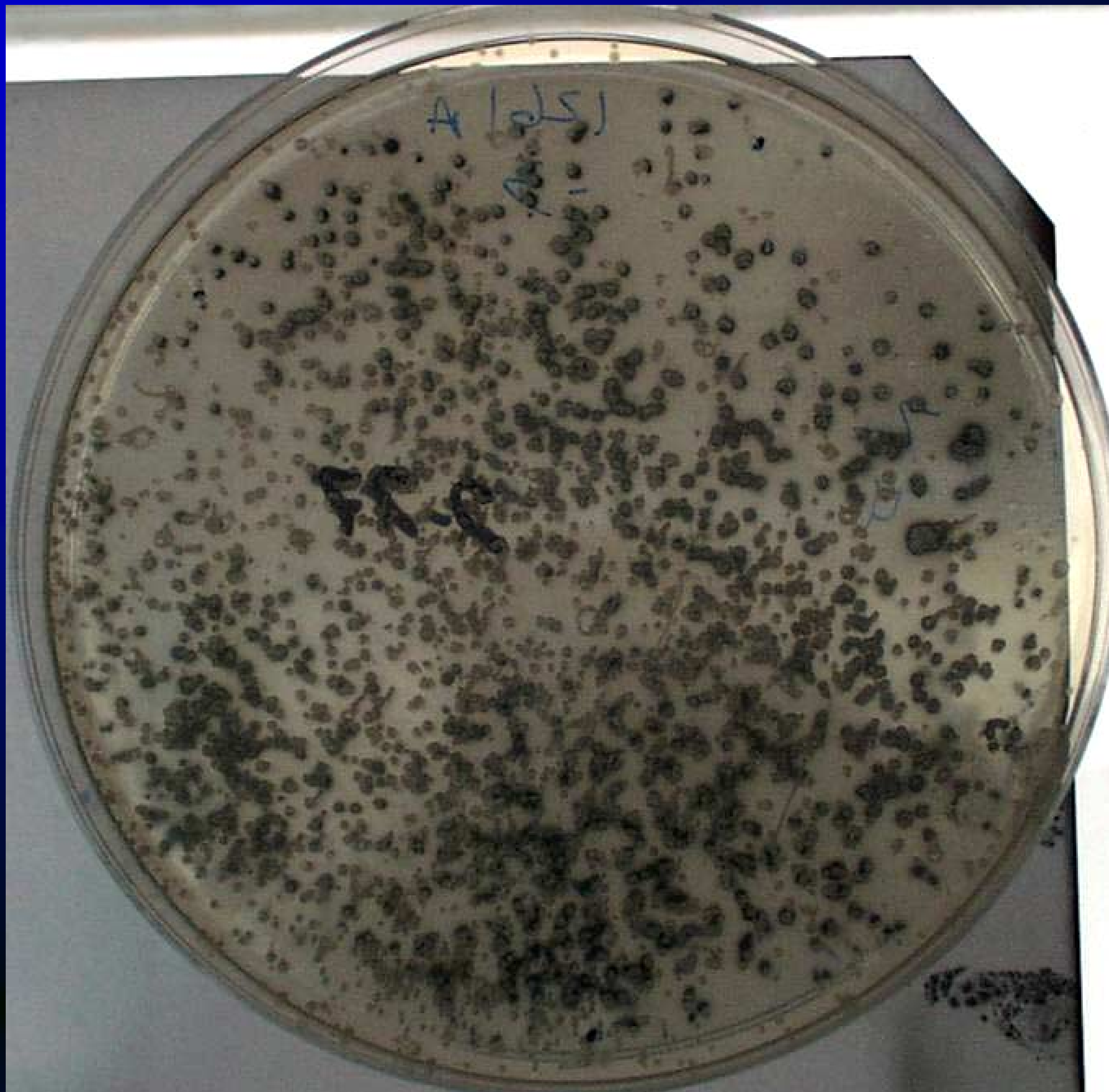
STEC Plating

-EC agar with 1% glucose


WHEATON

Before

After

Confirmation of STEC

Biochemical tests are required to confirm isolates are *E. coli*

Shigella dysenteriae, Citrobacter freundii, and *Enterobacter cloacae* have been found to produce Shiga toxins.

Prevalence of non-O157 STEC in Cattle

Country	Prevalence	Method	Reference
United States	5.9%	colony hybridization	Cray et al. 1996
	19%	vero cell assay	Wells et al. 1991
Argentina	37%	vero cell assay	Blanco et al. 1997
Canada	38.2%	vero cell assay	Van Donkersgoed et al. 1999
France	70%	PCR	Pradel et al. 2000
Japan	78.9%	PCR	Shinagawa et al. 2000
	100%	nested PCR	Kobayashi et al. 2001

Prevalence of STEC in Cattle

Calves appear to be more susceptible to STEC colonization than older cows.

STEC strains harboring *stx*1 are more commonly isolated from cattle than those harboring *stx*2.

Bovine-related STEC isolates lack accessory virulence factors intimin and hemolysin

EHEC serotypes (O157:H7, O111:H8, and O26:H11) are infrequently isolated form cattle when using unbiased methods.

Prevalence of non-O157 STEC on beef carcasses at processing

Sampled beef cattle carcasses at four large beef processing plants in Midwestern U.S. during the summer months

Samples collected for a survey of *E. coli* O157:H7 prevalence

Carcasses were followed through processing

Samples were taken after dehiding but prior to evisceration (preevisceration) and after all antimicrobial interventions when carcasses were in cooler (postprocessing)

PCR Screening

Surveyed for *E. coli* O157

E. coli O157 negative samples

Screen sample enrichments by PCR for *stx1* & *stx2* genes

All positive for *stx1* or *stx2* genes proceed to colony blotting

E. coli O157 positive samples

Cannot use PCR to screen

Proceed to colony blotting

Plant	Sample Period	Lot	*n*	Preevisceration Positive Samples	Postprocessing Positive Samples
A	1	1	84	6/18 (33.3)	0/18 (0)
		2	35	5/8 (62.5)	1/7 (14.3)
		3	68	1/15 (6.7)	0/16 (0)
	2	1	80	14/18 (77.8)	2/18 (11.1)
		2	41	8/10 (80.0)	2/9 (22.2)
		3	62	11/14 (78.6)	2/13 (15.4)
		4	44	8/13 (61.5)	1/13 (7.6)
B	1	1	46	0/11 (0)	0/9 (0)
		2	37	0/9 (0)	0/9 (0)
		3	39	6/9 (66.7)	1/9 (11.1)
		4	48	3/12 (25.0)	2/12 (16.7)
	2	1	36	2/9 (22.2)	0/9 (0)
		2	36	6/8 (75.0)	0/9 (0)
		3	36	8/9 (88.9)	0/7 (0)
		4	40	10/10 (100)	1/10 (10.0)
C	1	1	41	6/10 (60.0)	0/10 (0)
		2	76	10/17 (58.8)	1/17 (5.9)
		3	42	7/10 (70.0)	1/10 (10.0)
		4	38	10/10 (100)	2/9 (22.2)
	2	1	38	10/10 (100)	0/10 (0)
		2	44	5/9 (55.6)	1/10 (10.0)
		3	40	9/11 (81.8)	2/11 (18.2)
		4	46	9/12 (75.0)	4/12 (33.3)
D	1	1	37	4/7 (57.1)	1/8 (12.5)
		2	39	4/7 (57.1)	1/5 (20.0)
		3	38	2/8 (25.0)	0/8 (0)
		4	42	1/8 (12.5)	2/8 (25.0)
	2	1	65	4/14 (28.6)	0/13 (0)
		2	42	2/11 (18.2)	0/11 (0)
		3	58	9/17 (52.9)	0/16 (0)
Cumulative Totals				180/334 (53.9)	27/326 (8.3)

Samples	Preevisceration	Postprocessing
Total	334	326
Total non-O157 STEC positive	180 (54%)	27 (8%)
O157:H7 positive	144	6
Non-O157 STEC positive	84 (58%)	0 (0%)
O157:H7 negative	190	320
Non-O157 STEC positive	96 (51%)	27 (8%)
stx PCR Positive	146 (77%)	43 (13%)
Total STEC positive	240 (72%)	33 (10%)
Total *stx* positive	290 (87%)	49 (15%)

Prevalence of Non-O157 Contamination of Beef Cattle Carcasses

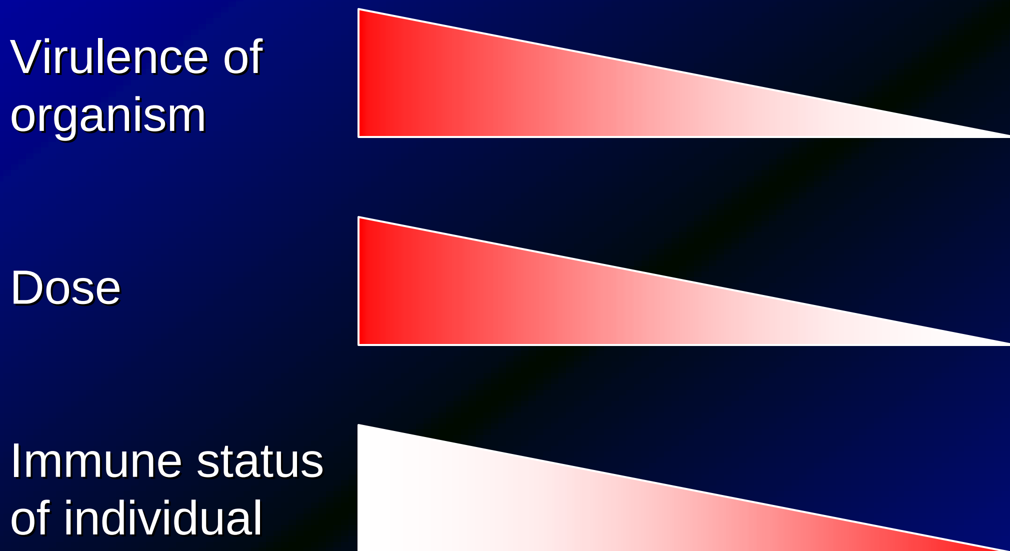
	U.S.	France[1]	Hong Kong[2]
Total Samples	326	851	986
Total non-O157 STEC positive	27 (8.3)	16 (1.9)	17 (1.7)
PCR positive for *stx* genes	43 (13.4)[3]	91 (10.7)	112 (11.4)

[1]Rogerie et al., 2001.

[2]Leung et al., 2001.

[3]PCR performed on 320 of the 326 samples.

<u>Determinants of infection</u>



Virulence attributes

- Shiga toxin 2 has lower LD_{50} for mice than Shiga toxin 1.

- Individuals infected with strains producing Shiga toxin 2 are more likely to develop severe disease than those infected with strains carrying Shiga toxin 1.

- STEC strains producing intimin and EHEC-hemolysin frequently associated with severe disease in humans.

STEC virulence factors	# of Isolates	Preevis	Post
*stx*1	152	135	17
*stx*2	93	78	15
*stx*1, *stx*2	15	15	0
*stx*1, *eae*	2	2	0
*stx*1, *hlyA*	8	3	5
*stx*2, *hlyA*	19	17	2
*stx*1, *stx*2, *hlyA*	31	23	8
*stx*1, *stx*2, *eae*	1	1	0
*stx*1, *eae*, *hlyA*	8	6	2
*stx*2, *eae*, *hlyA*	20	20	0
*stx*1, *stx*2, *eae*, *hlyA*	12	10	2
Total	361	310	51

Serotypes

- In the U.S., O26 and O111 are the two most common serogroups of clinical STEC isolates.

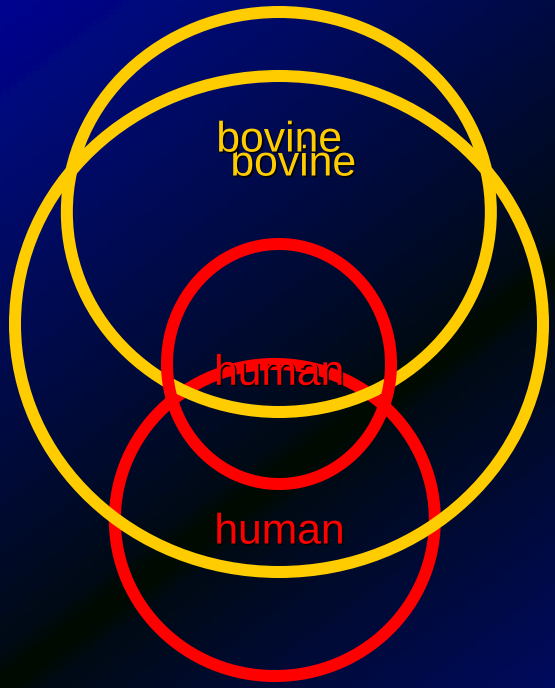
- Other serogroups commonly associated with human disease in the U.S. and abroad are O91, O103, O113, & O121

www.sciencenet.com.au/vtectable.htm

Serogroup distribution of non-O157 STEC isolates

Serogroup	# of isolates	Preevis	Post	Plants			
				A	B	C	D
O142	54	46	8	47		2	5
O121	31	31	0	2		27	2
O2	22	19	3	4	13	5	0
O171	18	18	0		6	12	
O113	15	12	3	1	10		4
O132	14	13	1		11	3	
O8	13	11	2	2	1	4	6
O88	10	10	0	5	2	2	1
O6	9	8	1		1	1	7
O139	9	5	4			4	5
O172	9	7	2			9	
OX3	9	3	6	6		3	
O104	5	1	4		2	3	
O117	5	5	0		4		1
O15	4	4	0	3			1
O165	4	4	0		2	1	1
O3	3	3	0	2			1
O55	3	3	0		2	1	
O153	3	3	0	1		2	
O168	3	0	3	3			
O10	2	2	0			2	
O45	2	2	0		2		
O103	2	2	0		2		
O109	2	0	2			2	
O119	2	2	0			2	
O145	2	2	0			2	
OX25	2	2	0	2			

Conclusion



- Majority of bovine STEC strains lack accessory virulence factors and are potentially less virulent.

- Cannot distinguish between virulent and nonvirulent STEC.

Acknowledgements

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Samples	Preevisceration	Postprocessing
Total Samples	334	326
O157 (+) Samples	144 / 334 (43%)	6 / 326 (2%)
non-O157 STEC (+)	180 / 334 (54%)	27 / 326 (8%)
O157 (+) & Non-O157 STEC (-)	60 / 334 (18%)	6 / 326 (2%)
O157 (+) & Non-O157 STEC (+)	84 / 334 (25%)	0 / 326 (0%)
O157 (-) & Non-O157 STEC (+)	96 / 334 (29%)	27 / 326 (8%)
Total STEC pos	240 /334 (72%)	33 / 326 (10%)