INTERNATIONAL COMPARISON OF SHIGA TOXIN-ENCODING BACTERIOPHAGE INSERTION SITE GENOTYPES OF CLINICAL, BOVINE AND ENVIRONMENTAL *E. COLI* O157 ISOLATES

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation/thesis of JOSHUA HERBERT WHITWORTH find it satisfactory and recommend that it be accepted.

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Abstract

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Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC O157) is a major cause of bloody diarrhea and hemolytic uremic syndrome (HUS) worldwide, although the annual incidence of EHEC O157 associated HUS varies from 0.01 to 0.41 cases per 100,000 population in different countries. Cattle are considered the principal reservoir of EHEC O157 and some genotypes of EHEC O157 commonly isolated from US cattle are rarely associated with human disease. We compared the genotype distribution of EHEC O157 in the cattle reservoir with human EHEC O157 disease incidence internationally to test the hypothesis that EHEC O157 disease incidence is due to differential exposure to genotypes of differing virulence. In this study, genotypes were defined by Shiga toxin-encoding bacteriophage insertion sites (Stx insertion genotypes). The relative frequencies of Stx insertion genotypes in isolates from the bovine reservoir were unrelated to HUS incidence, internationally ($P>0.05$). The distribution of Stx insertion genotypes of clinical isolates from Australia differed from those of the US ($P<0.017$), while clinical isolates from Japan and Germany were intermediate between them. The Stx insertion genotypes of US isolates obtained along a putative transmission chain from cattle, ground beef, clinically ill

humans and untreated municipal sewage demonstrated clear differences in distribution, with genotypes associated with human disease found in higher proportions in ground beef and, as expected, clinical isolates. These differing distributions are consistent with differences among EHEC O157 genotypes related to virulence, infectivity and/or environmental survival of this agent.

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INTRODUCTION

Infection with Enterohemorrhagic *Escherichia coli* (EHEC) produces disease in humans ranging from diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS), a leading cause of acute renal failure worldwide (13, 25). Cattle are considered the principal reservoir of these zoonotic agents (19). Enterohemorrhagic *Escherichia coli* O157 (EHEC O157) is the predominant EHEC associated with severe human disease in North America and Europe, and includes a widely distributed ß-glucuronidase-negative, non-sorbitol-fermenting clade as well as a ß-glucuronidase-positive, sorbitol-fermenting clade which is most commonly isolated in Germany (45).

The cardinal virulence determinants of EHEC O157 are the ability to cause intimate adherence to the colonic epithelium, encoded by a genomic island termed the locus of enterocyte effacement (LEE) and the ability to produce one or more Shiga toxins (Stx1 and/or Stx2), encoded by temperate lambdoid bacteriophages (24, 28, 34, 45). In the sequenced EHEC O157 strains EDL933 and Sakai, a Stx1 encoding phage is inserted in *yehV* and a Stx2 encoding phage is inserted in *wrbA* (20, 40). Shaikh and Tarr (44) used diversity in the Stx-encoding bacteriophage insertion sites to define three predominant genotypes among clinical isolates that they termed clusters 1, 2 and 3, which we will refer to as genotypes 1, 2 and 3 in this study. The predominance of these same genotypes among a larger set of US clinical isolates was subsequently confirmed (5).

Additional diversity in Stx-encoding bacteriophage insertion sites was detected in the US cattle reservoir (5). Clinical genotypes were less frequent among cattle isolates than among human clinical isolates, and numerous other Stx-encoding bacteriophage insertion site genotypes were identified among bovine isolates that were rarely or never identified among clinical isolates

(non-clinical genotypes). As the frequency of non-clinical genotypes in cattle suggests that the human population of the US is exposed to non-clinical genotypes approximately as frequently as to clinical genotypes; the reason(s) that these genotypes are not represented in clinical disease is of great interest, but remains undetermined.

The incidence of reported EHEC O157 associated disease (including diarrhea, bloody diarrhea, and HUS) differs markedly in different countries. For example, the reported incidence (infections per 100,000 population annually) of EHEC O157 associated disease was 4.1 for Scotland (2004), 0.9 for the USA (2004), 0.87 for Japan (2004), 0.13 - 1.6 for Germany (2004 or 1997-2003 data), 0.11 for the Republic of Korea (2003) and 0.08 for Australia (2004) (8, 11, 14-16, 31, 38). Because EHEC O157 infections are thought to be underreported and because more severe disease is less likely to go unreported, EHEC O157 associated HUS may be a more accurate indicator of the relative incidence of EHEC O157 associated disease (32). Accordingly, many national health agencies routinely report HUS associated with EHEC O157 separately from enteric EHEC O157 disease. Recent reports of EHEC O157 associated HUS incidence vary 40-fold internationally: 0.41 (Scotland), 0.1 (US), 0.05 (Republic of Korea), and 0.01 (Japan and Australia) (1, 9, 15, 16, 21, 37). Germany's reported HUS incidence was 0.002 in 2005 and 0.2 in 1997-2003, similar to the variation in its reported incidence of *E. coli* O157 infections (1, 14).

To test the hypothesis that the diverse international EHEC O157 disease incidence rates are due to differences in the proportion of EHEC O157 in the bovine reservoir that belong to clinical genotypes, we compared EHEC O157 associated disease rates (both enteric and HUS) with the proportion of clinical genotypes among cattle isolates in Australia, Japan, Korea, the USA, Scotland and Germany. Additionally, where available, we analyzed clinical EHEC O157 isolates from several countries to determine whether clinical genotypes predominated as in the

USA. Lastly, we compared genotypes of EHEC O157 isolated from a putative transmission chain from cattle, retail ground beef, clinically ill humans, and untreated human sewage.

MATERIALS AND METHODS

Bacterial isolates. Non-sorbitol-fermenting, β-glucuronidase-negative *Escherichia coli* O157 cattle isolates were selected. These strains originated from various production environments and different farms in geographically disseminated locations within the USA (1994-2002), Australia (1993-2003), Japan (1996-1997, provided by Dr. Masato Akiba), Scotland (1999, provided by Dr. Barti Synge), and Korea (1997, provided by Dr. B Young). Ground beef isolates were provided by Dr. Marcus Head. Additional isolates were obtained from untreated sewage at two municipal sewage treatment facilities in Washington State in 2006. Clinical isolates from the USA were acquired from the Washington Department of Health (2004-2005). Clinical isolates from Japan (1995-1996) were provided by Dr. Akiba. Clinical isolates from Australia were collected between 1986 and 1999 and were provided by Dr R. Robins-Browne and Dr D. Lightfoot, University of Melbourne, Parkville, Victoria, Australia. DNA from sorbitol and non-sorbitol fermenting human isolates obtained in Germany were kindly provided by Dr. Martina Bielaszewska. *E. coli* control strains used in this study included DH5α (negative) and EDL933 (positive).

Multiplex PCR genotyping. The Stx-encoding bacteriophage insertion site genotypes of *E. coli* O157 isolates were determined as previously described (5, 44) except that the reactions were combined into two multiplex PCR reactions and alternate *stx1* (36) and *stx2* (39) primers were used to improve compatibility with multiplex PCR. Isolated colonies were grown overnight at 37°C in LB broth with shaking and diluted 1:10 with water prior to PCR. The first multiplex reaction included primers for *stx1*, the right *wrbA*-bacteriophage junction and the left *yehV*-

bacteriophage phage junction. The second multiplex reaction contained primers for *stx2*, the left *wrbA*-bacteriophage junction and the right *yehV*-bacteriophage junction. Fragments were amplified in a total volume of 50 µl, consisting of 0.5 µl of 5 U/µl Taq polymerase, 2 µl of 50 mM $MgCl_2$, 2 µl of 10 mM dNTP, 5 µl of 10X buffer (Invitrogen, Carlsbad, CA) and 2 µl of template. Thermocycler parameters were 5 minutes at 95°C, followed by 35 cycles of 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 90 seconds, followed by a final cycle of 72°C for 10 minutes (iCycler, Bio-Rad, Hercules, CA). The PCR products were separated in a 1.2% agarose gel containing ethidium bromide submerged in 0.5x TBE buffer and photographed with UV illumination. Genotypes were assigned to the isolates based on the presence or absence of the PCR products (5).

Statistical analysis. Chi-square and Spearman's rank correlation analyses were performed using SAS (SAS Institute Inc., Cary, NC) and a web-based program produced by Kristopher J. Preacher at the University of Kansas [http://www.psych.ku.edu/preacher/chisq/chisq.htm]. Results were considered significant if the *P*-value was less than 0.05. Bonferroni adjustments were used when performing multiple pair-wise comparisons.

RESULTS

Genotyping results are presented in Table 1. There was no significant association between incidence of enteric EHEC O157 disease (r_s= 0.50, *P*=0.39) or HUS (r_s= 0.87, *P*=0.054) and the proportion of clinical genotypes among isolates from the bovine reservoir in the countries in this study. Isolates from Scottish cattle had the highest relative proportion of clinical genotypes (genotypes 1 – 3, 56%, Figure 1). The relative proportions of clinical genotypes in cattle isolates in the USA (38%), Korea (45%), Australia (37%), Japan (36%) and Scotland did not differ (χ^2= 5.1, 4 df, *P*= 0.28). Therefore, these data do not support the hypothesized

association between the genotype composition of *E. coli* O157 in the cattle reservoir and the incidence of human disease internationally.

The percentage of clinical genotypes among EHEC O157 clinical isolates differed significantly among countries (χ^2= 13.7, 3 df, *P*= 0.0034), ranging from 84% in the USA, 76% in Germany, 60% in Japan, and 47% in Australia. Pair-wise analyses demonstrated a significant difference only between the genotype distributions of clinical isolates from the USA and Australia (*P*<0.02). Isolates of the sorbitol fermenting, ß-glucuronidase positive EHEC O157 clade were negative for all markers except Stx 2, and hence Stx insertion genotyping does not appear to be informative with these isolates and they were excluded from further analyses.

As expected, the proportion of clinical genotypes in US clinical isolates was significantly higher than in isolates from cattle or ground beef specimens (Figure 2, *P*<0.01). Surprisingly, the proportion of clinical genotypes in cattle feces and retail ground beef specimens also differed significantly (χ^2=7.9, 1 df, *P*<0.01; Table 1, Figure 2), with higher proportions of genotype 3 and lower proportions of genotypes 5 and 6 in isolates from ground beef compared to cattle feces. Higher proportions of both genotypes 1 and 3 were also present in clinical isolates compared to ground beef isolates, although this was only significant for genotype 3 (χ^2=11.2, 1 df, *P*<0.001).

DISCUSSION

We found no simple association between the proportion of EHEC O157 clinical genotypes among cattle isolates from different countries and the respective incidence of human EHEC O157 associated disease. Nevertheless, these data support earlier observations that these bacteriophage insertion site genotypes are not distributed equally (5, 44), and specimen types expected to be related, such as isolates from cattle feces and from ground beef samples, show

differences in genotype distribution. Further research is needed to understand the biological basis of these differences.

One possible explanation of these results may lie in the amount of shedding of each genotype in bovine feces. If certain genotypes (e.g. 1 and 3) are characteristically shed in higher numbers, then we would expect greater representation of these genotypes in ground beef. At present, data is not available to evaluate this possibility. It is also possible that the genotype distribution in the cattle reservoir acts together with the prevalence of cattle infection to influence the incidence of human EHEC O157 related disease. Detection of EHEC O157 is known to be strongly affected by variables including sampling, culture and isolation methodology, so it is difficult to know whether the widely differing reported prevalences in cattle, both within and between countries, are due to true differences in prevalence or simply differences in detection sensitivity (10, 12, 17, 22, 23, 30, 33, 35, 42, 43, 46). Comparisons of the frequency of contamination of human food and water sources using internationally standardized methods and sampling frames, in conjunction with the genotype composition of the isolates in those sources, may better explain the marked differences in the incidence of *E. coli* O157 disease internationally.

Other researchers using different methodologies have reported unequal distribution of EHEC O157 genotypes from clinical infections and the bovine reservoir. Differential representation of alleles of a polymorphic locus in *tir,* which encodes the translocated intimin receptor, was recently reported in which one allele was consistently present in clinical isolates while both alleles were similarly represented in bovine isolates (7). Some phage types of EHEC O157 are over-represented among clinical isolates; for example phage type 21/28 which predominates in clinical isolates from Scotland (18, 26, 31). The bacteriophage anti-terminator Q

gene allele Q_{933} was detected more frequently among human isolates than among bovine isolates (29). Octamer Based Genomic Scanning (OBGS) revealed two genotypic groups, termed lineages I and II, which are over-represented among human clinical and bovine isolates, respectively (27). Both Stx insertion genotypes and OBGS classify most Australian isolates into groups less associated with US clinical disease (lineage II and non-clinical genotypes, respectively). Additionally, detection of the *tir* polymorphisms and Q_{933} allele have been shown to correlate with the clinical stx-insertion genotypes (5). Together these studies present a pattern of differential representation of some EHEC O157 genotypes in cattle compared to human disease isolates, which can be detected by diverse methods, and which remains unexplained.

Differential representation of specific EHEC O157 genotypes between clinical and non-clinical isolates raises the possibility that virulence or infectivity disparities may exist among different genotypes. If all genotypes of *E. coli* O157 had similar virulence or infectivity for humans and if cattle are the predominant source of human exposure, one might expect similar genotype distributions among the sources examined. Another possibility is that clinical genotypes better survive processing and better persist on hamburger and other meat products. Previous reports vary on the existence of strain-specific differences in survival on beef or in media, however, Avery and Buncic demonstrated that clinical isolates were more susceptible to drying than those from cattle (2, 3, 4, 6, 41).

Overall, genotypes 1 and 5 were the most broadly distributed genotypes detected in this study and they were the only genotypes detected in every specimen type from every country tested. These genotypes harbored the Stx2 gene, but not Stx1. These strains also had the Stx2 encoding bacteriophage inserted at a site other than *wrbA,* and the presence of bacteriophage sequences (lacking Stx1) in *yehV*. Additionally, the two strains are only differentiated in the Stx-

encoding bacteriophage insertion site typing scheme by the presence (genotype 1) or absence (genotype 5) of one bacteriophage-*yehV* junction. Genotype 1, the most common clinical isolate genotype detected from Australia, Germany and Scotland, has been proposed as the ancestral clinical genotype (44) and it is possible that genotype 5 is a closely related type.

These data do not support the hypothesized direct relationship between the proportions of EHEC O157 clinical genotypes in the bovine reservoir and the incidence of HUS in human populations. Because the large differences in HUS incidence remain unexplained, other factors such as the prevalence of EHEC O157 in the cattle reservoir, genotype-related differences in the fecal shedding by cattle, differences in survival in food products and environmental niches, and differential infectivity and virulence may contribute to the differing EHEC O157 disease incidence internationally as well as the differences in genotype distribution documented here among isolates from the bovine reservoir, ground beef, clinically ill humans, and untreated sewage.

Table 1: Stx-encoding bacteriophage insertion site genotypes of an international group of EHEC O157 clinical, cattle and environmental isolates.

Genotype[a]	Code[b]	Australia		Japan		Germany		Korea	Scotland	USA			
		Bovine	Human	Bovine	Human	Human[c]	Human	Bovine	Bovine	Bovine	Beef[d]	Human	Sewage[d]
Cluster 1	011100	21 (35.0)[e]	3 (20.0)	8 (18.2)	1 (20.0)	0 (0)	21 (72.4)	4 (12.9)	22 (56.4)	22 (15.3)	18 (17.5)	39 (26.4)	8 (66.7)
Cluster 2	011111	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.4)	2 (6.5)	0 (0)	2 (1.4)	5 (4.9)	4 (2.7)	0 (0)
Cluster 3	111111	2 (3.3)	4 (26.7)	8 (18.2)	2 (40.0)	0 (0)	0 (0)	8 (25.8)	0 (0)	31 (21.5)	35 (34)	82 (55.4)	0 (0)
4	010011	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.1)	0 (0)	1 (0.7)	0 (0)
5	011000	2 (3.3)	2 (13.3)	14 (31.8)	1 (20.0)	0 (0)	4 (13.8)	8 (25.8)	2 (5.1)	46 (31.9)	10 (9.7)	4 (2.7)	1 (8.3)
6	111000	15 (25.0)	2 (13.3)	3 (6.8)	0 (0)	0 (0)	0 (0)	0 (0)	7 (17.9)	31 (21.5)	10 (9.7)	3 (2.0)	0 (0)
7	111011	0 (0)	0 (0)	1 (2.3)	1 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	1 (0.7)	0 (0)
8	101100	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (9.7)	0 (0)	0 (0)	3 (2.9)	2 (1.4)	0 (0)
9	000000	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
10	001100	0 (0)	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)	1 (3.2)	3 (7.7)	1 (0.7)	2 (1.9)	1 (0.7)	2 (16.7)
11	010000	0 (0)	0 (0)	0 (0)	0 (0)	29 (100)	1 (3.4)	1 (3.2)	0 (0)	0 (0)	1 (1)	1 (0.7)	0 (0)
12	011011	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	1 (0.7)	0 (0)
13	101000	1 (1.7)	2 (13.3)	2 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7.7)	1 (0.7)	1 (1)	2 (1.4)	0 (0)
14	101011	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
15	110000	5 (8.3)	2 (13.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.4)	1 (1)	0 (0)	0 (0)
16	111100	12 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.9)	1 (3.2)	0 (0)	3 (2.1)	8 (7.8)	4 (2.7)	0 (0)
17	001000	0 (0)	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)	2 (6.5)	2 (5.1)	0 (0)	0 (0)	0 (0)	0 (0)
18	111101	0 (0)	0 (0)	6 (13.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.9)	1 (0.7)	0 (0)
19	010100	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.9)	0 (0)	1 (8.3)
20	100000	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)
21	011101	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.9)	0 (0)	0 (0)
22	101111	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)
23	111110	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)
Total		60	15	44	5	29	29	31	39	144	103	148	12

[a]Clusters 1-3 (44), genotypes 4-16 (5)

[b]PCR products for Stx1, Stx2, yehV-L, yehV-R, wrbA-L, wrbA-R concatenated; 1=present, 0=absent

[c] Sorbitol-fermenting, ß-glucuronidase-positive EHEC O157:H-; all other columns are non-sorbitol-fermenting, ß-glucuronidase-negative *Escherichia coli* O157:H7

[d] Beef=retail ground beef, Sewage=untreated municipal sewage

[e] Number of isolates (percent of column total)

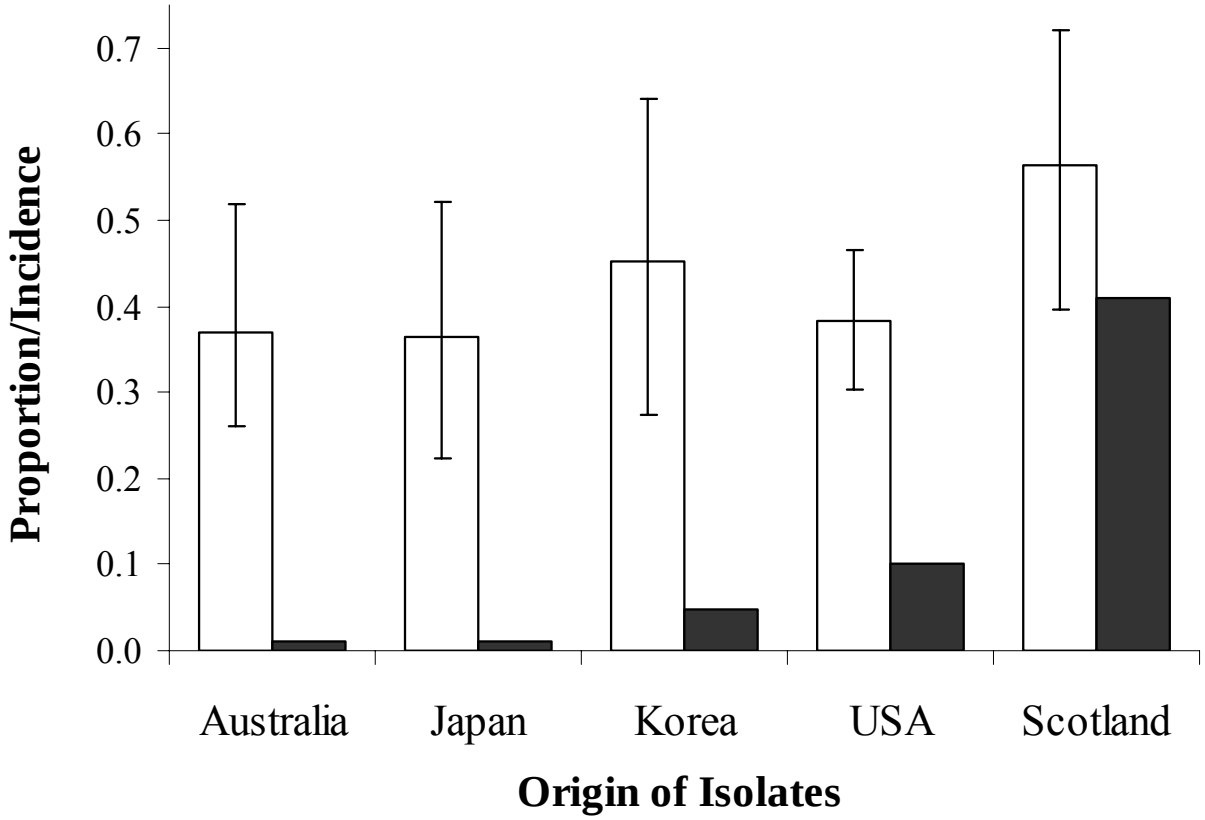

Figure 1: The proportion of bovine isolates with clinical genotypes (genotypes 1-3, unfilled bars) among isolates from cattle in the specified countries and the EHEC O157 HUS incidence (cases per 100,000 population per year, filled bars).

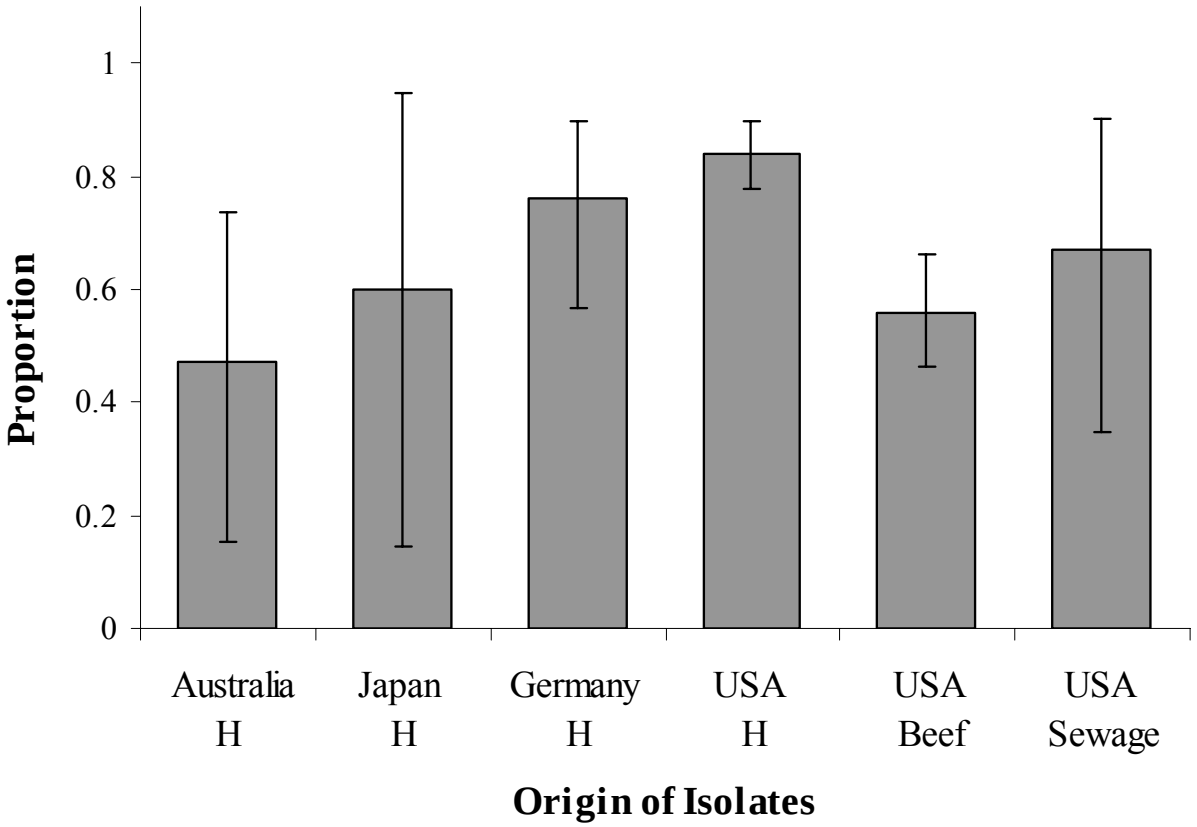

Figure 2: The proportion of clinical genotypes (genotypes 1-3) in clinical (H), retail ground beef (Beef) and untreated municipal sewage (Sewage) in the specified countries.

REFERENCES

1. **Anonymous.** 2007. Enter-net annual report: 2005 – surveillance of enteric pathogens in Europe and beyond. Enter-net surveillance hub, HPA, Centre for Infections, Colindale, London.
2. **Ansay, S. E., K. A. Darling, and C. W. Kaspar.** 1999. Survival of *Escherichia coli* O157:H7 in ground-beef patties during storage at 2, -2, 15 and then -2 degrees C, and -20 degrees C. J Food Prot **62:**1243-7.
3. **Avery, S. M., and S. Buncic.** 2003. *Escherichia coli* O157 diversity with respect to survival during drying on concrete. J Food Prot **66:**780-6.
4. **Barkocy-Gallagher, G. A., D. H. Kang, and M. Koohmaraie.** 2002. Fate of field-isolated *Escherichia coli* O157 in ground beef at different storage temperatures. J Food Prot **65:**1106-9.
5. **Besser, T. E., N. Shaikh, N. J. Holt, P. I. Tarr, M. E. Konkel, P. Malik-Kale, C. W. Walsh, T. S. Whittam, and J. L. Bono.** 2007. Greater diversity of Shiga toxin-encoding bacteriophage insertion sites among *Escherichia coli* O157:H7 isolates from cattle than in those from humans. Appl Environ Microbiol **73:**671-9.
6. **Bollman, J., A. Ismond, and G. Blank.** 2001. Survival of *Escherichia coli* O157:H7 in frozen foods: impact of the cold shock response. Int J Food Microbiol **64:**127-38.
7. **Bono, J. L., J. E. Keen, M. L. Clawson, L. M. Durso, M. P. Heaton, and W. W. Laegreid.** 2007. Association of *Escherichia coli* O157:H7 tir polymorphisms with human infection. BMC Infect Dis **7:**98.

8. **Centers for Disease Control and Prevention.** 2005. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 sites, United States, 2004. MMWR Morb Mortal Wkly Rep **54:**352-6.

9. **Centers for Disease Control and Prevention.** 2006. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 States, United States, 2005. MMWR Morb Mortal Wkly Rep **55:**392-5.

10. **Cobbold, R., and P. Desmarchelier.** 2000. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian diary herds. Vet Microbiol **71:**125-37.

11. **Disweb.** February 2007, access date. Communicable Disease Statistics. **http://dis.cdc.go.kr**.

12. **Dodson, K., and J. LeJeune.** 2005. *Escherichia coli* O157:H7, *Campylobacter jejuni*, and *Salmonella* Prevalence in cull dairy cows marketed in northeastern Ohio. J Food Prot **68:**927-31.

13. **Donnenberg, M. S., and T. S. Whittam.** 2001. Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic *Escherichia coli*. J Clin Invest **107:**539-48.

14. **Dreesman, J., and M. Pulz.** 2004. [The epidemiology of human enterohemorrhagic *Escherichia coli* infections in Lower Saxony]. Dtsch Tierarztl Wochenschr **111:**317-20.

15. **Energy Information Administration.** August 2006, posting date. International Energy Annual 2004; World Population, 1980-2004. **http://www.eia.doe.gov**.

16. **Fisher, I. S., and S. Meakins.** 2006. Surveillance of enteric pathogens in Europe and beyond: Enter-net annual report for 2004. Euro Surveill **11:**E060824 3.

17. **Gunn, G. J., I. J. McKendrick, H. E. Ternent, F. Thomson-Carter, G. Foster, and B. A. Synge.** 2007. An investigation of factors associated with the prevalence of verocytotoxin producing *Escherichia coli* O157 shedding in Scottish beef cattle. Vet J **174:**554-64.

18. **Halliday, J. E., M. E. Chase-Topping, M. C. Pearce, I. J. McKendrick, L. Allison, D. Fenlon, C. Low, D. J. Mellor, G. J. Gunn, and M. E. Woolhouse.** 2006. Herd-level risk factors associated with the presence of Phage type 21/28 *E. coli* O157 on Scottish cattle farms. BMC Microbiol **6:**99.

19. **Hancock, D., T. Besser, J. Lejeune, M. Davis, and D. Rice.** 2001. The control of VTEC in the animal reservoir. Int J Food Microbiol **66:**71-8.

20. **Hayashi, T., K. Makino, M. Ohnishi, K. Kurokawa, K. Ishii, K. Yokoyama, C. G. Han, E. Ohtsubo, K. Nakayama, T. Murata, M. Tanaka, T. Tobe, T. Iida, H. Takami, T. Honda, C. Sasakawa, N. Ogasawara, T. Yasunaga, S. Kuhara, T. Shiba, M. Hattori, and H. Shinagawa.** 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res **8:**11-22.

21. **Hong, I. H., and JH Koo.** 2001. Clinical Characteristics and Prognostic Factors of Hemolytic Uremic Syndrome in Korean Children. Korean J Nephrol. **20:**486-492.

22. **Hussein, H. S., and L. M. Bollinger.** 2005. Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. J Food Prot **68:**2224-41.

23. **Hussein, H. S., and T. Sakuma.** 2005. Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. J Dairy Sci **88:**450-65.

24. **Karch, H., H. Schmidt, C. Janetzki-Mittmann, J. Scheef, and M. Kroger.** 1999. Shiga toxins even when different are encoded at identical positions in the genomes of related temperate bacteriophages. Mol Gen Genet **262:**600-7.

25. **Karmali, M. A.** 2004. Infection by Shiga toxin-producing *Escherichia coli*: an overview. Mol Biotechnol **26:**117-22.

26. **Khakhria, R., D. Duck, and H. Lior.** 1990. Extended phage-typing scheme for *Escherichia coli* O157:H7. Epidemiol Infect **105:**511-20.

27. **Kim, J., J. Nietfeldt, J. Ju, J. Wise, N. Fegan, P. Desmarchelier, and A. K. Benson.** 2001. Ancestral divergence, genome diversification, and phylogeographic variation in subpopulations of sorbitol-negative, beta-glucuronidase-negative enterohemorrhagic *Escherichia coli* O157. J Bacteriol **183:**6885-97.

28. **Law, D.** 2000. Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*. J Appl Microbiol **88:**729-45.

29. **Lejeune, J. T., S. T. Abedon, K. Takemura, N. P. Christie, and S. Sreevatsan.** 2004. Human *Escherichia coli* O157:H7 genetic marker in isolates of bovine origin. Emerg Infect Dis **10:**1482-5.

30. **LeJeune, J. T., T. E. Besser, D. H. Rice, J. L. Berg, R. P. Stilborn, and D. D. Hancock.** 2004. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. Appl Environ Microbiol **70:**377-84.

31. **Locking, M., L. Allison, L. Rae, K. Pollock, and M. Hanson.** 2006. VTEC infections and livestock-related exposures in Scotland, 2004. Euro Surveill **11:**E060223 4.

32. **Mahon, B. E., P. M. Griffin, P. S. Mead, and R. V. Tauxe.** 1997. Hemolytic uremic syndrome surveillance to monitor trends in infection with *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli*. Emerg Infect Dis **3:**409-12.

33. **Meyer-Broseta, S., S. N. Bastian, P. D. Arne, O. Cerf, and M. Sanaa.** 2001. Review of epidemiological surveys on the prevalence of contamination of healthy cattle with *Escherichia coli* serogroup O157:H7. Int J Hyg Environ Health **203:**347-61.

34. **O'Brien, A. D., J. W. Newland, S. F. Miller, R. K. Holmes, H. W. Smith, and S. B. Formal.** 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science **226:**694-6.

35. **Ogden, I. D., M. MacRae, and N. J. Strachan.** 2004. Is the prevalence and shedding concentrations of *E. coli* O157 in beef cattle in Scotland seasonal? FEMS Microbiol Lett **233:**297-300.

36. **Olsvik, O., E. Rimstad, E. Hornes, N. Strockbine, Y. Wasteson, A. Lund, and K. Wachsmuth.** 1991. A nested PCR followed by magnetic separation of amplified fragments for detection of *Escherichia coli* Shiga-like toxin genes. Mol Cell Probes **5:**429-435.

37. **OzFoodNet Working Group.** 2006. Burden and causes of foodborne disease in Australia: Annual report of the OzFoodNet network, 2005. Commun Dis Intell **30:**278-300.

38. **OzFoodNet Working Group.** 2005. Reported foodborne illness and gastroenteritis in Australia: Annual report of the OzFoodNet network, 2004. Commun Dis Intell **29:**164-190.

39. **Paton, A. W., and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. J Clin Microbiol **36:**598-602.

40. **Perna, N. T., G. Plunkett, 3rd, V. Burland, B. Mau, J. D. Glasner, D. J. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Posfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anantharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409:**529-33.

41. **Sage, J. R., and S. C. Ingham.** 1998. Survival of *Escherichia coli* O157:H7 after freezing and thawing in ground beef patties. J Food Prot **61:**1181-3.

42. **Sargeant, J. M., M. W. Sanderson, R. A. Smith, and D. D. Griffin.** 2003. *Escherichia coli* O157 in feedlot cattle feces and water in four major feeder-cattle states in the USA. Prev Vet Med **61:**127-35.

43. **Sekiya, J.** 1997. *Escherichia coli* O157:H7 in livestock in Japan. Rev Sci Tech **16:**391-4.

44. **Shaikh, N., and P. I. Tarr.** 2003. *Escherichia coli* O157:H7 Shiga toxin-encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. J Bacteriol **185:**3596-605.

45. **Wick, L. M., W. Qi, D. W. Lacher, and T. S. Whittam.** 2005. Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. J Bacteriol **187:**1783-91.

46. **Woerner, D. R., J. R. Ransom, J. N. Sofos, G. A. Dewell, G. C. Smith, M. D. Salman, and K. E. Belk.** 2006. Determining the prevalence of *Escherichia coli* O157 in cattle and beef from the feedlot to the cooler. J Food Prot **69:**2824-7.