Shiga toxin-producing Escherichia coli (STEC), also called verotoxin-producing E. coli (VTEC), is the most important recently emerged group of foodborne pathogens. STEC can produce serious human illness linked to the consumption of contaminated foods, mainly of bovine origin. Argentina has the highest rate of hemolytic uremic syndrome (HUS) globally and HUS is the leading cause of acute renal failure among children. E. coli O157:H7 is the most common cause of HUS, but a substantial and growing proportion of this illness is caused by infection due to non-O157 strains. Multiple locus variable-number tandem repeat analysis (MLVA) has become an established technique to subtype STEC. This review will address the use of routine STEC subtyping by MLVA in order to type this group of isolates and to get insight into the genetic diversity of native STEC. With regard to these objectives we modified and adapted two MLVA protocols, one exclusive for O157 and the other, a generic E. coli assay. A total of 202 STEC isolates, from different sources and corresponding to 20 serotypes, have been MLVA genotyped in our laboratory. In our experience, MLVA constitutes a very sensitive tool and enables us to perform an efficient STEC subtyping. The diversity found in many serotypes may be useful for future epidemiological studies of STEC clonality, applied to O157 as well as to non-O157 isolates.

Keywords: STEC, MLVA, genotyping, O157:H7, non-O157
In relation with the MLVAc we applied it in order to analyze both non-O157:H7 and O157:H7 isolates (Bustamante et al., 2009b, 2010; Fernández et al., 2010b; Franci et al., 2011). In a total of 174 samples we detected 66 (37.9%) different MLVA profiles, being 41 of them unique. To our knowledge, we subtyped by MLVA for the first time 14 out of the 20 serotypes studied: O8:H19, O20:H19, O91:H21, O112:H2, O130:H11; O145:NM, O171:H2, O174:H21, O171:NM, ONT:H7, ONT:H19, and ONT:H21. Also, we observed several alleles which have not been previously described. The locus CVN004 was the most variable among serotypes and among isolates from a same serotype (Table 1), coincidently with the results of Lindstedt et al. (2007) and Gorgé et al. (2008). Among non-O157:H7 serotypes, the loci which presented the lowest variability were CVN002, CVN007, CVN015, and CVN003. Furthermore, this last locus presented null alleles (no PCR amplification) in all isolates except for those belonging to O157:H7 and O145:NM serotypes (Bustamante et al., 2010). Similarly, Laberśli et al. (2012) found this locus was absent in several serotypes and they only confirmed the presence of this locus among E. coli O145, O157, and O55:H7 isolates. The results obtained performing MLVAO157 and MLVAc showed a high genetic diversity in the STEC isolates analyzed, and five or more MLVA profiles were found in the serotypes O20:H19, O117:H7, O157:H7, O171:H2, O174:H21, and O178:H19 (Figure 1). On the contrary, preliminary data in regard to O130:H11 serotype, showed a unique profile in all the studied isolates which could be indicating that it is an emergent serotype or, on the contrary, that the chosen VNTR loci are not variable enough (Fernández et al., 2010b).

Another laboratory from Argentina has begun to evaluate the use of the MLVA for the epidemiological surveillance of E. coli O157:H7, as a complementary technique to pulsed-field-gel-electrophoresis (PFGE) in order to solve difficult cases (Chisen et al., 2010). The chosen protocol implies the study of eight VNTRs described by Hyytiä-Trees et al. (2010), some of which are also analyzed in the MLVAO157. Using that MLVA approach they were able to distinguish between sporadic cases and outbreaks, with higher discrimination than PFGE. Other authors who also applied MLVA for STEC typing obtained a higher number of MLVA than PFGE profiles and observed that MLVA was particularly useful to discriminate epidemiologically unrelated isolates (Keys et al., 2005; Hyytiä-Trees et al., 2010; Izumiya et al., 2010; Konno et al., 2011).

MLVAc worked well with the majority of STEC serotypes. However, in the case of some serotypes it was not possible to discriminate enough and, in consequence, this method could be

| Table 1 | Alleles detected by MLVAc: distribution by serotype and locus. |
|---|---|
| **Serotypes** | **Loci** |
| O8:H19 | CVN001 CVN002 CVN003 CVN004 CVN007 CVN014 CVN015 |
| O20:H19 | 7, 9 1 NA 12 6 9, 10, 11, 12 5 |
| O112:H2 | 7, 9 1 NA 12 6 5, 6, 7 5 |
| O113:H21 | 7, 9 1 NA 9, 12 NA, 6 5, 6, 7, 9, 10 NA, 5 |
| O113:NM | 7, 9 1 NA 12 6 5, 6, 7 NA, 5 |
| O117:H7 | 7, 9 1 NA 12 6 6, 8, 10, 11 5 |
| O117:NT | 5, 2 1 NA 9 8 NA 6 |
| O145:NM | 8, 1 2 12 6 3 5 |
| O157:H7 | 7, 9, 10, 11, 12, 13, 14, 15 5 |
| O171:H2 | 1, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18 |
| O171:NT | 8, 1 1 NA 12 6 7 5 |
| O171:NM | 5, 1 1 NA 12 6 7 5 |
| O174:H21 | 6, 7, 8, 9 1 NA 12 6 5, 7, 8, 9, 11, 12, 14, 15, 16, 18 |
| O178:H19 | 7, 9, 11, 12, 13, 14, 15 5 |
| ONT:H7 | 7, 9, 11, 12, 13, 14, 15 5 |
| ONT:H19 | 9, 1 1 NA 12 6 5 5 |
| ONT:H21 | 7 NA NA 12 6 8, 11 5 |
| ONT:NT | 7, 9 1 NA 12 6 5, 7, 10 5 |

NA, null allele.
improved by incorporating more loci. Recently, Labenski et al. (2012) improved that method by adding three new repeat-loci to a total of 10. They applied it and observed a considerable increase in resolution, of 71%, using the three new loci. Now, we are in process of adapting this proposed method in our laboratory and using it to subtype STEC. Regarding O157:H7 serotype, both MLVA protocols allowed to find high genetic diversity. In addition, they showed variability in all the VNTR loci analyzed in the MLVA O157. This protocol was the one that better reflected the epidemiological relationship among the isolates.

CONCLUDING REMARKS

In our experience, MLVA works well at our laboratory and enables us to perform an efficient O157:H7 and non-O157 STEC subtyping. The obtained results showed a high genetic diversity in the analyzed STEC isolates. The approach also allowed us to establish possible associations between MLVA genotypes and parameters such as source and virulence characteristics. The diversity found in many serotypes may be useful for future epidemiological studies of STEC strains, of both O157 as well as non-O157 serogroups.

ACKNOWLEDGMENTS

This work was supported by grants from CONICET, FONCYT, and SECAT-UNICEN. The authors thank M. R. Ortiz for her technical assistance. Ana V. Bustamante, Andrea M. Sanso, and Paula M. A. Lucchesi are members of the Research Career of CONICET.

REFERENCES

Bustamante, A. V., Lucchesi, P. M. A., and Parma, A. E. (2009a). Molecular characterization of verocytotoxigenic Escherichia coli O157:H7 isolates from Argentina by multiple-locus VNTR analysis (MLVA). Braz. J. Microbiol. 40, 927–932.

Bustamante, A. V., Sanso, A. M., Fernández, D., Padola, N. L., Lucchesi, P. M. A., and Parma, A. E. (2009b). “Genetic diversity of verocytotoxigenic Escherichia coli O178:H19 isolated from dairy farms in Argentina” in 7th International Symposium on Shiga Toxin-Producing Escherichia coli Infections (VTEC2009), Buenos Aires (Abstract 72).

Bustamante, A. V., Sanso, A. M., Lucchesi, P. M. A., and Parma, A. E. (2010). Genetic diversity of O157:H7 and non-O157 verocytotoxigenic Escherichia coli from Argentina inferred by multiple-locus variable-number tandem repeat analysis (MLVA). Rev. Argent. Microbiol. 42(Suppl. 1), 194–195.

Franci, T., Sanso, A. M., Bustamante, A. V., Lucchesi, P. M. A., and Parma, A. E. (2011). Genetic characterization of non-O157 verocytotoxigenic Escherichia coli isolated from raw beef products using multiple-locus variable-number tandem repeat analysis (MLVA). Folia Histochem. Cytobiol. 49, 1019–1023.

Gorgé, O., Lopez, S., Hilaire, V., Lisanti, O., Ramisse, V., and Vergnaud, G. (2008). Selection and validation of a multilocus variable-number tandem repeat analysis panel for typing Shigella spp. J. Clin. Microbiol. 46, 1026–1035.

Franci, T., Sanso, A. M., Bustamante, A. V., Lucchesi, P. M. A., and Parma, A. E. (2009a). Genetic diversity of verocytotoxigenic Escherichia coli O157:H7 isolated from dairy farms in Argentina by multiple-locus VNTR analysis (MLVA). Braz. J. Microbiol. 40, 927–932.
multiple-locus variable-number tandem repeat analysis protocol for shiga toxin-producing Escherichia coli O157: a novel approach to normalize fragment size data between capillary electrophoresis platforms. Foodborne Pathog. Dis. 7, 129–136.
Hyrýl-Tiipe, E., Smole, S. C., Field, P. A., Swanmanathan, B., and Bütler, E. M. (2006). Second generation subtyping of a proposed PulseNet protocol for multiple locus variable-number tandem repeat analysis of shiga toxin-producing Escherichia coli O157 (STEC). Foodborne Pathog. Dis. 3, 118–131.
Imamura, H., Poi, Y., Terajima, J., Ohnuki, M., Hayashi, T., Joda, S., and Watanabe, H. (2010). New systems for multiple variable-number tandem-repeat analysis of the enterohemorrhagic Escherichia coli strains belonging to three major serogroups: O157, O26, and O111. Microbiol. Immunol. 54, 569–577.
Johnson, K. E., Thorpe, C. M., and Sears, C. L. (2006). The emerging clinical importance of non-O157 Shiga toxin-producing Escherichia coli. Clin. Infect. Dis. 43, 1387–1399.
Keys, C., Kompey, S., and Keim, P. (2005). Highly diverse variable number tandem repeat loci in the E. coli O157:H7 and O111:H11 genomes for high-resolution molecular typing. J. Appl. Microbiol. 98, 926–940.
Kornos, T., Yatazunagenth, J., and Sains, S. (2011). Application of a multilocus variable number of tandem repeats analysis to regional outbreak surveillance of enterohemorrhagic Escherichia coli O157:H7 infections. Jpn. J. Infect. Dis. 64, 65–69.
Lindahl, B. A., Brandal, L. T., Aan, L., Vardal, T., and Kapperud, G. (2007). Study of polymorphic variable number of tandem repeats lost in the ECOR collection and in a set of pathogenic Escherichia coli and Shigella isolates for use in a genotyping assay. J. Microbiol. Methods 69, 197–205.
Lindahl, B. A., Heir, E., Gjemen, E., Vardal, T., and Kapperud, G. (2005). DNA fingerprinting of Shiga-toxin producing E. coli O157 based on multiple locus variable-number tandem repeat analysis (MIXA). Ann. Clin. Microbiol. Antimicrob. 4, 12.
Labediri, I., Haugum, K., and Lindahl, B. A. (2012). Rapid and high resolution genotyping of all E. coli Shiga-toxin producing serotypes using 10 genomic repeat-containing loci. J. Microbiol. Methods 89, 154–159.
Nolle, A. C., McEllistrem, M. C., Shutt, K. A., and Harrison, L. H. (2006). Locus-specific mutational events in a multilocus variable-number tandem repeat analysis of Escherichia coli O157:H7. J. Clin. Microbiol. 44, 576–577.
Padula, N. L., Sains, M. E., Blancs, J., Blanco, M., Blancs, J., Echavarria, A. I., Arroyo, G. H., Ureña, M. A., and Parma, A. E. (2004). Serotyping and virulence gene of bovine Shiga-toxinigenic Escherichia coli (STEC) isolated from a feedlot in Argentina. Vet. Microbiol. 100, 5–9.
Parma, A. E., Sains, M. E., Blancs, J., Blanco, M., Vitan, M. R., Blancs, M., Padula, N. L., and Echavarria, A. I. (2000). Virulence genes and serotypes of verotoxigenic Escherichia coli isolated from cattle and foods in Argentina. Importance in public health. Eur. J. Pediatr. 160, 57–62.
Powers, M., Bustamante, A. V., Santos, A. M., and Parma, A. E. (2001). "Estudio de la diversidad genética de Escherichia coli O157:H7 aisladas de niños con diarrea aguda de la infancia mediante el método de MIXA," in XIII Jornadas Argentinas de Microbiologia. Rosario. Buenos Aires, 195.
Powers, M. A., Ponsacco, J. A., Rodriguez, E. M., and Parma, A. E. (2010). Role and clinical course of verotoxigenic Escherichia coli infections in childhood acute diarrhea in Argentina. J. Med. Microbiol. 59, 345–352.
Sant, M. E., Villablloso, C., Elizaldebuky, E., and Arroyo, G. H. (2007). Prenatal detection of Escherichia coli non-entero- toxigenic in products lácticos de la ciudad de Tandil. La Ind. Cárnea Lat. 146, 56–58.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 March 2012; accepted: 02 August 2012; published online: 22 August 2012.
Citation: Bustamante, A. V., Sanso, A. M., Parma, A. E. and Lucchesi, P. M. (2012). Subtyping of STEC by MLVA in Argentina. Front. Cell. Inf. Microbiol. 2:111. doi: 10.3389/fcell.2012.00111
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