Epidemiology and Infection

Genetically diverse Shiga toxin-producing *Escherichia coli* in cattle can be categorized into several potentially virulent subgroups

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**Supplementary materials**

**TABLE S1** Questionnaire for cattle feces sampling used for epidemiological analyses

|  |  |
| --- | --- |
| Question | Answers |
| Farm level |  |
|  | No. of cattle |  |
|  | Farm type | Breeding/Fattening/Breeding & Fattening/Milking/Other |
|  | Type of breed | Japanese black/Japanese brown/Holstein/Jersey/F1/Other |
|  | Housing type | Pen/Tie stall/Free stall/Free barn/Pasturing |
|  | Bedding | Sawdust/Straw/Wood chip/Manure/Sand |
|  | Frequency of bedding exchange |  |
|  | Ventilation | Natural/Mechanical |
|  | Presence of animals in the same farm | Pig/Dog/Cat/Horse/Other |
|  | Use of probiotics | Yes/No |
|  | Type of feedbox | Flat/Indent/Box/Other |
|  | Watering equipment | Water cup/Fountain/Other |
|  | Boot exchange/washing in entering feed storage | Yes/No |
|  | Fence around the farm | Yes/No |
|  | Disinfection of vehicles before entering the farm | Yes/No |
|  | Change of working clothes everyday | Yes/No |
|  | Washing of cattle hide upon cattle's entering the farm | Yes/No |
|  | Cleaning of the cattle house after shipment to slaughterhouse | Yes/No |
| Sample level |  |
|  | Sex |  |
|  | Age |  |
| 　 | Breed | 　 |

**TABLE S2** Primers used in this study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Target |  | Primer | Sequence | Product size | Product/Function | Reference |
| Shiga toxin |  |  |  |  | 　 |
|  | All *stx1* subtypes | stx1-det-F1 | GTACGGGGATGCAGATAAATCGC | 209 | Shiga toxin 1 | 1 |
|  |  | stx1-det-R1　 | AGCAGTCATTACATAAGAACGYCCACT |  |  |
|  | All *stx2* subtypes | stx2-det-F4　 | GGCACTGTCTGAAACTGCTCCTGT | 627 or 625 | Shiga toxin 2 | 1 |
|  |  | stx2-det-R1　 | ATTAAACTGCACTTCAGCAAATCC |  |  |
|  |  | stx2-det-F4-f　 | CGCTGTCTGAGGCATCTCCGCT |  |  |
|  |  | stx2-det-R1-e/f | TAAACTTCACCTGGGCAAAGCC |  |  |
|  | *stx1a* | stx1a-F1　 | CCTTTCCAGGTACAACAGCGGTT | 478 |  | 1 |
|  |  | stx1a-R2　 | GGAAACTCATCAGATGCCATTCTGG |  |  |
|  | *stx1c* | stx1c-F1　 | CCTTTCCTGGTACAACTGCGGTT | 252 |  | 1 |
|  |  | stx1c-R1　 | CAAGTGTTGTACGAAATCCCCTCTGA |  |  |
|  | *stx1d* | stx1d-F1　 | CAGTTAATGCGATTGCTAAGGAGTTTACC | 203 |  | 1 |
|  |  | stx1d-R1　 | CTCTTCCTCTGGTTCTAACCCCATGATA |  |  |
|  | *stx2a* | stx2a-F2　 | GCGATACTGRGBACTGTGGCC | 349 or 347 |  | 1 |
|  |  | stx2a-R3　 | CCGKCAACCTTCACTGTAAATGTG |  |  |
|  |  | stx2a-R2　 | GGCCACCTTCACTGTGAATGTG |  |  |
|  | *stx2b* | stx2b-F1　 | AAATATGAAGAAGATATTTGTAGCGGC | 251 |  | 1 |
|  |  | stx2b-R1　 | CAGCAAATCCTGAACCTGACG |  |  |
|  | *stx2c* | stx2c-F1　 | GAAAGTCACAGTTTTTATATACAACGGGTA | 177 |  | 1 |
|  |  | stx2c-R2　 | CCGGCCACYTTTACTGTGAATGTA |  |  |
|  | *stx2d* | stx2d-F1　 | AAARTCACAGTCTTTATATACAACGGGTG | 179 and/or 235 |  | 1 |
|  |  | stx2d-R1　 | TTYCCGGCCACTTTTACTGTG |  |  |
|  |  | stx2d-R2　 | GCCTGATGCACAGGTACTGGAC |  |  |
|  | *stx2e* | stx2e-F1　 | CGGAGTATCGGGGAGAGGC | 411 |  | 1 |
|  |  | stx2e-R2　 | CTTCCTGACACCTTCACAGTAAAGGT |  |  |
|  | *stx2f* | stx2f-F1　 | TGGGCGTCATTCACTGGTTG | 424 |  | 1 |
|  |  | stx2f-R1　 | TAATGGCCGCCCTGTCTCC |  |  |
|  | *stx2g* | stx2g-F1　 | CACCGGGTAGTTATATTTCTGTGGATATC | 573 |  | 1 |
|  |  | stx2g-R1　 | GATGGCAATTCAGAATAACCGCT |  |  |
| Adhesin |  |  |  |  |  |
|  | *eae* | 4Meae-F | GACCCGGCACAAGCATAAGC | 384 | Intimin | 2 |
|  |  | 4Meae-R | CCACCTGCAGCAACAAGAGG |  |  |
|  | *eae* subtype | EaeVF  | AGYATTACTGAGATTAAG |  |  | 3 |
|  |  | EaeVR  | AAATTATTYTACACARAY |  |  |  |
|  | *saa* | SAADF　 | CGTGATGAACAGGCTATTGC | 119 | STEC autoagglutinating adhesin | 4 |
|  |  | SAADR　 | ATGGACATGCCTGTGGCAAC |  |  |  |
|  | *bfpA* | bfpA\_114F　 | GTCTGCGTCTGATTCCAATA | 408 - 414 | Bundle forming pilus | 5 |
|  |  | bfpA\_521R　 | TCAGCAGGAGTAATAGC |  |  |  |
|  | *aggR* | aggR-F　 | CTAATTGTACAATCGATGTA | 308 | Transcriptional activator of aggregative adherence fimbriae | 6 |
|  |  | aggR-R　 | AGAGTCCATCTCTTTGATAAG |  |  |
|  | *lpfA*O113 | lpfAO113-F | ATGAAGCGTAATATTATAG | 573 | Long polar fimbriae | 7 |
|  |  | lpfAO113-R | TTATTTCTTATATTCGAC |  |  |  |
|  | *lpfA*O157/OI-141 | lpfA1-F | CTGCGCATTGCCGTAAC | 412 | Long polar fimbriae | 8 |
|  |  | lpfA1-R | ATTTACAGGCGAGATCGTG |  |  |  |
|  | *f5* | F5-P1 | TGCGACTACCAATGCTTCTG | 450 | Fimbriae: F5 | 9 |
|  |  | F5-P2 | TATCCACCATTAGACGGAGC |  |  |  |
|  | *f17* | F17-P1 | GCAGAAAATTCAATTTATCCTTGG | 537 | Fimbriae: F17 | 10 |
|  |  | F17-P2 | CTGATAAGCGATGGTGTAATTAAC |  |  |  |
|  | *f41* | F41-P1 | GAGGGACTTTCATCTTTTAG | 431 | Fimbriae: F41 | 9 |
|  |  | F41-P2 | AGTCCATTCCATTTATAGGC |  |  |  |
|  | *fedA* | FedA 1 | GTGAAAAGACTAGTGTTTATTTC | 510 | Fimbriae: F18 | 11 |
|  |  | FedA 2 | CTTGTAAGTAACCGCGTAAGC |  |  |  |
|  | *clpG* | clpG1 | GGGCGCTCTCTCCTTCAAC | 385 | Afimbrial adhesin: CS31A | 12 |
|  |  | clpG2 | CGCCCTAATTGCTGGCGAC |  |  |  |
| Plasmid-encoded virulence marker |  |  |  |  |
|  | *ehxA* | hlyAF | AATGAGCCAAGCTGGTTAAGCT | 534 | Hemolysin | 2 |
|  |  | hlyAR | GCATCATCAAGCGTACGTTCC |  |  |  |
|  | *stcE* | stcE Fw | GGCTCCGGAGGTGGGGGAAT | 399 | Metalloprotease | 13 |
|  |  | stcE Rv | GAAGCCGGTGGAGGAACGGC |  |  |  |
|  | *katP* | katP Fw | GCGCCAGTGGTGGTCAGCAA | 914 | Catalase-peroxidase | 13 |
|  |  | katP Rv | ATATCGGGCTGCCGGTCCCA |  |  |  |
| Pathogenicity islands |  |  |  |  |  |
|  | *terC* | TerC1 | TCCTGGCGCTGAAAGAT | 1240 | Tellurite resistance (on OI-43/48) | 14 |
|  |  | TerC2 | GAAACACTCATAAAATAACCTCTT |  |  |  |
|  | *espK* | espK-F | GTAGCGGACACTCTCTGG | 385 | Type III effector (on OI-50) | 15 |
|  |  | espK-R | GACATTCTGCTCCTATTCCG |  |  |  |
|  | *nleG2-3* | nleG-F | GGATGGAACCATACCTGG | 551 | Type III effector (on OI-57) | 16 |
|  |  | nleG-R | CGCAATCAATTGCTAATGC |  |  |  |
|  | *efa1* | efa1-F | AAGGTGTTACAGAGATTA | 266 | *E. coli* factor for adherence (on OI-122) | 17 |
|  |  | efa1-R | TGAGGCGGCAGGATAGTT |  |  |  |
|  | *ihaB*-*btuB* region | iha-B | CGTGATGGTGATAACAAAGG | 1325 | Core region of locus of proteolytic activity (LPA) | 18 |
|  |  | btuB-A | CGGAAAAGAGTAAACAGTGG |  |  |  |
|  | ms-1 | ms-1F | GCTGATGCGTTACCACACTG | 684 | Pathogenicity island CL3 (PAI ICL3) | 19 |
|  |  | ms-1R | GTAATCCTCAACCGCACCAG |  |  |  |
|  | *irp2* | irp2 FP | AAGGATTCGCTGTTACCGGAC | 280 | High pathogenicity island (HPI) | 20 |
|  |  | irp2 RP  | TCGTCGGGCAGCGTTTCTTCT |  |  |  |
| O-antigen gene |  |  |  |  |  |
|  | *rfb*O157 | rfbO157-F | CAGGTGAAGGTGGAATGGTTGTC | 296 | O157-specific region | 21 |
|  |  | rfbO157-R | TTAGAATTGAGACCATCCAATAAG |  |  |  |
|  | *wzx*O26 | wzxO26-F | GGGGGTGGGTACTATATTGG | 241 | O26-specific region | 21 |
| 　 |  | wzxO26-R | AGCGCCTATTTCAGCAAAGA |  |  | 　 |



**Fig. S1**. Delta K in each predefined number of clusters (K) by the Bayesian clustering.



**Fig. S2.** Antimicrobial resistant rate of STEC strains stratified by the Bayesian cluster. Abbreviations of antimicrobials were as follows: AMP, ampicillin; CHL, chloramphenicol; TET, tetracycline; STR, streptomycin; KAN, kanamycin; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid. All strains were susceptible to cefoxitin, cefotaxime, ciprofloxacin, fosfomycin and gentamicin.

**References for supplementary materials**

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