PREVALENCE OF ESCHERICHIA ALBERTII IN RACCOONS (PROCYON LOTOR), JAPAN

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Natural reservoirs of Escherichia albertii remain unclear. In this study, we detected E. albertii by PCR in 248 (57.7%) of 430 raccoons from Osaka, Japan, and isolated 143 E. albertii strains from the 62 PCR-positive samples. These data indicate that raccoons could be a natural reservoir of E. albertii in Japan.

Escherichia albertii is a gram-negative facultative anaerobic bacterium and an emerging human enteropathogen. This bacterium belongs to the group of attaching and effacing pathogens, which can form pedestal-structured lesions on intestinal epithelium by using an eae-encoded adhesin called intimin and a type 3 secretion system. E. albertii commonly carries cytolethal distending toxin genes; in addition, certain strains carry Shiga toxin 2 (stx2a, stx2f) genes (1), suggesting that E. albertii has a potential to cause severe diseases such as hemorrhagic colitis and hemolytic uremic syndrome in humans, similar to Shiga toxin-producing E. coli. An increase in human outbreaks and sporadic cases of E. albertii have been reported recently from several countries, including Japan (1–3). However, the reservoir and transmission routes of E. albertii to humans have not yet been identified. We surveyed wild raccoons (Procyon lotor) captured in Osaka, Japan, for the presence of E. albertii to determine if raccoons could be a reservoir of E. albertii in Japan.

The Study
We collected 430 rectal swabs from wild raccoons in Osaka during 2016–2017 (Appendix, https://wwwnc.cdc.gov/EID/article/26/6/19-1436-App1.pdf). To determine the presence of E. albertii, we first subjected fecal specimens to an E. albertii–specific cdlt (Eacdt) gene-based PCR assay (4) after enrichment in tryptic soy broth. Of these 430 specimens, 248 (57.7%) yielded a 449-bp PCR amplicon specific for E. albertii (Table 1). By using XRM-MacConkey agar developed for the isolation of E. albertii (Appendix), we isolated and selected 143 E. albertii isolates from the 62 PCR-positive specimens (1–8 isolates/sample) with species identity confirmed by 2 different E. albertii–specific PCRs using primers targeting Eacdt (4), and yejH and yejK (5).

To determine the phylogenetic relationships among the isolates, we performed pulsed-field gel electrophoresis (PFGE) using XbaI-digested genomic DNA. The 143 isolates showed 59 pulsotypes (Figure), indicating that E. albertii isolates from raccoons were genetically diverse. We obtained 2–7 E. albertii isolates, which were determined to be clonal by PFGE, from 26 of 29 raccoons. The isolates from each of 3 raccoons (R305, R318, R419) showed 2–3 different DNA fingerprints with >3 bands different from each other, indicating that multiclonal E. albertii strains coexisted in the intestine of each of these 3 raccoons (Figure). In addition, we frequently observed that the isolates from different raccoons displayed exactly the same PFGE pattern (e.g., R7, R8, and R335; Figure), although the raccoons were usually captured in different locations in Osaka.

To evaluate the human pathogenic potential of E. albertii isolated from raccoons, we selected 1 isolate from each pulsotype (n = 59) and tested for the presence of virulence determinants in clinical E. albertii isolates (Appendix). We detected the eae gene in 59 strains (100%), Eccdt-I in 5 strains (8.5%), and stx2f genes in 2 strains (3.4%). By sequencing the entire eae gene in 59 strains (Appendix), we determined the intimin subtypes to be ρ (n = 8), ρ2 (n = 5), o (n = 4), ρ3 (n = 4), γ5 (n = 2), ρ5 (n = 2), α8 (n = 1), β3 (n = 1), and unknown (n = 32) (Appendix Table 3). Among the 32 unknown subtypes, 16 were grouped into the 5 subtypes (N1–N5) that were recently identified in clinical E. albertii strains from Japan (6). Two subtypes were homologous to those identified in clinical E. albertii strains 1251–6/89, 2 were homologous to strain 4281–7/89 (7), and 3 were homologous to those identified in strain 2013C-4143 (GenBank accession no. CP030787). We also identified 2 novel subtypes (UT1 and UT2;
Table 1. Prevalence of *Escherichia albertii* in Japanese wild raccoon fecal specimens and number of isolates

| Sampling year and month | No. specimens | No. (%) PCR positive | No. specimens from which *E. albertii* was isolated | No. *E. albertii* isolates |
|-------------------------|---------------|----------------------|---------------------------------------------------|---------------------------|
| 2016                    |               |                      |                                                   |                           |
| Jun                     | 57            | 25 (43.9)            | 7                                                 | 17                        |
| Jul                     | 55            | 34 (61.8)            | 4                                                 | 8                         |
| Aug                     | 22            | 14 (63.6)            | 0                                                 | 0                         |
| Sep                     | 7             | 2 (28.6)             | 0                                                 | 0                         |
| Oct                     | 8             | 3 (37.5)             | 0                                                 | 0                         |
| Dec                     | 14            | 7 (50.0)             | 0                                                 | 0                         |
| 2017                    |               |                      |                                                   |                           |
| Feb                     | 3             | 2 (66.7)             | 0                                                 | 0                         |
| Mar                     | 16            | 3 (18.8)             | 0                                                 | 0                         |
| Jul                     | 88            | 56 (63.6)            | 14                                                | 21                        |
| Aug                     | 104           | 63 (60.6)            | 21                                                | 56                        |
| Sep                     | 56            | 39 (69.6)            | 16                                                | 41                        |
| Total                   | 430           | 248 (57.7)           | 62                                                | 143                       |

**Figure.** Phylogenetic analysis of raccoon *Escherichia albertii* strains by pulsed-field gel electrophoresis (PFGE). XbaI-digested genomic DNA of 143 raccoon *E. albertii* strains isolated in this study were analyzed by PFGE. The dendrogram was constructed based on DNA fingerprints obtained (Appendix, https://wwwnc.cdc.gov/EID/article/26/6/19-1436-App1.pdf). The number in each strain name represents a specific raccoon identification number.
Appendix Table 3) in the remaining 9 strains; each showed <95% nt and aa identities with any known subtypes. We identified complete stx2f genes in the stx2f gene-positive strains RAC-199 and RAC-247. The culture supernatants caused Vero cell deaths, which were neutralized by anti-Stx2fA serum, indicating that both strains produced biologically active Stx2f. The toxin activity was enhanced in the presence of mitomycin C, indicating that the stx2f genes could be located on inducible prophage genomes (Table 2). The fold change of Stx2f production by mitomycin C in the strains RAC-199 and RAC-247 were comparable to that of Stx2 production in human clinical strains E. albertii AKT5 and EHEC O157:H7 Sakai. These data suggest that the E. albertii strains isolated from raccoons have a potential to cause serious human diseases.

Conclusions

E. albertii is known to be an emerging zoonotic pathogen and has been isolated from various animals, such as pigs, cats, and birds (6,8,9). Although much effort has been devoted to identify the natural reservoir, E. albertii was not detected in vertebrate animals such as fish (n = 138), amphibians (n = 106), reptiles (n = 447), and mammals (n = 1,063) (3) but was found in 1.4% (9/634) of birds in Australia and 0.9% (9/1,204) of birds in Korea. Thus, the natural reservoir of E. albertii is still unclear; this information would be essential to determine transmission dynamics and prevent E. albertii infections. Given that patients in clinical outbreaks in Japan might be infected thorough waters (spring and well waters) or vegetables, but not meats (3), the natural reservoir of E. albertii might not be major food animals (e.g., cattle and chickens, the reservoirs for Shiga toxin-producing E. coli and Campylobacter jejuni, respectively). Another possibility is wild animals, which may contaminate environmental water and vegetables. Among the wild animals, the raccoon is a synanthropic animal with the ability to reside in a wide range of habitats, including agricultural, forested, and urban areas. Raccoons are omnivorous and forage within vegetable fields. They also prefer riparian environments. Furthermore, raccoons are known to carry various pathogenic microorganisms (10–12). Thus, they can contaminate vegetables and waters with pathogens, possibly including E. albertii, leading to human infections. Therefore, we performed a survey targeting raccoons and found that E. albertii was highly prevalent (248/430; 57.7%) in wild raccoons in Japan, indicating that carriage of E. albertii by raccoons is not incidental. The E. albertii strains isolated from raccoons also possessed virulence determinants (eae, Eacdt, Eccdt-I, or stx2f) present in human clinical strains. Almost all the intimin subtypes of the raccoon strains were those identified in human clinical E. albertii strains. Two strains produced functional Stx2f, which may have a potential to cause severe diseases in humans. Taken together, these data suggested that raccoons constitute a major reservoir of E. albertii and could be a source of human infection in Japan.

Raccoons originated from North America and were introduced as pets or game animals into other countries, including Japan and countries in Europe. Some of these have escaped and settled in the wild. The number of raccoons has increased because of their adaptability to various environments, omnivorous feeding habits, high reproductive potential, and lack of predators in the environment (13).

In addition to Japan, E. albertii has been clinically isolated in other countries where raccoons reside (9,14,15). Interactions between raccoons and other animals, such as wild mice and wild boars, can also be possible. Therefore, further epidemiologic studies to survey raccoons and other wild animals in Osaka, other areas of Japan, and other countries are highly warranted to evaluate the significance of raccoons as a natural reservoir of E. albertii.

| Table 2. Stx2 production by stx2f gene-positive Escherichia albertii raccoon strains. |
|-----------------|-----------------|-----------------|-----------------|
| Species         | Strains         | Toxin gene      | Mitomycin C     | Toxin titer*   | Neutralization† |
| E. albertii     | RAC199          | stx2f           | Negative        | 4              | Yes            |
|                 | RAC247          | stx2f           | Positive        | 512            | Yes            |
|                 | AKT5            | stx2f           | Negative        | 32             | Yes            |
|                 |                 |                 | Positive        | 1,024          | Yes            |
|                 |                 |                 | Negative        | 512            | Yes            |
|                 |                 |                 | Positive        | 32,768         | Yes            |
| E. coli         | Sakai           | stx1, stx2a     | Negative        | 256            | Not done       |
|                 | C600            | None            | Positive        | 16,384         | Not done       |
|                 |                 |                 | Negative        | <1             | Not done       |
|                 |                 |                 | Positive        | <1             | Not done       |

*Reciprocal of highest dilution that resulted in death in >50% of cells is shown as toxin titer.
†Neutralization of cytotoxic effect by anti-Stx2f rabbit serum. Filtered culture supernatants in LB-broth with and without mitomycin C were used as toxin samples.
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Prevalence of *Escherichia albertii* in Raccoons (*Procyon lotor*), Japan

Appendix

**Sample Collection**

We collected rectal specimens using cotton swabs (SEEDSWAB γ1, Eiken Chemical Co., http://www.eiken.co.jp) from 430 wild raccoons (*Procyon lotor*) in Osaka, Japan, during June 2016–March 2017 (n = 182) and July–September 2017 (n = 248). All the raccoons seemed to be asymptomatic. Raccoons were captured to exterminate them throughout the year in Osaka. The samples were transported to the laboratory at ambient temperature and processed within 6 hours of collection. Fecal sampling in the present study was approved by Osaka Prefectural Government and performed according to the Guidelines for Animal Experimentation of Osaka Prefectural Animal Protection and Livestock Division.

**Detection of Eacdt Genes by PCR**

We suspended rectal swabs in 1 mL of sterilized Dulbecco’s phosphate-buffered saline (PBS). An aliquot (300 µL) of the suspension was inoculated into 3 mL of tryptic soy broth (Becton Dickinson, www.bd.com), and enriched them at 37°C for 14–16 h with shaking. We centrifuged 100 µL of the culture at 10,000 g at 4°C for 3 min. We suspended the resulting pellet in 85 µL of 50 mM NaOH, boiled it at 100°C for 10 min, and neutralized it by adding 15 µL of 1 M Tris-HCl buffer (pH 7.0). After centrifugation at 10,000 g at 4°C for 10 min, we subjected the supernatant to PCR analysis using a pair of *E. albertii* specific primers targeting *Eacdt* genes (Appendix Table 1).

**Isolation and identification of E. albertii**

We serially diluted the swab suspensions from PCR-positive specimens in PBS and spread 100 µL of each dilution on XRM-MacConkey agar, an *E. albertii*-selective medium ([1]), with composition of MacConkey agar base (Becton Dickinson) supplemented with 1% (w/v) each of xylose, rhamnose, and melibiose, and incubated them at 37°C for 20–24 hours. We examined colorless colonies (maximum 8 colonies) on the medium, which are typical feature of *E. albertii*, by PCR, targeting *Eacdt* genes. *Eacdt* gene-positive colonies were determined to be *E. albertii* by another *E. albertii*-specific PCR assay using a primer pair targeting *yejH* and *yejK* in *E. albertii*, which was developed by Ooka et al. ([2]).
Detection of Virulence Genes

We analyzed the presence of virulence genes by colony hybridization assay using $^{32}$P-labeled DNA probes targeting \textit{eae}, \textit{stx1}, \textit{stx2a}, \textit{stx2f}, \textit{Eccdt}-\text{IB}, and \textit{Eccdt}-\text{IVB} under high stringent conditions, as described previously (3). When \textit{stx2} and \textit{cdt} genes were detected by the colony hybridization assay, subtype-specific PCRs for \textit{stx2} (4), \textit{Eccdt}-I and \textit{Eccdt}-IV were carried out to determine their subtypes (Appendix Table 1). The entire nucleotide sequence of \textit{stx2f} genes was determined as described previously (5). PCR amplification was done by Veriti Thermal cycler (Thermo Fisher Scientific, https://www.thermofisher.com) using TaKaRa Taq DNA polymerase (Takara Bio, https://www.takarabio.com). We sequenced the PCR products by cycle sequencing method using BigDye Terminator v1.1 and ABI 3130 Genetic Analyzer (Thermo Fisher Scientific).

To determine each intimin subtype, we determined the entire \textit{eae} nucleotide sequence, as previously described (5). Predicted amino acid sequences of \textit{eae} genes were aligned with those of the reference intimin subtypes by the Clustal W program of MEGA6 (https://www.megasoftware.net). The reference intimin subtypes used were from Hinenoya et al. (5). If intimin subtypes of \textit{E. albertii} raccoon strains were determined to be untypable, the putative amino acid sequences were subjected to BLAST homology search using the tblastn module (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Pulsed-Field Gel Electrophoresis (PFGE)

We performed PFGE as described previously (6). Briefly, fresh bacterial cells were embedded in agarose plug and in situ lysis was carried out to isolate total genomic DNA. The genomic DNA embedded plug was subjected to restriction enzyme digestion with 30 U of \textit{XbaI} (Takara Bio), and electrophoretic separation of the DNA fragments was done in 1% pulsed-field certified agarose (Bio-Rad Laboratories, https://www.bio-rad.com) on a CHEF Mapper PFGE (Bio-Rad) using 0.5x TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA [pH 8.0]). Run conditions were generated by the autoalgorithm mode of the CHEF Mapper PFGE system for the sizes ranging between 20 and 300 kb, and the running time was 26.93 hours. \textit{XbaI}-digested genomic DNA of \textit{Salmonella} Braenderup strain H98121 was used as a molecular size marker. DNA fingerprints of \textit{E. albertii} strains were interpreted based on Tenover's criteria (7) and analyzed using Fingerprinting II Software (Bio-Rad) to know their phylogenetic relationships.

Detection of Stx2f Production in \textit{stx2f} Gene-Positive \textit{E. albertii}

Production of Stx2f by \textit{stx2f} gene-positive \textit{E. albertii} strains was determined by Vero cells cytotoxicity assay, as previously described (3). We prepared crude toxin samples as follows: \textit{E. albertii} was cultured in 3 mL of lysogenic broth (LB broth, Becton Dickinson) at 37°C for 14 hours. An aliquot of the culture was inoculated into 3 mL of fresh lysogenic broth
and cultured until early log phase (≈0.2 optical density at 600 nm). Mitomycin C (Kyowa Hakko Kirin, https://www.kyowakirin.com) was added to the culture at the final concentration of 0.5 µg/mL and further incubated at 37°C for 4 hours aerobically. Culture supernatant was passed through a sterile filter with 0.22-µm pore size (Merck Millipore, https://www.emdmillipore.com), and the filtrate was subjected to cytotoxicity assay. Neutralization assay of the toxin activity was also carried out using anti-Stx2fA rabbit serum (8), which was preincubated with crude toxin samples at 37°C for 30 min. The mixture was applied to the cytotoxicity assay.

Nucleotide Sequence Accession Numbers

All nucleotide sequences obtained in this study were registered into the DNA Data Bank of Japan database. The accession numbers are LC504574–LC504632 (for eae genes) and LC504633–LC504634 (for stx2f genes).

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**Appendix Table 1.** PCR primers for detection of *E. albertii*, and differentiating *Eccdt*-I and *Eccdt*-IV genes; PCR conditions (30 cycles).

| Target gene | Primer name | Sequence (5'-3') | PCR conditions (30 cycles) | Amplicon size (bp) | Reference |
|-------------|-------------|------------------|----------------------------|--------------------|-----------|
| *Eacdt*     | *EaCDT*sp-F2 | GCTTTAACTGGATGATTCTTG | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 449 | (9) |
| *Eacdt*-IA  | *EcCDT*1A-F | GAT GGG TGA TCC ACC TTC | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 499 | This study |
|             | *EcCDT*1A-R | TTT CTC AAG GGT GAT TGT AA | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 640 | This study |
| *Eacdt*-IB  | *EcCDT*1B-F | GAT TTT GCC GGG TAT TTC T | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 540 | This study |
|             | *EcCDT*1B-R | TCA AGA ACA CCA CCA CTA TG | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 540 | This study |
| *Eacdt*-IC  | *EcCDT*1C-F | TAC TGC TGA CAG GTT GTG | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 427 | This study (10) |
|             | *EcCDT*1C-R | CAG CTC GTT AAT GGA GAC | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 427 | This study (10) |
| *Eacdt*-IVA | *EcCDT*4A-F | TCT CCA ACA TTT GGG AG | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 286 | This study |
|             | *EcCDT*4A-R | GCT CCA GAA TCT ATA CCT | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 286 | This study |
| *Eacdt*-IVB | *EcCDT*4B-F | ACC ATC TTC ACG TAC ACT A | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 202 | This study |
|             | *EcCDT*4B-R | GTA AAT AAT GCA TTG CGA TTG | 94°C, 30 sec 50°C, 30 sec 72°C, 60 sec | 202 | This study |
Appendix Table 2. Information about raccoons from which *Escherichia albertii* strains were isolated.

| Strain   | Racoon ID | City captured* | Month of sampling |
|----------|-----------|----------------|-------------------|
| RAC-7A   | R7        | A              | 2016 Jun          |
| RAC-7B   |           |                |                   |
| RAC-8    | R8        | B              | 2016 Jun          |
| RAC-30   | R30       | C              | 2016 Jun          |
| RAC-33A  | R33       | B              | 2016 Jun          |
| RAC-33B  |           |                |                   |
| RAC-33C  | R34       | B              | 2016 Jun          |
| RAC-34A  |           |                |                   |
| RAC-34B  |           |                |                   |
| RAC-34C  |           |                |                   |
| RAC-34D  |           |                |                   |
| RAC-34E  |           |                |                   |
| RAC-44A  | R44       | B              | 2016 Jun          |
| RAC-44B  |           |                |                   |
| RAC-44C  |           |                |                   |
| RAC-44D  |           |                |                   |
| RAC-58   | R58       | D              | 2016 Jun          |
| RAC-81   | R81       | A              | 2016 Jul          |
| RAC-112  | R112      | E              | 2016 Jun          |
| RAC-116A | R116      | A              | 2016 Jul          |
| RAC-116B |           |                |                   |
| RAC-118A | R118      | B              | 2016 Jul          |
| RAC-118B |           |                |                   |
| RAC-118C |           |                |                   |
| RAC-118D |           |                |                   |
| RAC-199  | R199      | F              | 2017 Jul          |
| RAC-243  | R243      | G              | 2017 Jul          |
| RAC-244  | R244      | C              | 2017 Jul          |
| RAC-245  | R245      | C              | 2017 Jul          |
| RAC-247  | R247      | H              | 2017 Jul          |
| RAC-258  | R258      | A              | 2017 Jul          |
| RAC-261  | R261      | I              | 2017 Jul          |
| RAC-262A | R262      | J              | 2017 Jul          |
| RAC-262B |           |                |                   |
| RAC-262C |           |                |                   |
| RAC-262D |           |                |                   |
| RAC-263  | R263      | D              | 2017 Jul          |
| RAC-264A | R264      | H              | 2017 Jul          |
| RAC-264B |           |                |                   |
| RAC-264C |           |                |                   |
| RAC-264D |           |                |                   |
| RAC-264E |           |                |                   |
| RAC-264F |           |                |                   |
| RAC-264G |           |                |                   |
| RAC-264H |           |                |                   |
| RAC-266  | R266      | C              | 2017 Jul          |
| RAC-274A | R274      | F              | 2017 Jul          |
| RAC-274B |           |                |                   |
| RAC-274C |           |                |                   |
| RAC-274D |           |                |                   |
| RAC-274E |           |                |                   |
| RAC-278A | R278      | G              | 2017 Jul          |
| RAC-278B |           |                |                   |
| RAC-278C |           |                |                   |
| RAC-278D |           |                |                   |
| RAC-278E |           |                |                   |
| RAC-278F |           |                |                   |
| RAC-278G |           |                |                   |
| RAC-281  | R281      | C              | 2017 Jul          |
| RAC-300  | R300      | K              | 2017 Aug          |
| RAC-302A | R302      | L              | 2017 Aug          |
| RAC-302B |           |                |                   |
| RAC-303A | R303      | E              | 2017 Aug          |
| RAC-303B |           |                |                   |
| RAC-305A | R305      | K              | 2017 Aug          |
| RAC-305B |           |                |                   |
| RAC-306A | R306      | K              | 2017 Aug          |
| RAC-306B |           |                |                   |
| RAC-306C |           |                |                   |
| RAC-306D |           |                |                   |
| RAC-306E |           |                |                   |
| RAC-306F |           |                |                   |
| RAC-310A | R310      | G              | 2017 Aug          |
| RAC-310B |           |                |                   |
| RAC-313  | R313      | G              | 2017 Aug          |
| RAC-341  |           |                |                   |
| Strain     | Racoon ID | City captured* | Month of sampling |
|------------|-----------|----------------|-------------------|
| RAC-318A   | R318      | M              | 2017 Aug          |
| RAC-318B   |           |                |                   |
| RAC-318C   |           |                |                   |
| RAC-318D   |           |                |                   |
| RAC-318E   |           |                |                   |
| RAC-318F   |           |                |                   |
| RAC-324    | R324      | C              | 2017 Aug          |
| RAC-333A   | R333      | J              | 2017 Aug          |
| RAC-333B   |           |                |                   |
| RAC-334A   | R334      | F              | 2017 Aug          |
| RAC-334B   |           |                |                   |
| RAC-335    | R335      | F              | 2017 Aug          |
| RAC-336    | R336      | D              | 2017 Aug          |
| RAC-337    | R337      | D              | 2017 Aug          |
| RAC-342    | R342      | G              | 2017 Aug          |
| RAC-349    | R349      | N              | 2017 Aug          |
| RAC-351A   | R351      | A              | 2017 Aug          |
| RAC-351B   |           |                |                   |
| RAC-351C   |           |                |                   |
| RAC-351D   |           |                |                   |
| RAC-355    | R355      | A              | 2017 Aug          |
| RAC-357A   | R357      | M              | 2017 Aug          |
| RAC-357B   |           |                |                   |
| RAC-359A   | R359      | C              | 2017 Aug          |
| RAC-359B   |           |                |                   |
| RAC-365    | R365      | H              | 2017 Aug          |
| RAC-376    | R376      | D              | 2017 Aug          |
| RAC-381A   | R381      | K              | 2017 Sep          |
| RAC-381B   |           |                |                   |
| RAC-381C   |           |                |                   |
| RAC-382    | R382      | O              | 2017 Sep          |
| RAC-386    | R386      | P              | 2017 Sep          |
| RAC-393A   | R393      | B              | 2017 Sep          |
| RAC-393B   |           |                |                   |
| RAC-393C   |           |                |                   |
| RAC-393D   |           |                |                   |
| RAC-393E   |           |                |                   |
| RAC-393F   |           |                |                   |
| RAC-396    | R396      | N              | 2017 Sep          |
| RAC-400    | R400      | E              | 2017 Sep          |
| RAC-404    | R404      | L              | 2017 Sep          |
| RAC-406    | R406      | K              | 2017 Sep          |
| RAC-409A   | R409      | A              | 2017 Sep          |
| RAC-409B   |           |                |                   |
| RAC-410A   | R410      | N              | 2017 Sep          |
| RAC-410B   |           |                |                   |
| RAC-410C   |           |                |                   |
| RAC-410D   |           |                |                   |
| RAC-413A   | R413      | C              | 2017 Sep          |
| RAC-413B   |           |                |                   |
| RAC-413C   |           |                |                   |
| RAC-414    | R414      | I              | 2017 Sep          |
| RAC-419A   | R419      | A              | 2017 Sep          |
| RAC-419B   |           |                |                   |
| RAC-419C   |           |                |                   |
| RAC-419D   |           |                |                   |
| RAC-419E   |           |                |                   |
| RAC-423A   | R423      | B              | 2017 Sep          |
| RAC-423B   |           |                |                   |
| RAC-431A   | R431      | H              | 2017 Sep          |
| RAC-431B   |           |                |                   |
| RAC-431C   |           |                |                   |
| RAC-431D   |           |                |                   |
| RAC-431E   |           |                |                   |
| RAC-431F   |           |                |                   |
| RAC-439A   | R439      | H              | 2017 Sep          |
| RAC-439B   |           |                |                   |
| RAC-439C   |           |                |                   |

*City names are coded by alphabetical letters (A–P).

**Appendix Table 3.** Detailed information and characteristics of *E. albertii* raccoon strains.

| Strain* | Virulence genes | Intimins subtypes |
|---------|-----------------|-------------------|
|         | Eae/dt          | Eae/dt-I          | stx2f  |
| RAC-7A  | +               | +                 | –      | rho    |
| RAC-30A | +               | +                 | –      | xi     |
| RAC-33A | +               | +                 | –      | omicron|
| RAC-34A | +               | +                 | –      | beta3  |
| RAC-44A | +               | +                 | –      | xi     |

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| Strain  | Eacdt | eae  | Eecdt-1 | stx2f | Intimin subtypes  |
|---------|-------|------|---------|-------|-------------------|
| RAC-44D | +     | +    | +       | –     | gamma5            |
| RAC-58  | +     | +    | –       | –     | N4                |
| RAC-81A | +     | +    | –       | –     | sigma             |
| RAC-112 | +     | +    | –       | –     | N5                |
| RAC-116A| +     | +    | –       | –     | N1.3              |
| RAC-118A| +     | +    | –       | –     | alpha8            |
| RAC-199 | +     | +    | –       | +     | N1.2              |
| RAC-243 | +     | +    | –       | –     | N2                |
| RAC-244 | +     | +    | –       | –     | N5                |
| RAC-245 | +     | +    | –       | –     | Unknown (2013C-4143) |
| RAC-247 | +     | +    | +       | +     | gamma5            |
| RAC-258 | +     | +    | –       | –     | iota2             |
| RAC-263 | +     | +    | –       | –     | N2                |
| RAC-264A| +     | +    | –       | –     | Unknown (1261-6/89) |
| RAC-266 | +     | +    | –       | –     | rho               |
| RAC-274A| +     | +    | +       | –     | N3                |
| RAC-281 | +     | +    | –       | –     | sigma             |
| RAC-300 | +     | +    | –       | –     | rho               |
| RAC-302A| +     | +    | –       | –     | sigma             |
| RAC-303A| +     | +    | +       | –     | N3                |
| RAC-305A| +     | +    | +       | –     | unknown (1261-6/89) |
| RAC-305B| +     | +    | –       | –     | N3                |
| RAC-306A| +     | +    | –       | –     | Unknown (4281-7/89) |
| RAC-310A| +     | +    | –       | –     | omicron           |
| RAC-313 | +     | +    | –       | –     | UT1               |
| RAC-318A| +     | +    | –       | –     | UT2               |
| RAC-318F| +     | +    | –       | –     | UT2               |
| RAC-324 | +     | +    | –       | –     | N3                |
| RAC-333A| +     | +    | –       | –     | UT1               |
| RAC-336 | +     | +    | –       | –     | N3                |
| RAC-337 | +     | +    | –       | –     | N5                |
| RAC-342 | +     | +    | –       | –     | sigma             |
| RAC-349 | +     | +    | –       | –     | Unknown (4281-7/89) |
| RAC-355 | +     | +    | –       | –     | rho               |
| RAC-357A| +     | +    | –       | –     | UT1               |
| RAC-359A| +     | +    | –       | –     | UT1               |
| RAC-365 | +     | +    | –       | –     | rho               |
| RAC-376 | +     | +    | –       | –     | iota2             |
| RAC-383 | +     | +    | –       | –     | N4                |
| RAC-386 | +     | +    | –       | –     | UT2               |
| RAC-393A| +     | +    | –       | –     | N5                |
| RAC-396 | +     | +    | –       | –     | Unknown (2013C-4143) |
| RAC-404 | +     | +    | –       | –     | UT1               |
| RAC-406 | +     | +    | –       | –     | rho               |
| RAC-409A| +     | +    | –       | –     | iota2             |
| RAC-410A| +     | +    | –       | –     | rho               |
| RAC-413A| +     | +    | –       | –     | Unknown (2013C-4143) |
| RAC-414 | +     | +    | –       | –     | iota2             |
| RAC-419A| +     | +    | –       | –     | iota2             |
| RAC-419D| +     | +    | –       | –     | UT1               |
| RAC-419E| +     | +    | –       | –     | rho               |
| RAC-423A| +     | +    | –       | –     | N4                |
| RAC-431A| +     | +    | –       | –     | omicron           |
| RAC-439A| +     | +    | –       | –     | omicron           |

*RAC, number, letter (A-F) indicate racoon, racoon ID, and *E. albertii* colony ID, respectively. Strains with identical numbers were isolated from the same raccoons. +, present; –, not present.