The Journal of Microbiology (2011) Vol. 49, No. 5, pp. 747-752
DOI 10.1007/s12275-011-1133-y

Epidemiological Investigation of *eaeA*-Positive *Escherichia coli* and *Escherichia albertii* Strains Isolated from Healthy Wild Birds

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(Received March 15, 2011 / Accepted June 9, 2011)

***Escherichia coli* has commonly been associated with diarrheal illness in humans and animals. Recently, *E. albertii* has been reported to be a potential pathogen of humans and animals and to be carried by wild birds. In the present study, the prevalence and genetic characteristics of intimin-producing *E. coli* and *E. albertii* strains were evaluated in wild birds in Korea. Thirty one of 790 *Enterobacteriaceae* strains from healthy wild birds were positive for the intimin gene (*eaeA*) and twenty two of the 31 strains were identified as atypical enteropathogenic *E. coli* (aEPEC) that did not possess both *EAF* and *bfpA* genes. A total of nine lactose non-fermenting coliform bacterial strains were identified as *E. albertii* by PCR and sequence analysis of housekeeping genes. A total of 28 (90.3%) *eaeA*-positive strains were isolated from waterfowl. Fifteen aEPEC (68.2%) and two *E. albertii* (22.2%) strains had a β-intimin subtype and 14 aEPEC strains harboring *β*-intimin belonged to phylogenetic group B2. All *eaeA*-positive *E. albertii* and 3 aEPEC strains possessed the cytolethal distending toxin gene (*cdtB*). The *eaeA*-positive *E. coli* and *E. albertii* strains isolated from healthy wild birds need to be recognized as a potential pathogroup that may pose a potential threat to human and animal health. These findings indicate that *eaeA*-positive *E. coli* as well as *E. albertii* can be carried by wild birds, posing a potential threat to human and animal health.**

***Keywords*:** atypical enteropathogenic *E. coli*, *eaeA*-positive *E. albertii*, β-intimin subtype, cytolethal distending toxin gene (*cdtB*), phylogenetic group B2

Enteropathogenic *Escherichia coli* (EPEC) is frequently associated with *in vitro* and *in vivo* attaching and effacing lesions in epithelial cells and may be food-borne pathogens that cause diarrhea in humans (Zhang *et al*., 2002a). EPEC strains express a 94-97 kDa outer membrane protein virulence factor (intimin) encoded by the *eaeA* gene (Kobayashi *et al*., 2009). EPEC strains with the adherence factor (EAF) plasmid and plasmid-encoded type IV fimbrial gene, *bfpA* are classified as typical (Gunzburg *et al*., 1995), while those negative for the *EAF* and *bfpA* genes are atypical (Trabulsi *et al*., 2002).

Virulence studies for EPEC strains have been performed for humans and animals in many countries. Typical EPEC strains have been isolated only from humans, whereas atypical EPEC (aEPEC) has been found in both humans and animals (Trabulsi *et al*., 2002). In industrialized countries, aEPEC has become a more frequent cause of diarrhea than typical EPEC and also has been associated with prolonged diarrhea in children (Nguyen *et al*., 2006). The EPEC and Shiga toxin-producing *E. coli* strains from animals, such as cattle, typically possess major intimin subtypes (β, β_1, γ, and γ_1), which are prevalent in many Asian and European pathotype strains (Kobayashi *et al*., 2003; Blanco *et al*., 2004). A previous report detected both *eaeA*- and *stx*-possessing *E. coli* strains from wild bird cloacal swabs in Japan (Kobayashi *et al*., 2009). Those strains were identified as atypical *E. coli* strains containing various intimin subtypes (α, α_1, β, ε, λ_1, ν, μ, χ, ζ, and ξ), which were classified as a minor human intimin group (Kobayashi *et al*., 2009). Another previous study showed that 10 of 366 strains (2.7%) isolated from human and veterinary diarrheal patients were identified as atypical EPEC strains (including 2 *bfpA*-negative strains that carried the *cdtB* gene) (Kim *et al*., 2009).

Cytolethal distending toxins (CDTs) have been associated with gastroenteritidis in humans (Johnson and Lior, 1987; Pickett *et al*., 2004). CDT production has also been identified in a new *Escherichia* species, *E. albertii* (Hyma *et al*., 2005). *E. albertii* is classified by specific genetic polymorphism of the 16S rRNA gene and other housekeeping genes, including *lysP* (lysine-specific permease) and *mdh* (malate dehydrogenase) (Huys *et al*., 2003; Hyma *et al*., 2005). *E. albertii* also has specific virulence genes, *eaeA* and *cdtB* but does not have Shiga toxin genes (Oaks *et al*., 2010). *E. albertii* has been reported to be a potential pathogen of humans and animals and to be carried subclinically by various species of wild birds (Oaks *et al*., 2010).

The purpose of this study was to investigate the epidemiological characteristics of *eaeA*-positive *E. coli* and *E. albertii* strains isolated from healthy wild birds in Korea.

Materials and Methods

Sample collection

A total of 1,204 cloacal swab samples were collected from 58 wild bird species captured between 2009 and 2010 with cannon nets. The collection areas were located adjacent to 28 rivers, primarily from 26 cities and 9 provinces of the Republic of Korea. Individual birds were placed in metal trays for collection of feces with the BBL CultureSwab collection device (Beckton, Dickinson and Company,

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USA).

Isolation, identification, and virulence gene PCR of bacterial strains

Swab samples were enriched in Buffered Peptone Water (Beckton, Dickinson and Company) at 37℃ for 24 h. Bacterial suspensions (100 μl) were pipetted into 1.5 ml tubes, heated in a boiling water bath for 10 min, and centrifuged. Supernatants were used in the PCR reaction. Detection of seven virulence genes (verotoxins [VT1 and VT2], heat-labile enterotoxin [LT], and heat stable enterotoxin [ST] for enterotoxigenic *E. coli*; *eaeA* and *bfpA* for enteropathogenic *E. coli*; and *aggR* for enteroaggregative *E. coli*) associated with the diarrheal *E. coli* toxin was conducted by multiplex PCR, using a 7-plex detection kit (KOGENBIOTECH Co., Ltd., Korea). The *EAF* gene was also detected by simplex PCR (Wani *et al*., 2006). The *eaeA*-positive samples detected by multiplex PCR were serially diluted 10-fold in 1.5 ml eppendorf tubes. A 100 μl aliquot of each dilution (10^{-4} and 10^{-5}) was spread on a MacConkey agar plate (Beckton, Dickinson and Company) and was incubated at 37℃ for 18 h. Several colonies on the MacConkey agar plate were randomly selected and were tested by PCR to detect *eaeA* (863 bp, primers SK1 and SK2) as described previously (Zhang *et al*., 2002b). The *eaeA*-positive colonies were then subcultured on MacConkey agar plates (Beckton, Dickinson and Company)

The *eaeA*-positive isolates were inoculated into EC broth (Beckton, Dickinson and Company) with the Durham tube to differentiate fecal coliform bacteria from the rest of the coliform group. The presence of bacterial growth and gas production after Durham tube incubation (44.5℃ for 24 h) resulted in classification of the bacteria as fecal coliform bacteria (Hanai *et al*., 1997). The biochemical identification of *eaeA*-positive strains was performed using the VITEK2 system (bioMérieux sa. 69280, France).

Genetic identification for the lactose non-fermenting strains

Diagnostic multiplex PCR for the detection of the conserved housekeeping genes *clpX*, *lysP*, and *mdh* was performed as previously described to identify 9 *eaeA*-positive strains belonging to the lactose non-fermenting coliform group (Hyma *et al*., 2005), and PCR products of *lysP* and *mdh* were sequenced (Macrogen, Korea). The negative control for PCR was *E. coli* strain ATCC43893. The sequence of the bacterial 16S rRNA gene was analyzed by PCR and sequencing, using previously designed primer sets S-D-Bact-0008-a-S-20 and S-*-Univ-1492-b-A-21 (Suau *et al*., 1999).

CDT and phylogenetic grouping PCR

The cytolethal distending toxin gene, *cdtB* (from *eaeA*-positive strains) was amplified by PCR using the previously described primers VAT2 and WMI1 (Pickett *et al*., 1996). The PCR mixture consisted of 20 pmol of each primer, 2 U of TaKaRa *Ex Taq* DNA polymerase (TaKaRa Bio, Japan), 2.5 mM dNTPs, 5 μl of 10×*Ex Taq* buffer and 1 μl of genomic DNA template in a final volume of 50 μl. The PCR conditions were as follows: 30 cycles of denaturation at 94℃ for 30 sec, annealing at 48℃ for 30 sec, and extension at 72℃ for 45 sec.

A triplex PCR was performed to determine the phylogenetic grouping of *E. coli* isolates by targeting two genes, *chuA* and *yjaA*, and an anonymous DNA fragment, TSPE4.C2 (Clermont *et al*., 2000). Each 25 μl of PCR reaction mixture for multiplex PCR contained, in addition to 1 μl of genomic DNA, 1.5 mM of $MgCl_2$, 2.5 mM of each dNTP, and 2 U of TaKaRa *Ex Taq* DNA polymerase. The PCR conditions were as follows: 94℃ for 5 min followed by 30 cycles of 94℃ for 30 sec, 57℃ for 30 sec, and 72℃ for 30 sec. A final extension of 72℃ for 5 min was performed. A phylogenetic tree was used to classify *E. coli* isolates into four phylogenetic groups (A, B1, B2, and D).

Subtyping of the *eaeA* gene

The intimin-encoding *eaeA* genes were analyzed by PCR amplification of a portion of the LEE locus (enterocyte and effacement) using previously described primer pairs: SK1 for the 5′ conserved region and LP2 (*eae-α*), LP4 (*eae-γ*), LP5 (*eae-β*), LP6B (*eae-ζ*), LP7 (*eae-ι*), LP8 (*eae-η*), LP10 (*eae-κ*), or LP11B (*eae-θ*) for heterogeneous 3′ regions (Blanco *et al*., 2004; Wani *et al*., 2006, 2007). Strains negative for PCR with the known primers were analyzed by PCR and sequencing using a new primer set CesT-upper (5′-AGAGGAAATTAGTTC AAGCGATA-3′) and escD-lower (5′-TGATCCTAAAGATGGTCAA A-3′). Briefly, the PCR reaction mixture included 10 pmol of each primer, 1 U of TaKaRa *Ex Taq* DNA polymerase (TaKaRa Bio), 2.5 mM dNTPs, 1 μl of reaction buffer (20 mM Tris-HCl; pH 8.0, 100 mM KCl, 0.1 mM DTT, 0.5% Tween 20, 0.5% Nonidet P-40, 50% Glycerol) and 1 μl of genomic DNA template (100 ng/μl) in a final volume of 25 μl. The genomic DNA was purified using a QIAGEN Spin Miniprep kit (QIAGEN Strasse Hilden, Germany). The PCR amplifications were performed in a TP600 thermal cycler (TaKaRa Bio) as follows: an initial denaturation at 94℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 30 sec, annealing at 54℃ for 1 min, and extension at 72℃ for 3 min, and final extension at 72℃ for 10 min. PCR products were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized under UV light. PCR products were eluted from the gel using the QIAGEN Gel Extraction kit (QIAGEN), and were cloned into the vector (Invitrogen, USA). Cloned target fragments were sequenced (Macrogen).

Phylogenetic analysis

The nucleotide sequences of the 16S rRNA genes of *E. albertii* and other related bacterial species were determined in this study or obtained from GenBank. Sequence data were analyzed using Chromas software (Ver. 2.33) and were aligned in CLUSTAL X. Aligned sequences were examined with a similarity matrix. Phylogenetic trees were constructed by the unweighted pair group with arithmetic mean (UPGMA) method with 1,000 bootstrap resamplings using MEGA software to assess the relationships among individual pathogens.

Serotyping

Serotyping for the *eae*-positive strains was carried out as previously described using all available O antisera (O1-O181) produced by the Laboratorio de Referencia de *E. coli* (LREC) in Lugo, Spain (Rey *et al*., 2003). O antisera were kindly supplied by the Division of Enteric Bacterial Infections and Center for Infectious Disease, National Institute of Health, Korea.

Nucleotide sequence accession numbers

The nucleotide sequences of the 16S rRNA genes of *E. albertii* strains identified genotypically in this study were deposited into GenBank with accession numbers HM194877 through HM194886 (Fig. 1).

Results

Detection of *eaeA*-positive bacteria and virulence genes

We collected 790 enteric bacterial isolates from cloacal specimens of Korean wild birds during 2009-2010. The *eaeA*-pos-

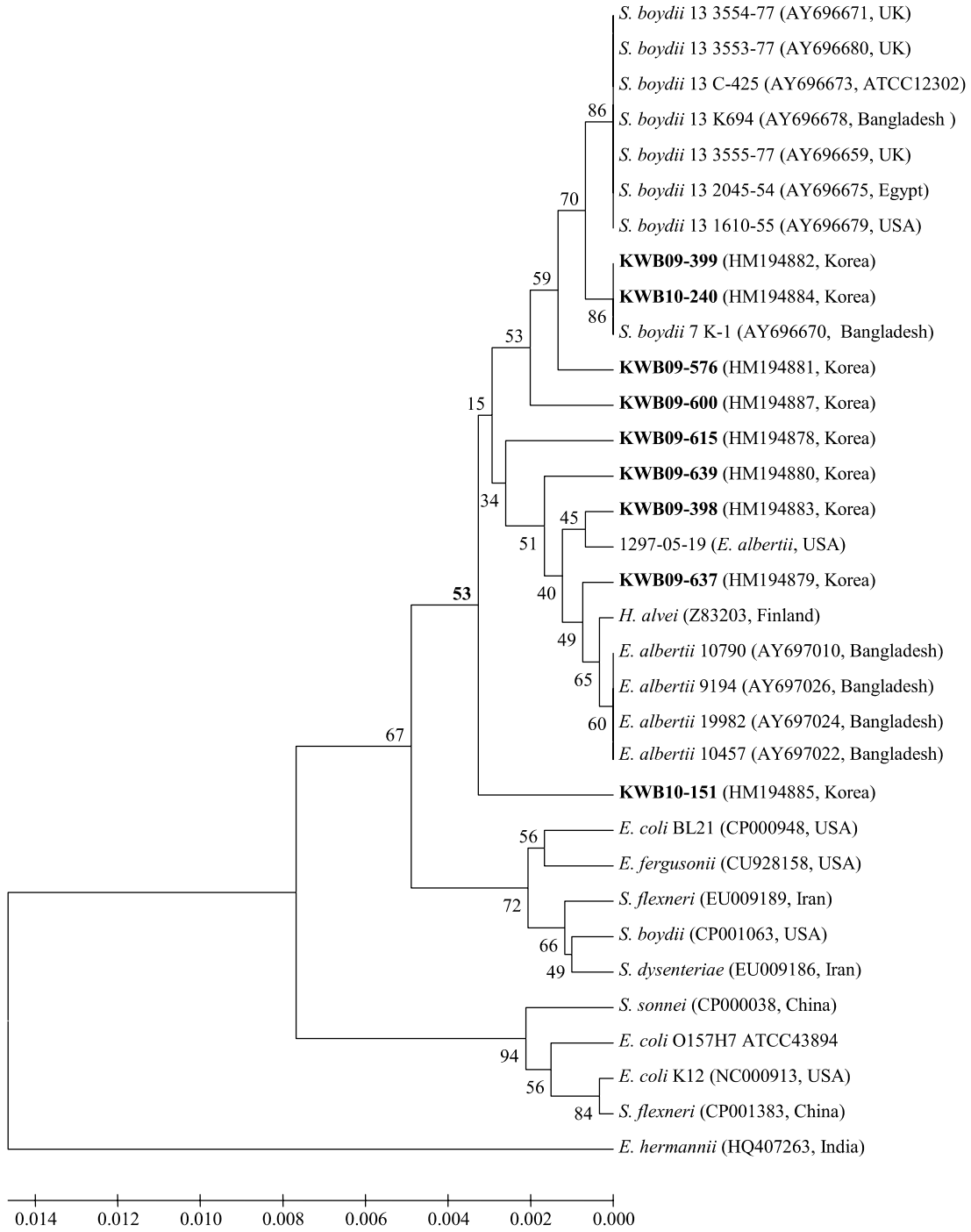

Fig. 1. A phylogenetic tree incorporating the 1,501 bp sequences of *E. albertii* strains isolated from wild birds in Korea.

itive strains were divided into two groups based on distinct colony differences with regard to being lactose fermenting or lactose non-fermenting on MacConkey agar, which included 22 (71.0%) *E. coli* strains and 9 (29.0%) *E. albertii* strains, respectively. The majority of the *eaeA*-positive strains (n=28, 90.3%) were isolated from water fowl (order Anseriformes, Table 1). The most frequently identified birds included the mandarin duck (*Aix galericulata*) (n=14, 45.2%), the European wigeon (*Anas penelope*) (n=7, 22.6%), the spot-billed duck (*Anas poecilorhyncha*) (n=3, 9.7%), the mallard (*Anas platyrhynchos*) (n=2, 6.5%), the pintail (*Anas acuta*) (n=1, 3.2%), and the common teal (*Anas crecca*) (n=1, 3.2%). The remaining 3 strains (9.7%) were isolated from the grey-backed thrush (*Turdus hortulorum*), the grey wagtail (*Motacilla cinerea*), and the great spotted woodpecker (*Dendrocopos major*). Twenty-two *eaeA*-positive *E. coli* strains were atypical enteropathogenic

Table 1. Virulence characteristics of *eaeA*-positive *E. coli* (n=22) strains and *E. albertii* (n=9) strains isolated from Korean wild birds between 2009 and 2010

Strain	Bird	Bacterial species	Virulence genes		Subtype of intimin	Phylogenetic grouping PCR	Serotype of O antigen[a]
			cdtB	*eaeA*			
KWB09-385	Grey-Backed Thrush	*E. coli*	-	+	*eae-γ*	B2	O113
KWB09-603	Mandarin Duck	*E. coli*	-	+	*eae-β*	B2	UT
KWB09-613	Mandarin Duck	*E. coli*	+	+	*eae-θ*	B1	UT
KWB09-628	Mandarin Duck	*E. coli*	-	+	*eae-β*	B2	UT
KWB09-689	Mandarin Duck	*E. coli*	+	+	*eae-β*	B2	UT
KWB09-696	Mandarin Duck	*E. coli*	-	+	*eae-ζ*	B2	UT
KWB09-749	Mandarin Duck	*E. coli*	-	+	*eae-β*	B2	O56
KWB09-754	Mandarin Duck	*E. coli*	-	+	*eae-β*	B1	O109
KWB09-810	Mandarin Duck	*E. coli*	-	+	*eae-β*	B2	UT
KWB09-875	Mandarin Duck	*E. coli*	-	+	*eae-β*	B2	UT
KWB09-1043	Mallard	*E. coli*	+	+	*eae-β*	B2	UT
KWB09-1423	Pintail	*E. coli*	-	+	*eae-β*	B2	UT
KWB10-180	Mandarin Duck	*E. coli*	-	+	*eae-β*	B2	UT
KWB10-204	Mandarin Duck	*E. coli*	-	+	*eae-θ*	B1	O105
KWB10-238	Mallard	*E. coli*	-	+	*eae-β*	B2	UT
KWB10-246	European Wigeon	*E. coli*	-	+	*eae-θ*	B1	UT
KWB10-264	European Wigeon	*E. coli*	-	+	*eae-β*	B2	UT
KWB10-271	European Wigeon	*E. coli*	-	+	*eae-ζ*	B1	O8
KWB10-274	European Wigeon	*E. coli*	-	+	*eae-δ*	A	O49
KWB10-283	European Wigeon	*E. coli*	-	+	*eae-β*	B2	UT
KWB10-284	European Wigeon	*E. coli*	-	+	*eae-β*	B2	UT
KWB10-285	European Wigeon	*E. coli*	-	+	*eae-β*	B2	O113
KWB09-398	Great spotted woodpecker	*E. albertii*	+	+	new	D	O115
KWB09-399	Grey Wagtail	*E. albertii*	+	+	*eae-γ*	D	UT
KWB09-576	Spot-billed Duck	*E. albertii*	+	+	*eae-β*	B1	O103
KWB09-600	Common Teal	*E. albertii*	+	+	*eae-β*	D	O115
KWB09-615	Mandarin Duck	*E. albertii*	+	+	*eae-ρ*	D	UT
KWB09-637	Mandarin Duck	*E. albertii*	+	+	*eae-ρ*	D	UT
KWB09-639	Mandarin Duck	*E. albertii*	+	+	*eae-ξ*	D	UT
KWB10-151	Spot-billed Duck	*E. albertii*	+	+	new	D	UT
KWB10-240	Spot-billed Duck	*E. albertii*	+	+	*eae-ρ*	D	UT

[a] UT, untypeable

E. coli, because they were negative for the *bfpA* and *EAF* genes. Other diarrheal toxin genes (VT1, VT2, LT, ST, *bfpA*, and *aggR*) were not identified in *eaeA*-positive strains by 7-plex PCR. Further, the *cdtB* gene was detected in 3 atypical EPEC strains and 9 *eaeA*-positive *E. albertii* strains.

Identification of *E. albertii* strains

A total of 31 *eaeA*-positive strains were identified as *E. coli* by biochemical testing using the VITEK2 system. The range of probability of being *E. coli* for the 9 lactose non-fermenting strains was 93% to 95%, in contrast to 22 lactose fermenting strains, which were *E. coli* at 99% probability. In addition, there was no distinct difference between lactose fermenting and lactose non-fermenting strains in their biochemical activity.

In order to distinguish the lactose non-fermenting strains from *E. coli*, the conserved housekeeping genes of the *eaeA*-positive strains were confirmed by diagnostic multiplex PCR and sequencing analysis. The *eaeA*-positive lactose non-fermenting isolates (n=9) were positive for all three genes (*clpX*, *lysP*, and *mdh*), which is a unique pattern for *E. albertii*, in contrast to *E. coli* ATCC43893 and the twenty-two lactose fermenting strains, which were positive only for the *clpX* gene. Furthermore, the nucleotide sequences of the *lysP* or *mdh* genes among the 9 lactose non-fermenting strains were identical to the nucleotide sequences of *lysP* or *mdh* genes of *E. albertii* TW07627 (GenBank accession no. ABKX01000030).

A similarity matrix score was generated by comparing the nucleotide sequences of 16S rRNA genes from the 9 *E. albertii* strains isolated in this study with 16S rRNA sequences of the deposited *E. albertii* lineage groups (*E. albertii*, *S. boydii* 7, and *S. boydii* 13) and related strains from the GenBank database. The similarity among 9 *E. albertii* strains ranged from 99.3% to 99.8%, and four strains (KWB10-240, KWB09-399, KWB09-576, and KWB09-600) were very similar to the *S. boydii* group 7 (K-1, Bangladesh). The nucleotide sequences of two *E. albertii* strains [KWB09-399, from the spot-billed duck (HM194884) and KWB10-240, from the grey wagtail (HM194882)] were identical to *S. boydii* 7, and these three together exhibited the greatest similarity to the seven *S. boydii* 13 groups, with 99.8% homology. The KWB09-637 (HM194879) strain had 99.8% homology to four *E. albertii* strains (9194, 10457, 10790, and 19982) from Bangladesh. Similarity of the

KWB09-398 isolate was very high (99.8%) when compared to the 1297-05-19 strain isolated from redpoll finches in Alaska, USA.

Intimin (*eae*) subtyping, phylogenetic grouping PCR, and serotyping analysis

The atypical EPEC and *E. albertii* strains were analyzed by *eae* subtyping, phylogenetic grouping PCR, and serotyping (Table 1). Most aEPEC strains (15/22, 68.2%) possessed an *eae-β* subtype, followed by *θ* (13.6%), *ζ* (9.1%), *γ* (4.6%), and *δ* (4.6%). Three (33.3%) *eaeA*-positive *E. albertii* strains possessed the *ρ*-intimin subtype, followed by *β* (22.2%), *ξ* (11.1%), and *γ* (11.1%). However, two *E. albertii* strains had a new *eae* subtype. Sixteen aEPEC strains were classified into the B2 group by phylogenetic grouping PCR. A total of 14 (63.6%) strains were classified into the *eae-β* subtype and classified into the B2 group. However, most *E. albertii* isolates (n=8, 88.9%) were assigned to phylogenetic group D. A total of 10 (32.3%) *eaeA*-positive *E. coli* and *E. albertii* strains were serotyped to groups O8, O49, O56, O103, O113, and O115, while the remaining 21 (67.7%) strains were classified as untypeable. The aEPEC strains belonging to both the *β*-intimin subtype and the phylogenetic group B2 were mostly untypeable (two isolates were typed). Three *E. albertii* strains were classified as either serotype O115 (n=2, 22.2%) or O103 (n=1, 11.1%).

Discussion

EPEC strains cause diarrheal illness and utilize the intimin adhesin to bind host intestinal cells. They possess an array of virulence factors that are similar to those found in *Shigella* that may possess a Shiga toxin. Studies for pathogenic *Enterobacteriaceae* in wild birds have not previously been reported in Korea. We characterized the *eaeA*-positive isolates obtained from wild birds in Korea by genetic identification, virulence genotyping, phylogenetic grouping, and serotyping. We identified *E. coli* and *E. albertii* isolates possessing virulence factors in both seasonal migratory and resident birds.

Significant numbers of *eaeA* genes found in isolates from migratory birds were confirmed to be *eae-β* subtypes although most isolates harboring the *eae-β* subtype were serologically untypeable. Intimins of both typical and atypical EPEC strains isolated from patients with gastrointestinal disease have commonly been detected as *eae-β* subtypes (Zhang *et al*., 2002b; Jenkins *et al*., 2006; Johnson *et al*., 2007). The aEPEC strains harboring the *eae-β* subtype have been distributed quite widely in migratory Korean wild birds although the serotypes are different. Moreover, those aEPEC strains were classified as phylogenetic group B2. The phylogenetic group B2 *E. coli* strains have been shown to be predominant agents in infants with neonatal bacterial meningitis and adult patients with urinary tract infection (Johnson *et al*., 2002; Zhang *et al*., 2002a; Nowrouzian *et al*., 2005; Kanamaru *et al*., 2006). This phylogenetic group was peviously found in humans and domestic animals in Jeonnam Province of Korea (Unno *et al*., 2009). The *E. coli* strains belonging to phylogenetic group B2 and harboring the *eaeβ* subtype found in this study, therefore, may be potentially pathogenic. Also, three aEPEC strains found in the present study carried the *cdtB* gene. CDT activity demonstrated in other bacterial strains, such as *Campylobacter* spp. has been associated with cytotoxic activity and involves three closely linked genes (*cdtA*, *cdtB*, and *cdtC*) (Johnson and Lior, 1988). Among them, *cdtB* damages host cell, DNA through DNase I-like activity *in vitro* (Lara-Tejero and Galán, 2000, 2001; Hyma *et al*., 2005). Kim *et al*. (2009) demonstrated cytotoxicity of the CDT-producing aEPEC strains, although the CDT-roducing typical EPEC strains appeared more toxic.

E. albertii strains were previously classified as novel *Escherichia* species (formly *Hafnia alvei*) derived from stool specimens of a diarrheic child in Bangladesh using pairwise comparisons of the 16S rRNA sequence (Huys *et al*., 2003). However, the genome of *E. albertii* has recently been sequenced and analyzed in a pathogenic strain (TW07627) isolated from children in Bangladesh. In this study, we identified *E. albertii* by PCR detection of two virulence genes, *eaeA* and *cdtB* and sequence analysis of the 16S rRNA gene and other specific housekeeping genes, *lysP* and *mdh*. The nine *E. albertii* strains identified in this study were classified into the *E. albertii* lineage group, along with the *S. boydii* strains (serotypes 7 and 13), *E. albertii* 1297-05-19 isolated from Redpoll finche from Alaska (USA), and *Hafnia alvei* from Finland, by 16S rRNA sequence similarity analysis. Consequently, we were able to inosculate the nine *E. albertii* strains isolated from wild birds in South Korea with the *E. albertii*/*S. boydii* lineage group by phylogenetic analysis. We have, therefore, demonstrated the presence of *eaeA*-positive *E. albertii* strains harboring the *cdtB* gene in Korean wild birds. *E. albertii* has been associated with diarrheal disease in humans and has also been associated with subclinical infection in wild birds (Huys *et al*., 2003; Oaks *et al*., 2010), although its prevalence and pathogenic role in humans and animals are not currently known.

In conclusion, *eaeA*-positive *E. coli* as well as *E. albertii* can be carried by healthy wild birds, supporting the widespread distribution of such bacteria in wild birds. The *eaeA*-positive *E. coli* strains possessing the *β*-intimin gene, as well as those belonging to phylogenetic group B2, and the *cdtB*- and *eaeA*-positive *E. coli* and *E. albertii* strains isolated from healthy wild birds, need to be recognized as a potential pathogroup that may pose a significant threat to human and animal health. Further studies are needed to assess virulence and clonal diversity of these strains.

Acknowledgements

This work was supported by grants from the Ministry for Food, Agriculture, Forestry and Fisheries, Government of Korea.

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