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Phylogenetic background, virulence gene profiles, and genomic diversity in commensal *Escherichia coli* isolated from ten mammal species living in one zoo

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Abstract

Three hundred commensal *Escherichia coli* recovered from healthy herbivorous, carnivorous, and omnivorous mammals from one zoo were characterized for their phylogenetic origin, intestinal virulence gene (VG) prevalence, and genomic diversity. The phylogenetic structure of the *E. coli* (groups A, B1, B2, and D) from the herbivores was homogenous, with a prevailing representation of group B1. In the carnivores and omnivores, the phylogenetic diversity was species specific with a higher representation of group A compared to the herbivores. Of 16 intestinal VGs in the whole set, 8 were detected and they formed 13 VG profiles. In the herbivores, all the VG-positive isolates belonged to group B1 and harboured the genes *eaeA*, *eastI*, *ehxA*, *stx1*, *and stx2*, which separately or in combination formed 8 VG profiles. In the carnivores and omnivores, the VG-positive isolates frequently belonged to group A and harboured the *estI* and *estII* genes or a combination of *eastI* and *estI*, forming three VG profiles. Single genes *cnf*2, in group B2, and *eastI*, in group D, were found. Similarity analysis of pulsed-field gel electrophoresis (PFGE) patterns revealed closer relatedness between the isolates from carnivores and omnivores than those from herbivores. The comparison between the prevalence of phylogenetic groups and the phylogenetic origin of VG-positive isolates in the examined *E. coli* suggested, that *E. coli* from group B1 in herbivores and *E. coli* from group A rather than B1 in carnivores and omnivores are ''best adapted'' to the host organism. The groups revealed different preferences in the acquisition and maintenance of intestinal VGs.

Keywords: Zoo animals; Commensal *E. coli*; Phylogenetic group of *E. coli*; Intestinal virulence genes

1. Introduction

Escherichia coli is a very diversified species comprising both commensals of mammals (Selander et al., 1987) as well as intestinal or extraintestinal

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pathogens (Ørskov and Ørskov, 1992). So many different ways of interaction with the host organism result from the structure of the *E. coli* genome, which consists of a universal core of genes and a flexible gene pool which is clone specific and includes virulence genes (VGs). VGs are carried by mobile genetic elements such as pathogenicity islands, transposons, bacteriophages, and plasmids (Dobrindt et al., 2002). The pathogenicity in *E. coli* is closely associated with the possession of certain combinations of VGs conditioning attachment and the production of toxins. On the basis of the association of various combinations of VGs and clinical symptoms, diarrhoeagenic *E. coli* in animals are classified as enteropathogenic (EPEC), enterohaemorrhagic (EHEC), or enterotoxigenic (ETEC) (DebRoy and Maddox, 2001). Moreover, some combinations of VGs connected with particular categories of diarrhoeagenic *E. coli* occur more frequently in some animal species or humans than in others, which indicates their species specificity (Hart et al., 1993).

Although commensal *E. coli* are not overtly connected with the occurrence of disease they carry some of these VGs. In order to obtain a better understanding of the role of commensals in the acquisition and maintenance of various VGs, it is essential to investigate their evolutionary origin. Phylogenetic analysis of the *E. coli* species revealed that the majority of strains belong to four phylogenetic groups: A, B1, B2, and D. Commensal strains fall into groups A and B1, whereas extraintestinal *E. coli* (ExPEC) belong mainly to group B2 (Escobar-Páramo et al., 2004; Picard et al., 1999). Diarrhoeagenic strains fall into groups A, B1, and D (Girardeau et al., 2005; Wu et al., 2007). The aim of this study was to analyse the relationship between the phylogenetic origin of *E. coli* and the prevalence of intestinal VGs as well as the genomic diversity in commensal isolates derived from healthy herbivorous, carnivorous, and omnivorous mammalian species.

2. Materials and methods

2.1. Bacterial isolates

Details about the source animals, the methods of isolation, and the identification of the *E. coli* isolates are described in Baldy-Chudzik and Stosik (2007). Briefly, the samples were obtained from herbivorous, carnivorous, and omnivorous mammals living on the Safari Zoo in Świerkocin area (Western Poland). The study comprised 10 animal species, each represented by three healthy adult individuals (i.e. 30 animals). The herbivorous animals were represented by five species: the Defassa waterbuck (*Kobus ellipsiprymus*), eland (*Taurotragus oryx*), yak (*Bos mutus graniens*), aurochs (*Bos primigenius*), and buffalo (*Bubalus bubalis*), the carnivorous by three species: the lion (*Panthera leo*), lynx (*Felis lynx*), and wildcat (*Felis silvetris*), and the omnivorous by two species: the racoon (*Procyon lotor*) and dingo (*Canis familiaris*). The species were selected because of their different diets and the location of their walks on the grounds of the Safari Zoo. The walks for the herbivorous animals were shared or neighbouring, whereas those for the carnivorous and omnivorous species were separated from each other and from those of the herbivorous animals. A faeces sample was obtained from each animal. In each faeces sample, 6–12 non-identical commensal isolates were identified basing on the BOX-PCR fingerprinting method (Baldy-Chudzik and Stosik, 2007). A total of 300 *E. coli* isolates were analysed in this study. The numbers of *E. coli* isolates recovered from each individual representing each of the examined species are shown in Table 1. All the isolates were grown on Luria–Bertani (LB) media and routinely stored at −80 °C in 15% glycerol. PCR template DNA was prepared by boiling 1 ml of overnight LB broth culture for 10 min, and 2 μl aliquots of the supernatant were subjected to PCR.

2.2. Phylogenetic group determination

The main phylogenetic groups (A, B1, B2, and D) of *E. coli* isolates were determined by triplex PCR amplification as described by Clermont et al. (2000).

2.3. Detection of virulence determinants by PCR

A group of 16 virulence genes reported in the literature to be associated with intestinal *E. coli* pathotypes in animals were selected as the panel to be used in the analysis (Table 2) (Chapman et al., 2006). The individual PCR amplifications were performed for the *east1*, *bfpA*, *cnf1*, and *cnf2* genes as described

Table 1
Distribution of the 300 animal commensal *E. coli* isolates among the four phylogenetic groups

Trophic level	Host species	Number of isolates from individuals			All number	Number of isolates in phylogenetic groups			
		a	b	c	*n*	A	B1	B2	D
Herbivorous	Aurochs	12	10	10	32	2	26	2	2
	Buffalo	12	12	11	35	5	25	4	1
	Eland	12	11	11	34	2	27	3	2
	Waterbuck	12	11	11	34	5	21	6	2
	Yak	7	7	6	20	5	11	2	2
	All				155	19	110	17	9
Carnivorous	Lion	12	12	12	36	7	19	6	4
	Lynx	12	11	10	33	24	4	3	2
	Wildcat	7	7	7	21	9	9	2	1
	All				90	40	32	11	7
Omnivorous	Dingo	10	6	6	22	10	3	2	7
	Racoon	12	12	9	33	7	18	3	5
	All				55	17	21	5	12
Total					300	76	163	33	28

by Yamamoto and Echeverria (1996), Gunzburg et al. (1995), and Blanco et al. (1996). The multiplex PCR amplifications were performed for four sets of genes: (1) *eaeA*, *ehxA*, *stx1*, *stx2* (Paton and Paton, 2002), (2) *eltA*, *estII*, *fasA*, (3) *estI*, *faeG*, *fanC*, and (4) *fedA* and $fimF_{41}$, as described by Chapman et al. (2006). The amplified products were visualised by electrophoresis in 2% agarose gel and ethidium bromide staining.

Table 2
Virulence genes associated with diarrhoea-related *E. coli* in animals

Virulence gene (VG)/activity	Phenotype/function	Genomic location of elements encoding virulence gene	*E. coli* pathotype
Adhesins			
eaeA	Intimin	Pathogenicity island LEE	EPEC, EHEC
bfpA	Type IV bundle-forming pili	EAF plasmid	EPEC
faeG	F4 fimbrial adhesin	Plasmid	ETEC
fanC	F5 fimbrial adhesin	Plasmid	ETEC
fasA	F6 fimbrial adhesin	Plasmid	ETEC
fedA	F18 fimbrial adhesin	Plasmid	ETEC
$fimF_{41}$	F41 fimbrial adhesin	Chromosome	ETEC
Toxins			
eastI	EaggEC heat-stable enterotoxin 1 (EAST1)	Plasmid; chromosome	EHEC, EPEC (EaggEC in human)
ehxA	Enterohemolysin (Ehx)	Plasmid	EPEC, EHEC
eltA	Heat-labile toxin (LT)	Plasmid	ETEC
cnf1	Cytotoxic necrotizing factor 1 (CNF1)	Chromosome	ExPEC
cnf2	Cytotoxic necrotizing factor 2 (CNF2)	Plasmid	EPEC, ExPEC
estI	Heat-stable enterotoxin a (STa)	Plasmid; transposons	ETEC
estII	Heat-stable enterotoxin b (STb)	Plasmid; transposons	ETEC
stx1	Shiga toxin I (Stx 1)	Bacteriophage	EHEC
stx2	Shiga toxin II (Stx 2)	Bacteriophage	EHEC

Amplified DNA fragments of specific sizes were located by UV illumination and imaged with BioCapt computer software (Vilber Lourmat). Their lengths were verified by a 100-base pair ladder (Fermentas) run simultaneously. Control DNA samples from reference strains were included in each reaction. The reference *E. coli* strains used as positive controls were B2 (O157:H7 *ehxA*, *eaeA*, *stx1*, *stx2*) (National Veterinary Research Institute, Puławy, Poland), 256 (O141:K81, *estI*, *estII*), 281 (O149:K91, *eltA*, *faeG*), 453 (O78:K89, *eastI*), 107/86 (*fedA*, *stx2*), FVL2 (*cnf1*), B62 (*cnf2*) (Department of bacteriology, National Institute of Hygiene, Warsaw, Poland), B41 (O101, *estI*, *fanC*, $fimF_{41}$), and ND1 (O64, *estI*, *fasA* (P987)) (Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain) and as a negative control PCM 467 C600 (K12, non-toxigenic) (Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wrocław, Poland) was used.

2.4. Serotyping

The serotyping of the VG-carrying isolates was performed by bacterial agglutination (Guinée et al., 1972). The panel, including 174 antisera (Statens Serum Institute, Copenhagen, Denmark), was used for O typing of *E. coli*. The strains negative for all antisera were considered to be non-typeable (NT).

2.5. Pulsed-field gel electrophoresis (PFGE)

Genomic DNA for PFGE was prepared using the GenePath Group 2 Reagent Kit according to the manufacturer's instructions (Bio-Rad). The restriction digestion of plugs was performed using *NotI* endonuclease. The resulting fragments were separated using 1.2% PFGE-grade agarose gel (Bio-Rad) in a CHEF MAPPER system (Bio-Rad) with a 5–50 s pulse time and at 200 V for 22 h in 0.5× TBE buffer at 14 °C. Gel images were saved in TIFF format and processed using Fingerprinting II Informatix software (Version 3.0, Bio-Rad) for computer analysis. The PFGE fingerprints were compared using the criteria of Tenover et al. (1995). Similarity between the fingerprints was further determined on the basis of the Dice coefficient, and dendrograms were generated by the unweighted pair group method with arithmetic mean analysis (UPGMA).

2.6. Statistical analysis

The distributions of the phylogenetic groups of *E. coli* among the isolates as well as the distributions of the virulence genes were compared by the Chi-squared test (Sneath and Sokal, 1973).

3. Results

3.1. Phylogenetic grouping

The distribution of the 300 *E. coli* isolates within the four phylogenetic groups is presented in Table 1. In animals of the same species, the phylogenetic structures of the isolates did not reveal any significant differences. The four phylogenetic groups of *E. coli* were not uniformly distributed ($p < 0.005$) in the particular species as well as in the whole set of examined isolates. Moreover, the distribution of the *E. coli* isolates among the host species was statistically different ($p < 0.001$) from the uniform distribution for the A and B1 groups (with the exception of wildcat). Group A was the most frequent one in lynx (carnivorous) and dingo (omnivorous) and B1 in all the herbivorous species and in lion and racoon. These differences were statistically significant, with $p < 0.001$. Within the group B2, no significant differences were found and the number of isolates from group D was significantly higher in the omnivores than in the carnivores ($p < 0.01$) as well as in the herbivores ($p = 0.03$).

3.2. Prevalence of the virulence genes and serogroups

The 300 isolates were tested using the PCR method for the presence of the 16 intestinal virulence genes (VGs) (Table 2). In the whole set of isolates, 8 different VGs were identified. Among the gene-positive isolates, 13 different VG profiles were found, with up to two virulence genes identified in a single *E. coli* isolate (Tables 3 and 4). Both the same and different VG profiles occurred in isolates from individuals of the same species. The frequency of VG-positive isolates was different in the various host species: from three positive *E. coli* per individual (aurochs) (Fig. 2) to one positive *E. coli* per individual (wildcat) (Figs. 1 and 4).

Table 3
Occurrence of virulence gene profiles in *E. coli* isolates from five herbivorous species

Virulence gene profile[a]	Number, O serogroup and phylogenetic group of isolates found in samples from					Total number of VG-positive isolates (%), $n = 300$
	Aurochs, $n = 32$	Buffalo, $n = 35$	Eland, $n = 34$	Waterbuck, $n = 34$	Yak, $n = 20$	
eaeA	4, O26, B1	2, O111, B1	5, O111, B1	3, O26, B1	1, NT, B1	15 (5.0)
eastI	–	–	–	–	3, O25, B1	3 (1.0)
ehxA	–	1, O55, B1	–	–	–	1 (0.3)
stx2	1, O40, B1	–	2, O2, B1	2, O8, B1	–	5 (1.7)
eaeA, *ehxA*	–	1, O44, B1	–	–	–	1 (0.3)
eaeA, *stx2*	2, NT, B1	1, O111, B1	1, O111, B1	–	–	4 (1.3)
eastI, *stx2*	–	–	–	1, O26, B1	–	1 (0.3)
stx1, *stx2*	2, O40, B1	2, O40, B1; 1, NT, B1	–	–	–	5 (1.7)
Total number of VG-positive isolates (%)	9 (28.1)	8 (22.9)	8 (23.5)	6 (17.6)	4 (20.0)	35 (11.7)

[a] VGs such as *cnf2*, *estI*, and *estII* were not found.

Eight VG profiles were found in the *E. coli* from the herbivorous species and all the VG-positive isolates fell into group B1 (Table 3). The profile of the *eae* gene occurred in 15 *E. coli* isolates and was present in the isolates from each of the herbivorous species. The *stx2* gene occurred in 5 *E. coli* isolates from three species (aurochs, buffalo, and waterbuck). The combination of the genes *eaeA* and *stx2* occurred in 4 isolates from aurochs, buffalo, and eland, whereas the combination *stx1* and *stx2* occurred in 5 isolates from aurochs and buffalo. A single *eastI* gene occurred in 3 *E. coli* isolates, exclusively from yak, and the *ehxA* gene and the combination *eaeA* and *ehxA* occurred in a single *E. coli* isolate from buffalo. Seven VG profiles were revealed in the *E. coli* from the carnivorous species, 5 of which also occurred in isolates from omnivorous species (Table 4). Single *eaeA* and *stx1* genes occurred in 2 *E. coli* isolates from lion (group B1) and lynx (group A), respectively. Within group A, the following VG-positive *E. coli* were found: 4 *E. coli* carrying the *estI* gene (lion, lynx, racoon), 5 *E. coli* with the gene *estII* (lynx, dingo), and 7 *E. coli* with the combination *eastI* and *estI* (wildcat, dingo, racoon). Three isolates from group B2 from

Table 4
Occurrence of virulence gene profiles in *E. coli* isolates from carnivores and omnivores species

Virulence gene profile[a]	Number, O serogroup, and phylogenetic group of isolates found in samples from					Total number of VG-positive isolates (%), $n = 300$
	Carnivores			Omnivores		
	Lion, $n = 36$	Lynx, $n = 33$	Wildcat, $n = 21$	Dingo, $n = 22$	Racoon, $n = 33$	
cnf2	2, O126, B2	–	–	1, O126, B2	–	3 (1.0)
eaeA	1, O26, B1	–	–	–	–	1 (0.3)
eastI	–	–	1, O8, D		1, O8, D	2 (0.7)
estI	2, O9, A	1, NT, A	–	–	1, NT, A	4 (1.3)
estII	–	2, O80, A 2, NT, A	–	1, NT, A	–	5 (1.7)
stx1	–	1, O5, A	–	–	–	1 (0.3)
eastI, *estI*	–	–	2, NT, A	2, O130, A	3, NT, A	7 (2.3)
Total number of VG-positive isolates (%)	5 (13.9)	6 (18.2)	3 (14.3)	4 (18.2)	5 (15.1)	23 (7.7)

[a] VGs such as *ehxA* and *stx2* were not found.

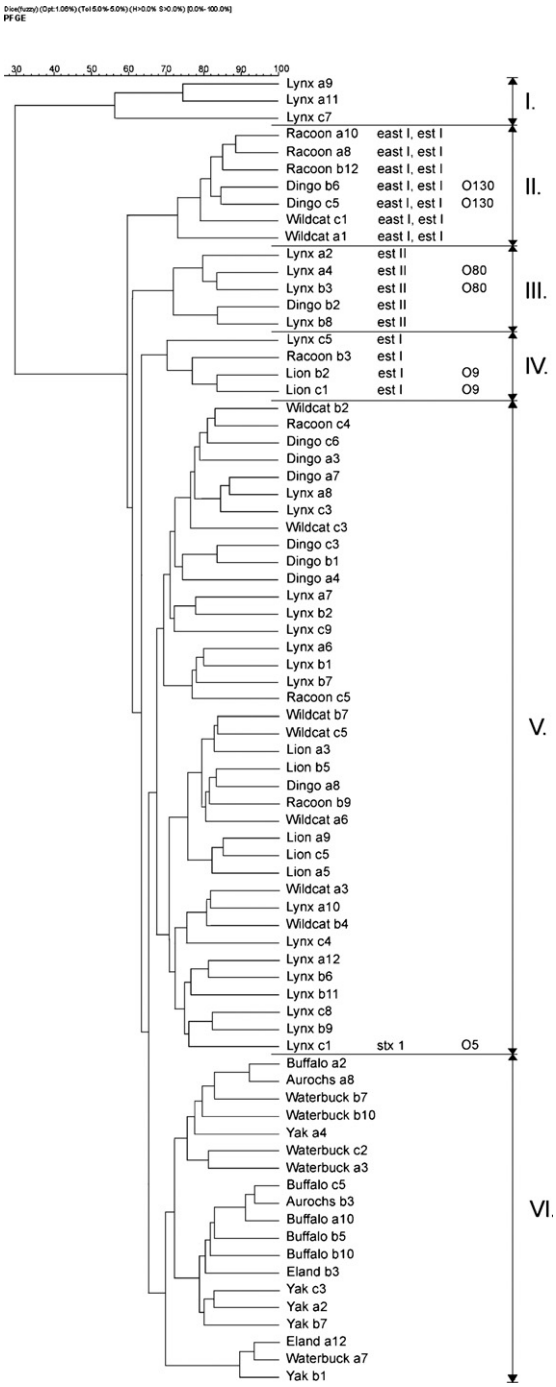

Fig. 1. Dendrogram of the similarity relation of PFGE *NotI* patterns of the 76 *E. coli* isolates belonging to the phylogenetic group A. Each of the isolates is defined by: taxonomic species of the host/a, b or c refers to an individual of a given species, an Arabic numeral refers to a number of an isolate identified in an individual. Roman numeral defines particular similarity clusters. The degree of similarity/relation (%) is shown on the scale.

lion and dingo carried the *cnf2* gene and 2 isolates from group D from wildcat and racoon carried the *eastI* gene. The VG-positive isolates from the carnivorous and omnivorous species fell into the phylogentic group A significantly more frequently ($p < 0.001$).

The total number of identified VG-positive isolates amounted at 58, each of them serotyped with the panel of 174 O antisera. Fourteen of the 58 *E. coli* were negative for all antisera and were considered non-typeable (NT). The VG-positive *E. coli* from the herbivores belonged to the serogroups O2, O8, O25, O26, O40, O44, O55, and O111, whereas those from the carnivores and omnivores belonged to O5, O8, O9, O26, O80, O126, and O130.

3.3. Cluster analysis—PFGE

Genetic diversity was analysed in 300 isolates by PFGE, which revealed 300 distinct restriction patterns, with a difference in at least a single band as the basis for discriminating between isolates. The similarity/relatedness of isolates belonging to the same phylogenetic group was analysed on the basis of dendrograms produced by UPGMA. High degrees of polymorphism were revealed in the isolates from groups A and B1. In group A, 76 isolates formed six clusters (from 3 to 38 isolates per cluster) with similarity from 75 to 30% (Fig. 1). In group B1, 163 isolates formed six clusters (from 4 to 72 isolates per cluster) with similarity from 60 to 55%, whereas 2 isolates carrying the *ehxA* gene and the combination of *eaeA* and *ehxA* were not classified to any of the clusters (Fig. 2). In group B2, 33 isolates fell into two clusters (17 and 16 isolates per cluster) with a similarity of 65% (Fig. 3). In group D, 28 isolates formed 2 clusters (9 and 19 isolates per cluster) with a similarity of 72% (Fig. 4). Within the dendrograms of each phylogenetic group, the isolates harbouring VGs generally formed separate clusters (group A: clusters II, III, and IV, group B1: clusters II, IV, V, and VI) or subclusters (group B2: Ia, group D: Ia). VG-positive isolates of the same O serogroup always revealed higher similarity in

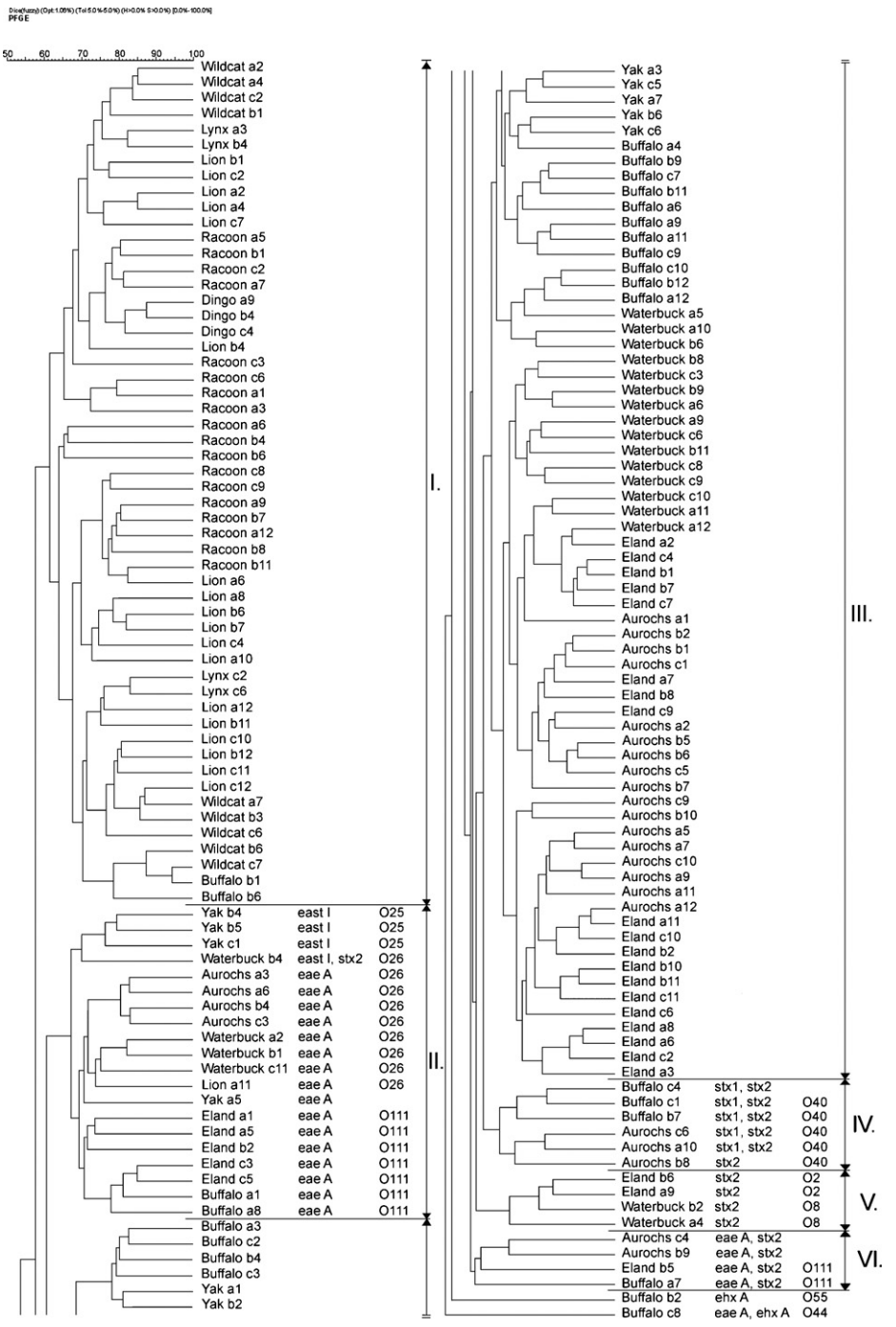

Fig. 2. Dendrogram of the similarity relation of PFGE *NotI* patterns of the 163 *E. coli* isolates belonging to the phylogenetic group B1.

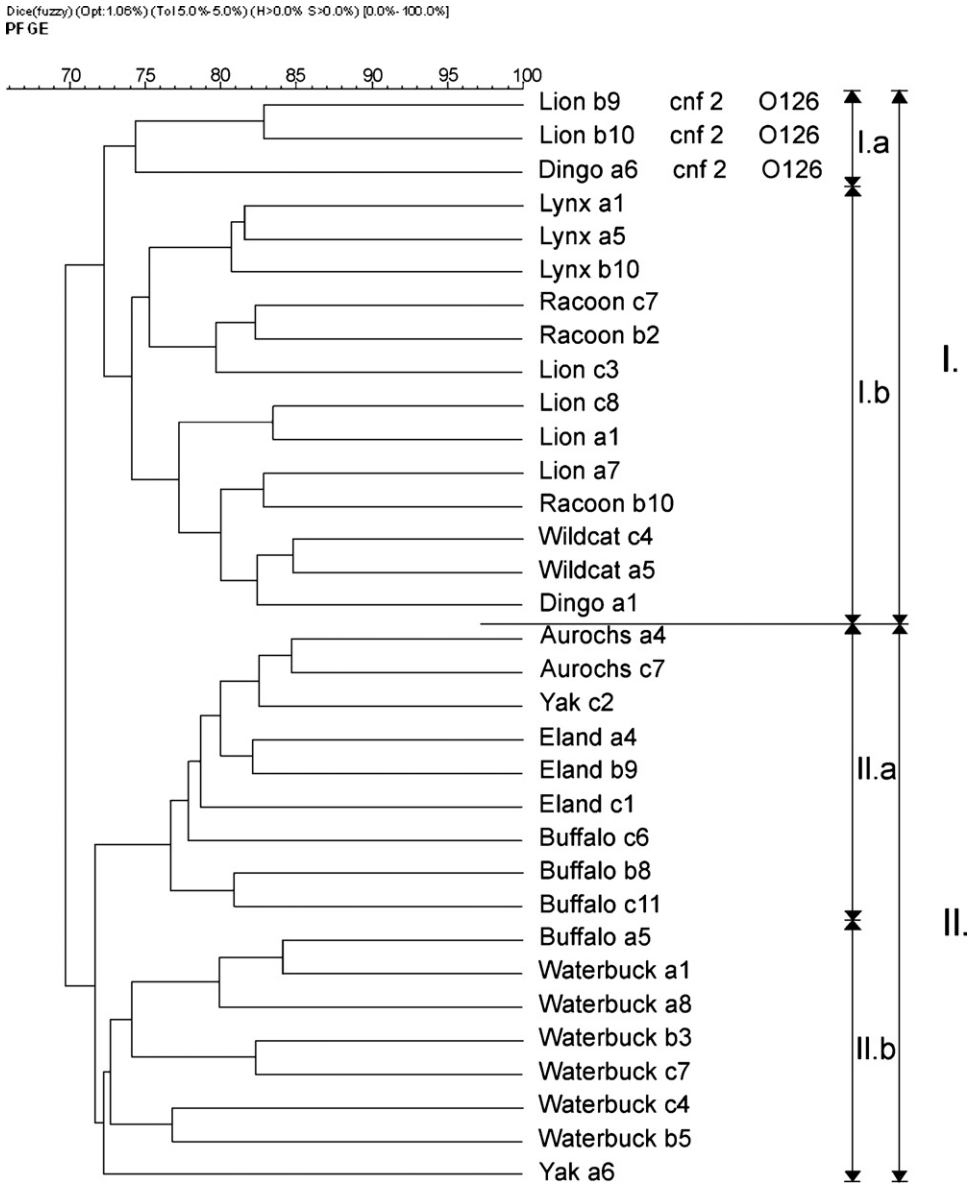

Fig. 3. Dendrogram of the similarity relation of PFGE *NotI* patterns of the 33 *E. coli* isolates belonging to the phylogenetic group B2. Roman numeral defines particular similarity clusters. Roman numeral with character defines particular subclusters.

relation to isolates of different or non-typeable O serogroup. Isolates from carnivorous and omnivorous species always formed common similarity clusters, whereas isolates from herbivorous species were grouped separately. Isolates derived from the same host species demonstrated greater similarity than isolates from different species.

4. Discussion

This study includes the comparative characteristics of commensal isolates of *E. coli* derived from five herbivorous, three carnivorous, and two omnivorous mammalian species from one zoo. Direct comparisons of *E. coli* diversity in different animal species are rare

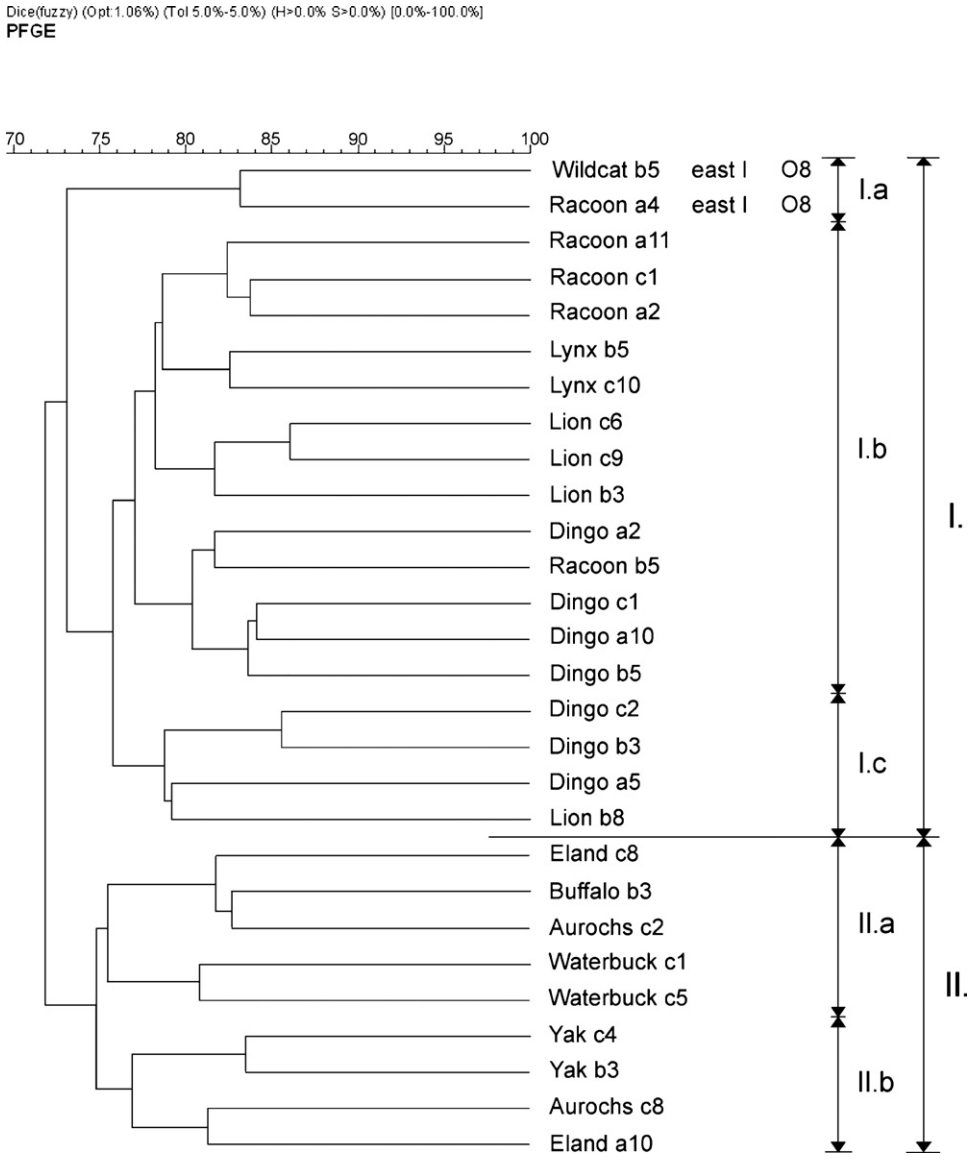

Fig. 4. Dendrogram of the similarity relation of PFGE *NotI* patterns of the 28 *E. coli* isolates belonging to the phylogenetic group D.

in the literature (Gordon and Crowling, 2003; Souza et al., 1999). Most of the data focus on the analyses of *E. coli* in a single species of domesticated animals and they report a considerable diversity in both diarrhoea-related and commensal isolates (Chapman et al., 2006).

In this study the diversity of *E. coli* in animals of the same species was expressed by both polymorphism of the PFGE patterns and different numbers of VG-positive isolates and/or the presence of various VG profiles, which indicates that in animals from the Zoo the degree of *E. coli* diversity is comparable to the one revealed for domestic animals (Wu et al., 2007).

The comparative analysis of the isolates from the different mammal species revealed that the diversity of *E. coli* is complex. In the five herbivorous species, a homogeneous phylogenetic structure of *E. coli* was revealed, with a significant prevalence of isolates from

group B1 with high genomic polymorphism. The VG-positive isolates from the herbivores fell exclusively into group B1, and four VG profiles of the eight identified were present at least in two species. The most frequently occurring genes were those associated with pathotypes EPEC/EHEC and they were: a single intimin (*eaeA*) gene, Shiga toxin 2 gene (*stx2*), enterohemolysin (*ehxA*) gene, and their combination. The occurrence of various combinations of these genes in isolates of the same O serogroup (e.g. the *stx2* gene and the combination *stx1*, *stx2* in *E. coli* O40 in aurochs or the *eaeA* gene and the combination *eaeA*, *stx2* in *E. coli* O111 in buffalo and eland) as well as the occurrence of the same VG profile in isolates of various O serogroups (e.g. the *eaeA* gene with serogroup O26 or O111 or the *stx2* gene with serogroup O2, O8, O40) is consistent with the horizontal transfer of the genetic elements harbouring these genes and indicates that undomesticated herbivorous species as well as domesticated ones comprise a reservoir of *E. coli* carrying VGs associated with the EPEC/EHEC pathotypes (Nataro and Kaper, 1998).

The isolates from the carnivorous and omnivorous species revealed a larger number of common features than different ones. The phylogenetic structure of the isolates varied and depended on the species and not on the trophic level of the host (carnivorous/omnivorous). The PFGE patterns of the isolates from the carnivores and omnivores formed common clusters in the dendrograms of each phylogenetic group, which indicates a closer relatedness, and simultaneously distinguished them explicitly from the isolates from the herbivorous species. Five VG profiles of the seven found in the *E. coli* from the carnivores coincided in at least one omnivorous species. The VG-positive isolates from the carnivores and omnivores fell most frequently into group A and harboured the genes of two heat-stable enterotoxins, STa (*estI*) and STb (*estII*), associated with ETEC. It is known that the genes *estI* and *estII* are most frequently carried by transpozones on plasmids and that enterotoxin plasmids from serotypes frequently associated with *E. coli* diarrhoea are generally nonconjugative, whereas toxin plasmids from serotypes rarely associated with diarrhoea are usually conjugative (Kaper et al., 1999). In the present study the isolates carrying the genes *estI* or *estII* belonged to serogroup O9, associated with ETEC, or to the non-pathogenic serogroups O80 and O130, or their serogroup was not typeable, which suggests their prevalence by conjugation.

In the *E. coli* of group B2 from the carnivorous and omnivorous species, the *cnf2* gene encoding cytotoxic necrotizing factor type 2 was found, which confirms earlier reports of the occurrence of isolates associated with extraintestinal pathogenicity (ExPEC) in the faeces of healthy animals (Orden et al., 2002). The gene *eastI*, encoding enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1), was revealed in groups A and D in carnivores and omnivores as well as in group B1 in herbivores. Isolates harbouring *eastI* belonged to serogroups O8 (carnivores and omnivores), O25 and O26 (herbivores), and O130 (dingo, omnivore) or their serogroup was not typeable (carnivore and omnivore), which supports recent surveys revealing that *eastI* occurring on the plasmid more frequently than on the chromosome is widely distributed among various *E. coli* (Ménard and Dubreuil, 2002). In the examined set of *E. coli*, no harbouring of fimbrial genes were found. This suggests that either there were no VGs in the *E. coli* of the examined animals or that they constituted a very small fraction of the intestinal microflora and were thus not identified in the randomly sampled faeces. This may suggest that fimbrial genes play a significant role in the development of the pathogenicity of *E. coli* (Chapman et al., 2006).

On the other hand, some data referring to the relationships between the phylogenetic structure of *E. coli* and the prevalence of VG in animals reveal a series of similarities to the results obtained here and indicate that Shiga toxin-producing *E. coli* from cattle (herbivorous) were classified as group B1 more frequently than as group D or A (Girardeau et al., 2005), whereas in swine (omnivorous), both commensal and ETEC isolates fell most frequently into group A (Wu et al., 2007).

5. Conclusion

The results of the comparative analysis of commensal *E. coli* in herbivorous, carnivorous and omnivorous species from one zoo revealed that in the phylogenetic structure of *E. coli* in herbivorous

species group B1 prevailed, and VG-positive isolates fell only into this group, whereas in carnivorous and omnivorous species representation of group A was significantly higher than in herbivorous, and VG-positive isolates belonged to group A more frequently. The differences suggest that in the examined herbivorous species, commensal *E. coli* from group B1 are ''best adapted'' to the hosts' organisms, whereas in carnivorous and omnivorous species, it is *E. coli* from group A rather than B1 which are ''best adapted''. The results also indicate that horizontal transfer plays an important role in VG prevalence in best adapted *E. coli* clones. Although clones from both group A and group B1 are able to acquire any kind of VG, it seems that each of the phylogentic groups reveals preferences to maintain a different repertoire of VGs. Within group A, these are VGs associated with ETEC pathotypes and within group B1, they are the ones associated with EHEC/EPEC pathotypes. The acquisition of VGs increases the genetic diversity within groups A and B1, enables virulence genes to survive within the *E. coli* clones ''best adapted'' to the host species, and makes the host organism their reservoir.

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