



HAL
open science

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

Katarzyna Baldy-Chudzik, Pawel Mackiewicz, Michal Stosik

► To cite this version:

Katarzyna Baldy-Chudzik, Pawel Mackiewicz, Michal Stosik. Phylogenetic background, virulence gene profiles, and genomic diversity in commensal isolated from ten mammal species living in one zoo. *Veterinary Microbiology*, 2008, 131 (1-2), pp.173. 10.1016/j.vetmic.2008.02.019 . hal-00532402

HAL Id: hal-00532402

<https://hal.science/hal-00532402>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

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

Authors: Katarzyna Baldy-Chudzik, Paweł Mackiewicz, Michał Stosik



PII: S0378-1135(08)00095-3
DOI: doi:10.1016/j.vetmic.2008.02.019
Reference: VETMIC 3977

To appear in: *VETMIC*

Received date: 4-12-2007
Revised date: 27-2-2008
Accepted date: 28-2-2008

Please cite this article as: Baldy-Chudzik, K., Mackiewicz, P., Stosik, M., Phylogenetic background, virulence gene profiles, and genomic diversity in commensal *Escherichia coli* isolated from ten mammal species living in one zoo, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2008.02.019

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

Katarzyna Baldy-Chudzik^{1*}, Paweł Mackiewicz², Michał Stosik¹

^{1*}Department of Microbiology and Genetics, Institute of Biotechnology and Environmental Science, University of Zielona Góra, Monte Cassino 21B, 65-561 Zielona Góra, , Poland, tel.: +48 068 328 73 33; Fax: +48 068 328 73 23;

e-mail: K.Baldy-Chudzik@ibos.uz.zgora.pl; * corresponding author

²Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

1 Abstract

2 Three hundred commensal *E.coli* recovered from healthy herbivorous, carnivorous, and
3 omnivorous mammals from one zoo were characterized for their phylogenetic origin,
4 intestinal virulence gene (VG) prevalence, and genomic diversity. The phylogenetic
5 structure of the *E. coli* (groups A, B1, B2, and D) from the herbivores was homogenous,
6 with a prevailing representation of group B1. In the carnivores and omnivores, the
7 phylogenetic diversity was species specific with a higher representation of group A
8 compared to the herbivores. Of 16 intestinal VGs in the whole set, 8 were detected and they
9 formed 13 VG profiles. In the herbivores, all the VG-positive isolates belonged to group B1
10 and harboured the genes *eaeA*, *eastI*, *ehxA*, *stx1*, and *stx2*, which separately or in
11 combination formed 8 VG profiles. In the carnivores and omnivores, the VG-positive
12 isolates frequently belonged to group A and harboured the *estI* and *estII* genes or a
13 combination of *eastI* and *estI*, forming three VG profiles. Single genes *cnf2*, in group B2,
14 and *eastI*, in group D, were found. Similarity analysis of pulsed-field gel electrophoresis
15 (PFGE) patterns revealed closer relatedness between the isolates from carnivores and
16 omnivores than those from herbivores. The comparison between the prevalence of
17 phylogenetic groups and the phylogenetic origin of VG-positive isolates in the examined *E.*
18 *coli* suggested, that *E. coli* from group B1 in herbivores and *E. coli* from group A rather
19 than B1 in carnivores and omnivores are “best adapted” to the host organism. The groups
20 revealed different preferences in the acquisition and maintenance of intestinal VGs.

21 **Keywords:** Zoo animals, Commensal *E. coli*, Phylogenetic group of *E. coli*, Intestinal
22 virulence genes

23 Introduction

24 *E. coli* is a very diversified species comprising both commensals of mammals (Selander
25 et al., 1987) as well as intestinal or extraintestinal pathogens (Ørskov and Ørskov, 1992). So

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

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

24 **Materials and methods**

25 **Bacterial isolates**

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

22 **Phylogenetic group determination**

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

25 **Detection of virulence determinants by PCR**

1 A group of 16 virulence genes reported in the literature to be associated with intestinal *E.*
2 *coli* pathotypes in animals were selected as the panel to be used in the analysis (Table 2)
3 (Chapman et al., 2006). The individual PCR amplifications were performed for the *eastI*,
4 *bfpA*, *cnf1*, and *cnf2* genes as described by Yamamoto and Echeverria (1996), Gunzburg et
5 al., (1995), and Blanco et al. (1996). The multiplex PCR amplifications were performed for
6 four sets of genes: 1) *eaeA*, *ehxA*, *stx1*, *stx2* (Paton and Paton, 2002), 2) *eltA*, *estII*, *fasA*, 3)
7 *estI*, *faeG*, *fanC*, and 4) *fedA* and *fimF*₄₁, as described by Chapman et al. (2006). The
8 amplified products were visualised by electrophoresis in 2% agarose gel and ethidium
9 bromide staining. Amplified DNA fragments of specific sizes were located by UV
10 illumination and imaged with BioCapt computer software (Vilber Lourmat). Their lengths
11 were verified by a 100-base-pair ladder (Fermentas) run simultaneously. Control DNA
12 samples from reference strains were included in each reaction. The reference *E. coli* strains
13 used as positive controls were B2 (O157:H7 *ehxA*, *eaeA*, *stx1*, *stx2*) (National Veterinary
14 Research Institute, Puławy, Poland), 256 (O141:K81, *estI*, *estII*), 281 (O149 :K91, *eltA*,
15 *faeG*), 453 (O78:K89, *eastI*), 107/86 (*fedA*, *stx2*), FVL2 (*cnf1*), B62 (*cnf2*) (Department of
16 bacteriology, National Institute of Hygiene, Warsaw, Poland), B41 (O101, *estI*, *fanC*, *fimF*-
17 ₄₁), and ND1 (O64, *estI*, *fasA* (P987)) (Faculty of Veterinary, University of Santiago de
18 Compostela, Lugo, Spain) and as a negative control PCM 467 C600 (K12, non-toxicogenic)
19 (Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wrocław,
20 Poland) was used.

21 Serotyping

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

1 **Pulsed-field gel electrophoresis (PFGE)**

2 Genomic DNA for PFGE was prepared using the GenePath Group 2 Reagent Kit
3 according to the manufacturer's instructions (Bio-Rad). The restriction digestion of plugs
4 was performed using *NotI* endonuclease. The resulting fragments were separated using
5 1.2% PFGE-grade agarose gel (Bio-Rad) in a CHEF MAPPER system (Bio-Rad) with a 5 to
6 50 s pulse time and at 200 V for 22 h in 0.5 x TBE buffer at 14°C. Gel images were saved in
7 TIFF format and processed using Fingerprinting II Informatix software (version 3.0, Bio-
8 Rad) for computer analysis. The PFGE fingerprints were compared using the criteria of
9 Tenover et al. (1995). Similarity between the fingerprints was further determined on the
10 basis of the Dice coefficient, and dendrograms were generated by the unweighted pair group
11 method with arithmetic mean analysis (UPGMA).

12 **Statistical analysis**

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

16 **Results**

17 **Phylogenetic grouping**

18 The distribution of the 300 *E. coli* isolates within the four phylogenetic groups is
19 presented in Table 1. In animals of the same species, the phylogenetic structures of the
20 isolates did not reveal any significant differences. The four phylogenetic groups of *E. coli*
21 were not uniformly distributed ($p < 0.005$) in the particular species as well as in the whole set
22 of examined isolates. Moreover, the distribution of the *E. coli* isolates among the host
23 species was statistically different ($p < 0.001$) from the uniform distribution for the A and B1
24 groups (with the exception of wildcat). Group A was the most frequent one in lynx
25 (carnivorous) and dingo (omnivorous) and B1 in all the herbivorous species and in lion and

1 racoon. These differences were statistically significant, with $p < 0.001$. Within the group B2,
2 no significant differences were found and the number of isolates from group D was
3 significantly higher in the omnivores than in the carnivores ($p < 0.01$) as well as in the
4 herbivores ($p = 0.03$).

5 **Prevalence of the virulence genes and serogroups**

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

14 Eight VG profiles were found in the *E. coli* from the herbivorous species and all the VG-
15 positive isolates fell into group B1 (Table 3). The profile of the *eae* gene occurred in 15 *E.*
16 *coli* isolates and was present in the isolates from each of the herbivorous species. The *stx2*
17 gene occurred in 5 *E. coli* isolates from three species (aurochs, buffalo, and waterbuck). The
18 combination of the genes *eaeA* and *stx2* occurred in 4 isolates from aurochs, buffalo, and
19 eland, whereas the combination *stx1* and *stx2* occurred in 5 isolates from aurochs and
20 buffalo. A single *eastI* gene occurred in 3 *E. coli* isolates, exclusively from yak, and the
21 *ehxA* gene and the combination *eaeA* and *ehxA* occurred in a single *E. coli* isolate from
22 buffalo. Seven VG profiles were revealed in the *E. coli* from the carnivorous species, 5 of
23 which also occurred in isolates from omnivorous species (Table 4). Single *eaeA* and *stx1*
24 genes occurred in 2 *E. coli* isolates from lion (group B1) and lynx (group A), respectively.
25 Within group A, the following VG-positive *E. coli* were found: 4 *E. coli* carrying the *estI*

1 gene (lion, lynx, racoon), 5 *E. coli* with the gene *estII* (lynx, dingo), and 7 *E. coli* with the
2 combination *eastI* and *estI* (wildcat, dingo, racoon). Three isolates from group B2 from lion
3 and dingo carried the *cnf2* gene and 2 isolates from group D from wildcat and racoon
4 carried the *eastI* gene. The VG-positive isolates from the carnivorous and omnivorous
5 species fell into the phylogenetic group A significantly more frequently ($p<0.001$).

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

12 **Cluster analysis – PFGE**

13 Genetic diversity was analysed in 300 isolates by PFGE, which revealed 300 distinct
14 restriction patterns, with a difference in at least a single band as the basis for discriminating
15 between isolates. The similarity/relatedness of isolates belonging to the same phylogenetic
16 group was analysed on the basis of dendrograms produced by UPGMA. High degrees of
17 polymorphism were revealed in the isolates from groups A and B1. In group A, 76 isolates
18 formed six clusters (from 3 to 38 isolates per cluster) with similarity from 75% to 30% (Fig.
19 1). In group B1, 163 isolates formed six clusters (from 4 to 72 isolates per cluster) with
20 similarity from 60% to 55%, whereas 2 isolates carrying the *ehxA* gene and the combination
21 of *eaeA* and *ehxA* were not classified to any of the clusters (Fig. 2). In group B2, 33 isolates
22 fell into two clusters (17 and 16 isolates per cluster) with a similarity of 65% (Fig. 3). In
23 group D, 28 isolates formed 2 clusters (9 and 19 isolates per cluster) with a similarity of
24 72% (Fig. 4). Within the dendrograms of each phylogenetic group, the isolates harbouring
25 VGs generally formed separate clusters (group A: clusters II, III, and IV, group B1: clusters

1 II, IV, V, and VI) or subclusters (group B2: Ia, group D: Ia). VG-positive isolates of the
2 same O serogroup always revealed higher similarity in relation to isolates of different or
3 non-typeable O serogroup. Isolates from carnivorous and omnivorous species always
4 formed common similarity clusters, whereas isolates from herbivorous species were
5 grouped separately. Isolates derived from the same host species demonstrated greater
6 similarity than isolates from different species.

7 **Discussion**

8 This study includes the comparative characteristics of commensal isolates of *E. coli*
9 derived from five herbivorous, three carnivorous, and two omnivorous mammalian species
10 from one zoo. Direct comparisons of *E. coli* diversity in different animal species are rare in
11 the literature (Gordon et al., 2003; Souza et al., 1999). Most of the data focus on the
12 analyses of *E. coli* in a single species of domesticated animals and they report a
13 considerable diversity in both diarrhoea-related and commensal isolates (Chapman et al.,
14 2006).

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

19 The comparative analysis of the isolates from the different mammal species revealed that
20 the diversity of *E. coli* is complex. In the five herbivorous species, a homogeneous
21 phylogenetic structure of *E. coli* was revealed, with a significant prevalence of isolates from
22 group B1 with high genomic polymorphism. The VG-positive isolates from the herbivores
23 fell exclusively into group B1, and four VG profiles of the eight identified were present at
24 least in two species. The most frequently occurring genes were those associated with
25 pathotypes EPEC/EHEC and they were: a single intimin (*eaeA*) gene, Shiga toxin 2 gene

1 (*stx2*), enterohemolysin (*ehxA*) gene, and their combination. The occurrence of various
2 combinations of these genes in isolates of the same O serogroup (e.g. the *stx2* gene and the
3 combination *stx1*, *stx2* in *E. coli* O40 in aurochs or the *eaeA* gene and the combination
4 *eaeA*, *stx2* in *E. coli* O111 in buffalo and eland) as well as the occurrence of the same VG
5 profile in isolates of various O serogroups (e.g. the *eaeA* gene with serogroup O26 or O111
6 or the *stx2* gene with serogroup O2, O8, O40) is consistent with the horizontal transfer of
7 the genetic elements harbouring these genes and indicates that undomesticated herbivorous
8 species as well as domesticated ones comprise a reservoir of *E. coli* carrying VGs associated
9 with the EPEC/EHEC pathotypes (Nataro and Kaper, 1998).

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

1 serogroups O80 and O130, or their serogroup was not typeable, which suggests their
2 prevalence by conjugation.

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

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

24 **Conclusion**

25

1 The results of the comparative analysis of commensal *E. coli* in herbivorous, carnivorous
2 and omnivorous species from one zoo revealed that in the phylogenetic structure of *E. coli*
3 in herbivorous species group B1 prevailed, and VG-positive isolates fell only into this
4 group, whereas in carnivorous and omnivorous species representation of group A was
5 significantly higher than in herbivorous, and VG-positive isolates belonged to group A more
6 frequently. The differences suggest that in the examined herbivorous species, commensal *E.*
7 *coli* from group B1 are “best adapted” to the hosts’ organisms, whereas in carnivorous and
8 omnivorous species, it is *E. coli* from group A rather than B1 which are “best adapted”. The
9 results also indicate that horizontal transfer plays an important role in VG prevalence in best
10 adapted *E. coli* clones. Although clones from both group A and group B1 are able to acquire
11 any kind of VG, it seems that each of the phylogentic groups reveals preferences to maintain
12 a different repertoire of VGs. Within group A, these are VGs associated with ETEC
13 pathotypes and within group B1, they are the ones associated with EHEC/EPEC pathotypes.
14 The acquisition of VGs increases the genetic diversity within groups A and B1, enables
15 virulence genes to survive within the *E. coli* clones “best adapted” to the host species, and
16 makes the host organism their reservoir.

17 **Acknowledgements**

18 This work was supported by a grant from The Voivodeship Fund for Environmental
19 Protection and Water Management in Zielona Góra (2006 /On-E/ Os-041/ D)

20

21

22

23

24

25

1 **References**

- 2
3 Baldy-Chudzik, K., Stosik, M., 2007. Diversity of *fliC* gene in commensal *Escherichia coli*
4 derived from various mammals. *Folia Microbiol.* 52, 261-272.
5
6 Blanco, M., Blanco, J. E., Blanco, J., Alonso, M. P., Balsalobre, C., Mouriño, M., Madrid,
7 C., Juárez, A., 1996. Polymerase chain reaction for detection of *Escherichia coli* strains
8 producing cytotoxic necrotizing factor type 1 and type 2 (CNF1 and CNF2). *J. Microbiol.*
9 *Methods.* 26, 95-101.
10
11 Chapman, T. A., Wu, X.-Y., Barchia, I., Bettelheim, K. A., Driesen, S., Trott, D., Wilson,
12 M., Chin, J. J.-C., 2006. Comparison of virulence gene profiles of *Escherichia coli* strains
13 isolated from healthy and diarrheic swine. *Appl. Environ. Microbiol.* 72, 4782-4795.
14
15 Clermont, O., Bonacorsi, S., Bingen, E., 2000. Rapid and simple determination of the
16 *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* 66, 4555-4558.
17
18 DebRoy, D., Maddox, C. W., 2001. Identification of virulence attributes of gastrointestinal
19 *Escherichia coli* isolates of veterinary significance. *Anim. Health. Res. Rev.* 2, 129-140.
20
21 Dobrindt, U., Hentschel, U., Kaper, J.B., Hacker, J., 2002. Genome plasticity in pathogenic
22 and nonpathogenic enterobacteria. *Curr. Top. Microbiol. Immunol.* 264, 157-175.
23
24 Escobar-Páramo, P., Clermont, O., Blanc-Potard, A. B., Bui, H., Le Bouguenec, C.,
25 Denamur, E., 2004. A specific genetic background is required for acquisition and
26 expression of virulence factors in *Escherichia coli*. *Mol. Biol. Evol.* 21, 1085-1094.

- 1 Girardeau, J. P., Dalmaso, A., Bertin, Y., Ducrot, C., Bord, S., Livrelli, V., Vernozy-
2 Rozand, C., Martin, C., 2005. Association of virulence genotype with phylogenetic
3 background in comparison to different seropathotypes of Shiga toxin-producing
4 *Escherichia coli* isolates. J. Clin. Microbiol. 43, 6098-6107.
- 5
- 6 Gordon, D. M., Crowling, A., 2003. The distribution and genetic structure of *Escherichia*
7 *coli* in Australian vertebrates: host and geographic effects. Microbiol. 149, 3575-3586.
- 8
- 9 Guinée, P.A.M., Agterberg, C.M., Jansen, W.H., 1972 *Escherichia coli* O antigen typing
10 by means of a mechanized microtechnique. Appl. Microbiol. 24:127-131
- 11
- 12 Gunzburg, S. T., Tornieporth, N. G., Riley, L. W., 1995. Identification of enteropathogenic
13 *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. J. Clin.
14 Microbiol. 33, 1375-1377.
- 15
- 16 Hart, C. A., Batt, R. M., Saunders J. R., 1993. Diarrhoea caused by *Escherichia coli*. Ann.
17 Trop. Paediatr. 13, 121-131.
- 18
- 19 Kaper, J. B., Mellies, J. L., Nataro, J. P., 1999. Pathogenicity islands and other mobile
20 genetic elements of diarrheagenic *Escherichia coli*. In: Kaper, J. B., Hacker, J.(Eds.),
21 Pathogenicity islands and other mobile virulence elements. American Society for
22 Microbiology, Washington, D. C., pp.33-58.
- 23
- 24 Ménard, L.-Ph., Dubreuil, J. D., 2002. Enteroaggregative *Escherichia coli* heat-stable
25 enterotoxin 1 (EAST1): a new toxin with an old twist. Critical Rev. Microbiol. 28, 43-60.

- 1 Nataro, J. P., Kaper, J. B., 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11,
2 142-201.
- 3
- 4 Orden, J. A., Cid, D., Ruia-Santa-Quiteria, J. A., Garcia, S., Martinez, S., de la Fuente, R.,
5 2002. Verotoxin- producing *Escherichia coli* (VTEC), enteropathogenic *E.coli* (EPEC) and
6 necrotoxicogenic *E.coli* (NTEC) isolated from healthy cattle in Spain. J. Appl. Microbiol 93,
7 29-35.
- 8
- 9 Ørskov, F., Ørskov, I., 1992. *Escherichia coli* serotyping and disease in man and animals.
10 Can. J. Microbiol. 38, 699-704.
- 11
- 12 Paton, A. W., Paton, J. C., 2002. Direct detection and characterization of Shiga toxigenic
13 *Escherichia coli* by multiplex PCR for *stx1*, *stx2*, *eae*, *ehxA*, and *saa*. J. Clin. Microbiol.
14 40, 271-274.
- 15
- 16 Picard, B., Garcia, j. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J.,
17 Denamur, E., 1999. The link between phylogeny and virulence in *Escherichia coli*
18 extraintestinal infection. Infect. Immun. 67, 546-553.
- 19
- 20 Selander, R. K., Caugant, D. A., Whittam, T. S., 1987. Genetic structure and variation in
21 natural populations of *Escherichia coli*. In: Neidhardt, F. C., Ingraham, J. L., Low, K. B.,
22 Magasanik, B., Schaechter, M., Umbager, H. E. (Eds.), *Escherichia coli* and *Salmonella*
23 *typhimurium*:cellular and molecular biology. American Society for Microbiology,
24 Washington,D.C., pp. 1625-1648.
- 25

1 Sneath, P. H. A., Sokal, R. R., 1973. Numerical Taxonomy. Freeman (Ed.), San Francisco,
2 pp. 423-438.

3

4 Souza, V., Rocha, M., Valera, A., Equiarte L. E., 1999. Genetic structure of natural
5 populations of *Escherichia coli* in wild hosts on different continents. Appl. Environ.
6 Microbiol. 65, 3373-3385.

7

8 Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D.
9 H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced
10 by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol.
11 33, 2233-2239.

12

13 Wu, X.-Y., Chapman, T, Trott, D. J., Bettelheim, K., Do, T. N., Driesen, S., Walker, M. J.,
14 Chin, J., 2007. Comparative analysis of virulence genes, genetic diversity, and phylogeny
15 of commensal and enterotoxigenic *Escherichia coli* isolates from weaned pigs. Appl.
16 Environ. Microbiol. 73, 83-91.

17

18 Yamamoto, T., Echeverria, P., 1996. Detection of the enteroaggregative *Escherichia coli*
19 heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E.coli* strains pathogenic for
20 humans. Infect. Immun. 64, 1441-1445.

21

22

23

24

25

1

2 **Figure captions**

3 Fig. 1. Dendrogram of the similarity relation of PFGE *Not I* patterns of the 76 *E. coli*
4 isolates belonging to the phylogenetic group A. Each of the isolates is defined by:
5 taxonomic species of the host / a, b or c refers to an individual of a given species, an Arabic
6 numeral refers to a number of an isolate identified in an individual. Roman numeral defines
7 particular similarity clusters. The degree of similarity/relation (%) is shown on the scale.

8

9 Fig. 2. Dendrogram of the similarity relation of PFGE *Not I* patterns of the 163 *E. coli*
10 isolates belonging to the phylogenetic group B1.

11

12 Fig. 3. Dendrogram of the similarity relation of PFGE *Not I* patterns of the 33 *E. coli*
13 isolates belonging to the phylogenetic group B2. Roman numeral defines particular
14 similarity clusters. Roman numeral with character defines particular subclusters.

15

16 Fig. 4. Dendrogram of the similarity relation of PFGE *Not I* patterns of the 28 *E. coli*
17 isolates belonging to the phylogenetic group D.

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 n	Number of isolates in phylogenetic groups:			
		a	b	c		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	

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:			
<i>eaeA</i>	Intimin	Pathogenicity island LEE	EPEC, EHEC
<i>bfpA</i>	Type IV bundle-forming pili	EAF plasmid	EPEC
<i>faeG</i>	F4 fimbrial adhesin	plasmid	EPEC
<i>fanC</i>	F5 fimbrial adhesin	plasmid	EPEC
<i>fasA</i>	F6 fimbrial adhesin	plasmid	EPEC
<i>fedA</i>	F18 fimbrial adhesin	plasmid	EPEC
<i>fimF</i> ₄₁	F41 fimbrial adhesin	chromosome	EPEC
Toxins:			
<i>eastI</i>	EaggEC heat-stable enterotoxin 1 (EAST 1)	plasmid; chromosome	EHEC, EPEC (EaggEC in human)
<i>ehxA</i>	Enterohemolysin (Ehx)	plasmid	EPEC, EHEC
<i>eltA</i>	Heat-labile toxin (LT)	plasmid	EPEC
<i>cnf1</i>	Cytotoxic necrotizing factor 1 (CNF1)	chromosome	ExPEC
<i>cnf2</i>	Cytotoxic necrotizing factor 2 (CNF2)	plasmid	EPEC, ExPEC
<i>est I</i>	Heat-stable enterotoxin a (STa)	plasmid; transposons	EPEC
<i>est II</i>	Heat-stable enterotoxin b (STb)	plasmid; transposons	EPEC
<i>stx 1</i>	Shiga toxin I (Stx 1)	bacteriophage	EHEC
<i>stx 2</i>	Shiga toxin II (Stx 2)	bacteriophage	EHEC

Table3. Occurrence of virulence gene profiles in *E. coli* isolates from five herbivorous species

Virulence gene profile*	Number, O serogroup and phylogenetic group of isolates found in samples from:					Total no. 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)
east I	-	-	-	-	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)
east I, stx2	-	-	-	1, O26, B1	-	1 (0.3)
stx1, stx2	2, O40, B1	2, O40, B1 1, NT, B1	-	-	-	5 (1.7)
Total no. of VG- positive isolates (%)	9 (28.1)	8 (22.9)	8 (23.5)	6(17.6)	4(20.0)	35 (11.7)

* VGs such as cnf2, estI, and est II were not found

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

Virulence gene profile*	Number, O serogroup, and phylogenetic group of isolates found in samples from :					Total no. of VG - positive isolates (%) n=300
	Carnivores			Omnivores		
	Lion n=36	Lynx n=33	Wildcat n=21	Dingo n=22	Raccoon n=33	
cnf2	2, O126, B2	-	-	1, O126, B2	-	3 (1.0)
eaeA	1, O26, B1	-	-	-	-	1 (0.3)
east I	-	-	1, O8, D	-	1, O8, D	2 (0.7)
est I	2, O9, A	1, NT, A	-	-	1, NT, A	4 (1.3)
est II	-	2, O80, A 2, NT, A	-	1, NT, A	-	5 (1.7)
stx1	-	1, O5, A	-	-	-	1 (0.3)
east I, est I	-	-	2, NT, A	2, O130, A	3, NT, A	7 (2.3)
Total no. of VG- positive isolates (%)	5(13.9)	6(18.2)	3 (14.3)	4 (18.2)	5 (15.1)	23 (7.7)

* VGs such as ehxA and stx2 were not found

Accepted Manuscript

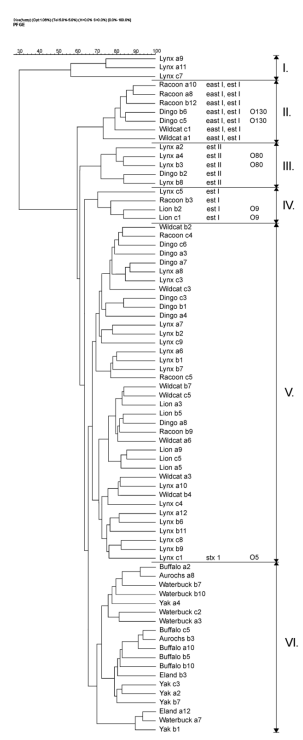


Fig. 1.

Accepted Manuscript

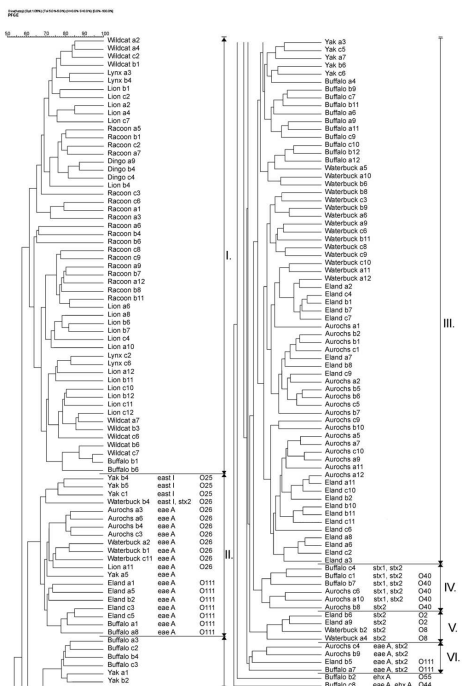


Fig. 2.

ed Manuscript

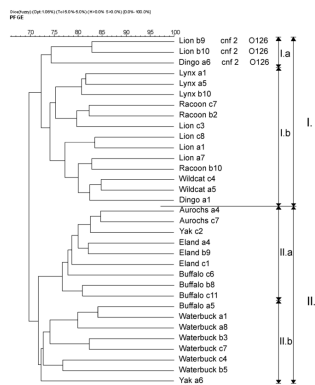


Fig.3.

Accepted Manuscript

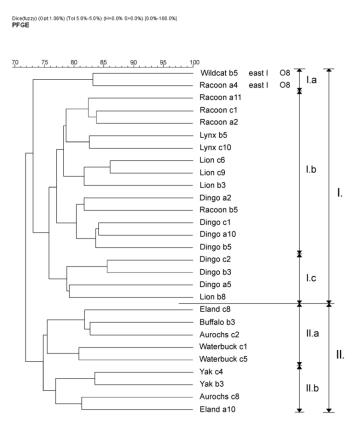


Fig 4.