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High prevalence iron receptor genes of avian pathogenic Escherichia coli

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High prevalence iron receptor genes of avian pathogenic *Escherichia coli* Ellen Ons^{*}, Nele Bleyen, Huruma Nelwike Tuntufye, Fréderic Vandemaele and Bruno Maria Goddeeris

Abstract

Avian Pathogenic *Escherichia coli* (APEC) are known to cause significant losses in the poultry industry worldwide. Although prophylactic measures based on vaccination are advisable, until now no full heterologous protection against colibacillosis has been achieved. Since iron is an essential nutrient to these bacteria, the aim of this study was to investigate the prevalence of 12 outer membrane iron receptor genes in 239 pathogenic strains isolated from clinical cases of colibacillosis in chickens. Five multiplex PCRs were developed as a tool for efficient screening. Among the 239 avian *E. coli* isolates, 100% were positive for *fhuE* and *fepA*, 96.2% for *fiu*, 92.9% for *cir*, 92.5% for *iroN*, 87.4% for *iutA*, 63.2% for *fecA*, 53.1% for *fyuA*, 46.9% for *fhuA*, 45.6% for *ireA*, 41.8% for *chuA* and 4.6% for *iha*.

Introduction

Avian pathogenic *Escherichia coli* (APEC) cause disease in poultry worldwide leading to major economic losses (Gross, 1994; Dho-Moulin & Fairbrother, 1999).

FepA, FhuA, FhuE, FiuA, FyuA, Iha, IreA, IroN and IutA) and one receptor for haem

(ChuA) (Wagegg & Braun, 1981; Hantke, 1981, 1983; Fecker & Braun, 1983; de

Lorenzo et al., 1986; Pierce & Earhart, 1986; Torres & Payne, 1997; Schubert et al.,

1998; Russo et al., 2001; Hantke et al., 2003; Léveillé et al., 2006).

To identify the most prevalent OM receptors involved in iron uptake, 5 multiplex PCRs were developed to investigate 239 APEC strains recovered from colibacillocisaffected chickens.

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Deleted: In the intestine where soluble ferrous iron is present, bacteria can take up iron by the Feo system or by the metal-ABC permease SitABCD (Kammler *et al.*, 1993, Sabi *et al.*, 2006). In the blood, an iron-sequestration system involving outer membrane (OM) receptors and siderophores is needed to take up the less soluble ferric iron (Andrews *et al.*, 2003).

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Materials and Methods

Bacterial strains and media. The 239 strains collected from different farms and outbreaks were isolated from blood or organs from chickens showing distinct clinical symptoms of APEC infection and were kindly provided by DGZ (Drongen, Belgium, 175 strains) and Intervet International (Boxmeer, The Netherlands, 64 strains). O serogrouping of the Belgian strains was performed as described by Vandemaele et al. Deleted: while (2003). The Dutch strains were O serogrouped by agglutination with reference antisera at the National Institute of Public Health (RIVM), Bilthoven. For 155 of the strains, the which they originate, chicken type of origin is known: 76 strains from layers (serogroups: 2 x O1, 11 x O2 and Deleted: strains 25 x O78), 64 from broilers (serogroups: 3 x O1, 6 x O2 and 25 x O78) and 15 from Deleted: strains breeders (serogroups: 4 x O1, 1 x O2 and 3 x O78). O1, O2 and O78 serogroup Deleted: All distribution in the remaining 84 strains was 1, 6 and 10 respectively. Strains were grown in standard Luria-Bertani (LB) medium at 37°C at 230 rpm and stored at -80°C in LB medium containing 15% glycerol.

Multiplex PCR. Five multiplex PCRs were designed to detect the jron receptor genes (Table 1) using APEC1 (Vandemaele et al., 2006) and APEC25, a strain of the O109 serotype isolated from the peritoneum of a deceased broiler, as reference strains. Two µl of bacterial LB overnight culture were added to a 18 µl PCR master mix composed of Supertaq buffer (SphaeroQ, Leiden, The Netherlands), 0.75U Supertaq DNA polymerase (SphaeroQ), 0.2 mM dNTP (Roche Diagnostics, Mannheim, Germany), 5% DMSO (Invitrogen, Paisley, Scotland), 1 µM of each primer (Table 1; Invitrogen)

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and DNase free water. The PCR conditions were initiated with 93°C for 10 min and followed by 35 cycles each consisting of denaturation at 94°C for 1 min, the annealing temperature given in Table 1 for 1 min and extension at 72°C for 1 min. The PCR was terminated by a final elongation for 10 min at 72°C. All PCR products were visualized on a 2% agarose gel containing SYBRsafe by transillumination. DNase free water was used as negative control.

Statistical analysis. All statistical analyses were performed using the SAS software vs. 8.2 (SAS institute Cary, NC, USA). The Univariate procedure and the Kruskal-Wallis analysis were applied and the significance level was set to $\alpha = 0.05$.

Results and Discussion

The multiplex PCRs gave clear, PCR products (Figure 1): 92.5% of the strains were positive for *iroN*, 87.1% for *iutA*, 41.8% for *chuA*, 45.6% for *ireA* and 4.6% for *iha*. Similar prevalence rates were reported by Rodriguez-Siek et al. (2005) (89.7% iroN, 82.2% iutA, 44.4% ireA and 3.4% iha), by Ewers et al. (2007) (83.7% iroN, 41.3% ireA, 3.1% iha and 50.3% chuA) and by Vandekerchove et al. (2005) (90% prevalence for jutA). FyuA could be found in 53.1% of our APEC strains while other research groups found this gene in 58.8% (Rodriguez-Siek et al., 2005), 66.4% (Ewers et al., 2007), 71.3% (Ewers et al., 2004) or 90% (Vandekerchove et al., 2005) of the APEC strains from chickens. The suggestion that fyuA is part of an unstable pathogenicity island (Rakin et al., 1995) might explain these differences. The prevalence of fepA (100%), fhuE

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(100%), *fiu* (96.2%), *cir* (92.9%), *fecA* (63.2%) and *fhuA* (46.9%) in APEC strains has not been published before.

As broilers are more sensitive to APEC compared to other *E. coli* (Gross 1994) and O1, O2 and O78 are considered to be the predominant serogroups associated with infection (Dho-Moulin & Fairbrother, 1999) (although this is contradicted by more recent studies (Rodriguez-Siek *et al.*, 2005)), the correlation between type of bird or serogroup on one hand and gene prevalence on the other hand was investigated. The prevalence of *chuA*, *fhuA*, *fyuA* and *ireA* was significantly higher in strains containing the O1 or O2 antigens than in strains containing the O78 antigen (Table 2). *Jha*, was significantly more present in strains of the O1 serogroup than in strains of the O2 or the O78 serogroups. In contrast, *fecA* was more prevalent in strains belonging to the O78 serogroup followed by strains from the O2 and eventually by strains from the O1 serogroup. The *fecA* and *iutA* genes were significantly more present in strains not belonging to these serogroups. *FhuA* was significantly less prevalent among the O1+O2+O78 group.

Both *fyuA* and *chuA* were more frequently detected in strains isolated from layers (56.6% and 47.4%) and breeders (66.7% and 73.3%) than in those from broilers (32.8% and 23.4%) while *fiu* was significantly more prevalent in strains from broilers (98.4%) than in those from breeders (86.7%). For *fyuA* and *chuA* these differences could be partially related to their correlation with the O1, O2 and O78 serogroups. In conclusion, *fhuE*, *fepA*, *fiu*, *cir*, *iroN* and *iutA* are the most abundant in APEC strains. Interfering with these OM receptors may prove valuable for the protection of chickens against colibacillosis.

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References

- Andrews, S.C., Robinson, A.K. & Rodriguez-Quinones, F. (2003). Bacterial iron homeostasis. *FEMS Microbiology Reviews*, 27, 215-237.
- de Lorenzo, V., Bindereif, A., Paw, B.H. & Neilands, J.B. (1986). Aerobactin biosynthesis and transport genes of plasmid ColV-K30 in *Escherichia coli* K-12. *Journal of Bacteriology*, 165, 570-578.
- Dho-Moulin, M. & Fairbrother, J.M. (1999). Avian pathogenic *Escherichia coli* (APEC). *Veterinary Research*, *30*, 299-316.
- Ewers, C., Janssen, T., Kiessling, S., Philipp, H.C. & Wieler, L.H. (2004). Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. *Veterinary Microbiology*, 104, 91-101.
- Ewers, C., Li, G., Wilking, H., Kiessling, S., Alt, K., Antao, E.M., Laturnus, C., Diehl, I., Glodde, S., Homeier, T., Böhnke, U., Steinrück, H., Philipp, H.C. & Wieler, L.H. (2007). Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: how closely related are they? *International Journal of Medical Microbiology*, 297, 163-176.
- Fecker, L. & Braun, V. (1983). Cloning and expression of the fhu genes involved in iron(III)-hydroxamate uptake by *Escherichia coli*. Journal of Bacteriology, 156, 1301-1314.
- Gross, W.G. (1994). Diseases due to *Escherichia coli* in poultry. In C.L. Gyles. *Escherichia coli* in Domestic Animals and Humans (pp.237-259). Wallingford:
 CAB International.

- Hantke, K. (1981). Regulation of ferric iron transport in *Escherichia coli* K12: isolation of a constitutive mutant. *Molecular and General Genetics*, *182*, 288-292.
- Hantke, K. (1983). Identification of an iron uptake system specific for coprogen and rhodotorulic acid in *Escherichia coli* K12. *Molecular and General Genetics*, 191, 301-306.
- Hantke, K., Nicholson, G., Rabsch, W. & Winkelmann, G. (2003). Salmochelins, siderophores of Salmonella enterica and uropathogenic Escherichia coli strains, are recognized by the outer membrane receptor IroN. Proceedings of the National Academy of Sciences of the United States of America, 100, 3677-3682.
- Kammler, M., Schön, C. & Hantke, K. (1993). Characterization of the ferrous iron uptake system of *Escherichia coli*. *Journal of Bacteriology*, 175, 6212-6219.
- Léveillé, S., Caza, M., Johnson, J.R., Clabots, C., Sabri, M. & Dozois, C.M. (2006). Iha from an *Escherichia coli* urinary tract infection outbreak clonal group A strain is expressed in vivo in the mouse urinary tract and functions as a catecholate siderophore receptor. *Infection and Immunity*, *74*, 3427-3436.
- Pierce, J.R. & Earhart C.F. (1986). *Escherichia coli* K-12 envelope proteins specifically required for ferrienterobactin uptake. *Journal of Bacteriology*, *166*, 930-936.
- Rakin, A., Urbitsch, P. & Heesemann, J. (1995). Evidence for two evolutionary lineages of highly pathogenic *Yersinia* species. *Journal of Bacteriology*, *177*, 2292-2298.
- Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J. & Nolan L.K. (2005). Characterizing the APEC pathotype. *Veterinary Research*, *36*, 241-256.

- Russo, T.A., Carlino, U.B. & Johnson, J.R. (2001). Identification of a new iron-regulated virulence gene, *ireA* in an extraintestinal pathogenic isolate of *Escherichia coli*. *Infection and Immunity*, 69, 6209-6216.
- Sabri, M., Léveillé, S. & Dozois, C.M. (2006). A SitABCD homologue from an avian pathogenic *Escherichia coli* strain mediates transport of iron and manganese and resistance to hydrogen peroxide. *Microbiology*, 152, 745-758.
- Schubert, S., Rakin, A., Karch, H., Carniel, E. & Heesemann, J. (1998). Prevalence of the "high –pathogenicity island" of *Yersinia* species among *Escherichia coli* strains that are pathogenic to humans. *Infection and Immunity*, 66, 480-485.
- Torres, A.G. & Payne, S.M. (1997). Haem iron-transport system in enterohaemorrhagic *Escherichia coli* O157:H7. *Molecular Microbiology*, 23, 825-833.
- Vandekerchove, D., Vandemaele, F., Adriaensen, C., Zaleska, M., Hernalsteens, J.P., De Baets, L., Butaye, P., Van Immerseel, F., Wattiau, P., Laevens, H., Mast, J., Goddeeris, B. & Pasmans F. (2005). Virulence-associated traits in avian *Escherichia coli*: comparison between isolates from colibacillosis-affected and clinically healthy layer flocks. *Veterinary Microbiology*, *108*, 75-87.
- Vandemaele, F., Bleyen, N., Abuaboud, O., vanderMeer, E., Jacobs, A. & Goddeeris,
 B.M. (2003). Predominance of the papGII allele with high sequence homology to that of human isolates among avian pathogenic *Escherichia coli* (APEC). *Veterinary Microbiology*, 97, 245-257.
- Vandemaele, F., Bleyen, N., Abuaboud, O., vanderMeer, E., Jacobs, A. & Goddeeris B.M. (2006). Immunization with the biologically active lectin domain of PapGII

induces strong adhesion-inhibiting antibody responses but not protection against avian pathogenic Escherichia coli. Avian Pathology, 35, 238-349.

Wagegg, W. & Braun, V. (1981). Ferric citrate transport in Escherichia coli requires outer membrane receptor protein FecA. Journal of Bacteriology, 145, 156-163.

Figure legends

Figure 1: Detection of the iron receptor genes by multiplex PCR. Each picture shows the negative control (DNase free water) in the first lane, the molecular marker Smartladder SF (Eurogentec) with 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp and 1000 bp fragments in the middle lane and the positive control (APEC25 for iha and fiu and APEC1 for all other genes) in the last lane.

Δ bp. a middle lane ar genes) in the last t.

Table 1 The primer sequences, sizes of amplified fragments, and PCR conditions used forthe detection of the iron receptor genes

Multiplex	Gene	Primers	Length of PCR product (bp)	Annealing temperature
	iroN	IroN_F: 5' ctctggtggtggaagccacc 3'	814	
1		Iron_R: 5 igicggiacaggcggiicgic 3		
	iutA	lutA R: 5' toottoogataaagcoctcatg 3'	470	64°C
	fhuA	FhuA_F: 5' agacactatcaccgttaccgctg 3' FhuA_R: 5' gccgcgaatgatcaggtggtc 3'	265	
2	fyuA	FyuA_F: 5' acggctttatcctctggccttgg 3' FyuA_R: 5' tgaaaacccagtcatcggtgg 3'	878	56 5 %
	fepA	FepA_F: 5' agctgactgacagcaccatcg 3' FepA_R: 5' cgggatgatcgacaaacggtcg 3'	554	30.3 0
3	chuA	ChuA_F: 5' gttacggcaacggggaatgc 3' ChuA_R: 5' agcagggtaatcggcagatcg 3'	1061	
	fecA	FecA_F: 5' aggttaatatcgcaccgggatcg 3' FecA_R: 5' atggcatccatgttgccgagc 3'	565	68°C
	ireA	IreA_F: 5' tgttactgcctccgggttcac 3' IreA_R: 5' ccggtttttcattcccaccagtg 3'	158	
4	cir fhuE	Cir_F: 5' tgtcactgcatcttccgtggaac 3' Cir_R: 5' tgtccagcaagtcggaatcacg 3'	704	56%
		FhuE_F: 5' gtcggtcactattgttagccagc 3' FhuE_R: 5' aattcacgactggtcgcgtgt 3'	330	50 0
5	fiu	Fiu_F: 5' ccggtctttgtattggcatcacg 3' Fiu_R: 5' acgattcgctgtaccaaggcca 3'	689	
	iha	Iha_F: 5' ttactggcatgccgaggcagt 3' Iha_R: 5' cggatgctgatttccagcccac 3'	410	55°C

iron rocontor	Iron receptor genes (%)			Iron recept	Iron receptor genes (%)	
gene	O1 (n=10)	O2 (n=24)	O78 (n=63)	O1+O2+O78 (n=97)	Other (n=142)	
chuA	100 ^(a)	87.5 ^(a)	7.9 ^(b)	37.1	45.0	
fhuA	90.0 ^(a)	66.7 ^(a)	11.1 ^(b)	33.0*	56.3*	
ireA	90.0 ^(a)	75 ^(a)	36.5 ^(b)	51.4	41.5	
fyuA	90.0 ^(a)	87.5 ^(a)	44.4 ^(b)	59.8	48.6	
iha	40.0 ^(a)	4.00 ^(b)	0.00 ^(b)	5.20	4.00	
fecA	20.0 ^(a)	75 ^(b)	93.7 ^(c)	81.4*	50.7*	
cir	90.0	100	90.5	92.8	93.0	
fiu	90.0	95.8	96.8	95.9	96.5	
iroN	90.0	100	95.2	95.9	90.1	
iutA	100	100	96.8	97.9*	80.3*	
fepA	100	100	100	100	100	
fhuE	100	100	100	100	100	

Table 2: Prevalence of iron receptor genes in relation to O-serogroup

Significant differences ($P \le 0.05$) in prevalence for each individual iron receptor gene between strains of the O1, O2 and O78 serogroups are indicated with a different letter. Significant differences ($P \le 0.05$) in prevalence for each individual iron receptor gene between the total of strains containing one of these O1, O2 or O78 LPS antigens and strains belonging to any other serogroup are indicated with an asterix.





