Veterinary Microbiology 225 (2018) 72–78

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Characterization of *Escherichia coli* causing cellulitis in broilers

Louise Ladefoged Poulsen[a], Magne Bisgaard[b], Steffen Lynge Jørgensen[a], Tommy Dideriksen[c], Jacob Roland Pedersen[c], Henrik Christensen[a,*]

[a] University of Copenhagen, Department of Veterinary Animal Science, Faculty of Health and Medical Sciences, Stigbojlen 4, DK- 1870, Frederiksberg, Denmark
[b] Bisgaard Consulting, Viby, Denmark
[c] Danpo A/S, Denmark

ARTICLE INFO

Keywords:
SNP
ST117
PFGE
MLST
aat
pic
upaG

ABSTRACT

The aim of the study was to investigate cellulitis caused by *Escherichia coli* which has been responsible for economic and welfare problems in Danish broiler production between 2014 and 2016. The study included 13 flocks with unusually high condemnation rates due to cellulitis during a period of approximately one year. From six flocks, 126 condemned carcasses were collected at a Danish slaughterhouse. Further 272 broilers dead on their own were collected on nine broiler farms from flocks with increased mortality and cellulitis (2 farms included both birds from the rearing period and broilers subsequently condemned). All broilers were subjected to *post mortem* investigation including bacteriology and 247 *E. coli* isolates were obtained in pure culture from typical lesions of cellulitis. Two-hundred-thirty six *E. coli* isolates were investigated by pulsed field gel electrophoresis for clonality and 21 selected strains representing major clones were subsequently multi locus sequence typed allowing comparison to sequence types (ST) in the databases. One dominating PFGE type (A) was found to cause cellulitis on all 13 flocks (67% of all isolates). The clone belonged to ST117, which is well described as a pathogen in poultry, and was the primary agent responsible for cellulitis. Whole genome sequencing of eight *E. coli* isolates confirmed the close genetic relationship between isolates from the outbreaks and showed the presence of genes predicted to encode for the autotransporter proteins *aatA*, *pic* and *upaG*, reported to be of importance for adhesion of *E. coli* to eukaryotic cells.

1. Introduction

Escherichia coli has been associated with most cases of avian cellulitis (Nolan et al., 2015). In Denmark, cellulitis caused by *E. coli* has recently been observed as a problem in broilers both in terms of welfare and economy (Bisgaard et al., 2015a,b; 2016). Cellulitis is characterized by subcutaneous fibrinonecrotic plaques and inflammation of the overlying chicken skin. Cellulitis is usually initiated by scratches and wounds and followed by infection (Macklin et al., 1999; Norton et al., 1999). Typical lesions include discoloration of the skin of the lower abdomen near the cloaca/vent sometimes also involving the thighs. Cellulitis is normally not considered to result in clinical disease or decreasing normal growth of the birds (Elfadil et al., 1996). It often passes unnoticed during production and is subsequently recognized by condemnation of carcasses at processing which might result in major economic losses.

Different types of *E. coli* have previously been associated with cellulitis. According to Peighambari et al. (1995) and Ngeleka et al. (1996), *E. coli* isolated from cellulitis share similarities with other avian pathogenic *E. coli* (APEC). However, under experimental conditions strains isolated from cellulitis more often resulted in cellulitis compared to strains obtained from other sources (Ngeleka et al., 1996; Norton et al., 1997, 2000; Peighambari et al., 1995). It has been assumed that scratches and wounds were the port of entry for *E. coli* associated with cellulitis, and that the *E. coli* had their origins in the bedding, and therefore were of polyclonal nature (Macklin et al., 1999; Norton et al., 1999). The investigation by Singer et al. (1999) compared *E. coli* isolated from cellulitis from different houses on different farms, and found that *E. coli* in different houses on the same farm were closer related than isolates from different farms. These authors suggested that *E. coli* populations were endemic to the single farm and originally shared a common origin in the hatchery. Further investigation by Singer et al. (2000) confirmed the observation and reported numerous pulsed field gel electrophoresis (PFGE) profiles on the same farm, which persisted independently of cleaning and disinfections strategies.

The aim of the present study was to investigate the clonal relationship of *E. coli* isolated from cellulitis and if a haematogenous spreading of these *E. coli* to internal organs might take place.

* Corresponding author.
E-mail address: hech@sund.ku.dk (H. Christensen).

https://doi.org/10.1016/j.vetmic.2018.09.011
Received 20 April 2018; Received in revised form 9 August 2018; Accepted 16 September 2018

Table 1
Investigation of 12 broiler farms affected by cellulitis.

									PFGE group (number of isolates) ST					
Time (months) period for the investigation	Farm	Flock	House	Origin of broilers [a]	Number of broilers	Number of broilers with *E. coli* isolated in pure culture	Numbers of *E. coli* analysed by PFGE	Condemned due to cellulitis at slaughter (%)	117	117	117	371	ND	Number of PFGE types
1	A	1	2	C	14	14	14	0.17	A (2)			R (12)		2
2	H	2	2	P	28	26	33	7.78	A (31)	M (2)				2
2	J	3	A	P	23	10	13	0.27	A (13)					1
2	J	3	B	P	23	7	8	0.24	A (7)				s (1) [b]	2
2	K	4	1	P	16	3	5	0.17	A (4)				s (1)	2
2	G	5	3	P	24	4	4	0.36	A (4)					1
2	B	6	1	C	10	10	7	0.10	A (5)				s (1), s (1) (ST101)	3
4	I	7	1	P	30	22	12	0.08	A (12)					1
4	D	8	1	P	24	18	5	0.29	A (1)				J (4)	2
4	D	8	2	P	25	17	5	0.18		M (5)				1
4	D	8	1	C	19	19	17	0.29	A (11)	M (3)			N (1), O (2)	4
4	D	8	2	C	19	19	16	0.18	A (14)	M (1)			s (1)	3
5	E	9	1	C	19	19	22	1.55	A (16)			R (3)	s (1), s (1), s (1)	5
6	F	10	1	P	21	7	6	0.52	A (6)					1
8	C	11	3	P [c]	17	4	12	0.36	A (12)					1
10	L	12	3	C	20	15	10	0.14			B (10)			1
11	L	12	3	P	20	15	8	0.14			B (8)			1
11	L	12	3	P	21	19	28	0.14	A (10)		B (8)		I (6), E (2), F (2)	5
15	G	13	3	C	25	10	11	0.92	A (11)					1
Total	12	13			398	258	236		159	11	26	15	25	

[a] P, production day 34 except where indicated; C, condemned at slaughter. The grey shading indicates chicken from the same flocks sampled both in production and slaughter.
[b] s, singleton.
[c] samples from 11 days old broilers.

2. Materials and methods

Dead chicken were collected for *post mortem* analysis from Danish broiler flocks with a high mortality at the end of production. The study included flocks in which cellulitis was confirmed by *post mortem* analysis and from which *E. coli* was isolated in pure culture with massive growth from the lesions (n = 272). From the slaughterhouse receiving broilers from the farms, condemned animals due to cellulitis (n = 126) were collected, and these birds were also subjected to *post mortem* analysis. On the average some 30 broilers were collected from each flock (Table 1). Birds collected at farms and at the slaughterhouse were immediately frozen at – 20 °C until *post mortem* and bacteriological examination.

Bacteriological samples were collected during *post mortem* examinations of dead broilers from subcutis of affected tissue as well as from the affected organs (mainly liver and spleen). Samples were plated on blood agar plates (BA) (5% calf blood in blood agar base, Oxoid, Basingstoke, UK) and grown aerobically overnight at 37 °C in a sealed plastic bag. From pure cultures demonstrating a typical colony morphology of *E. coli* (circular with an entire margin, low convex, medium size, with a light grey color on BA) a single colony was sub-cultured in Brain Heart Infusion Broth (BHI) (Difco, Brøndby, Denmark) from each plate showing abundant growth and stored in 15% glycerol at −80 °C. The combination of a distinct sequence type obtained by MLST and clonal relations obtained by PFGE analysis documented the correct identification of *E. coli*.

For PFGE, the protocol was modified after http://www.pulsenetinternational.org/protocols/ including the use of the restriction enzyme Xbal (Thermo Scientific). All gels were normalized according to an internal reference strain which was run together with the Low Range PFGE marker (New England Biolabs, Ipswich, USA). The gels were analysed by GelComparII (Applied Math, Sint-Martens-Latem, Belgium) (1% optimization, 1% tolerance), and the Dice similarity calculated and a dendrogram constructed by UPGMA (unweighted pair-group arithmetic average clustering). A PFGE profile was designated a capital letter if it included at least two strains, showing the same fragment pattern tolerating variation in one fragment, or was designated as a singleton, if only a single strain was found with a pattern.

The genomes of eight strains selected to represent different farms (6 out of 12), time points (5 out of 15) and PFGE types (4 out of 18) were whole genome sequenced. DNA was extracted using DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and sequencing was done by MiSeq (Illumina, San Diego, USA). De novo assembly was performed by CLC Genomic Workbench (QIAGEN, Aarhus A/S). The genomes were annotated in RAST (http://rast.nmpdr.org/rast.cgi) (Aziz et al., 2008). Proteins were further identified by BLAST search in UniProt (www.uniprot.org) and at NCBI (www.ncbi.nlm.nih.gov). Prediction of virulence genes, SNPs (single nucleotide polymorphisms), MLST (multilocus sequence type) and serotype of the whole genomic sequences was performed at Center for Genomic Epidemiology (DTU) (Larsen et al., 2012; Leekitcharoenphon et al., 2012; Joensen et al., 2014, 2015). The phylogeny of SNPs was analysed in MEGA7 (Kumar et al., 2016).

Thirteen *E. coli* strains selected to represent the PFGE clusters and not included with the whole genomic sequences, were typed by MLST using primers and PCR conditions as described at http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search (Wirth et al., 2006). The PCR products were sequenced in both directions (Macrogen Inc. Seoul, Korea) and the sequences obtained were analysed using CLC Main Workbench 7 (Qiagen Bioinformatics, Aarhus, Denmark), and compared to existing alleles in the *E. coli* MLST scheme, and each strain was assigned a sequence type.

3. Results

Out of the 398 broilers investigated from 12 farms representing 13 flocks (farm G was sampled twice), 246 animals (62%) demonstrated *E. coli* in pure culture from typical lesions of cellulitis (Table 1). The condemnation rates related to cellulitis at the slaughterhouse varied from 0.10 to 7.78% with an average of 0.73%. If the single outlier of 7.78% was excluded from the comparison, the average was 0.34%. Typical lesions included discoloration of the skin of the lower abdomen and the cloacal region in addition to subcutaneous fibrinopurulent