Veterinary Microbiology 166 (2013) 276–280

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

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

Short communication

Characterization of *Escherichia coli* O78 from an outbreak of septicemia in lambs in Norway

Cecilie K. Kjelstrup [a], Lotte P. Stenfors Arnesen [a], Erik G. Granquist [b], Trine M. L'Abée-Lund [a,*]

[a] Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, 0033 Oslo, Norway
[b] Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, P.O. Box 8146 Dep, 0033 Oslo, Norway

ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 14 May 2013
Accepted 15 May 2013

Keywords:
Escherichia coli
Septicemia
Sheep
Neonatal
Virulence

ABSTRACT

The aim of the study was to characterize isolates of *Escherichia coli* from an outbreak of septicemia in a Norwegian sheep flock in 2008 with emphasis on virulence, serological grouping, phylogenicity and homology. Six *E. coli* isolates from succumbed neonatal lambs and four *E. coli* isolates collected from healthy individuals were analyzed by Pulsed-Field Gel Electrophoresis (PFGE), miniaturized microarray, and polymerase chain reaction (PCR). The septicemic *E. coli* isolates showed identical pulsotypes (PTs), and belonged to serogroup O78, phylogenetic group A, and MLST ST 369. The virulence genes *f17G*, *bmaE*, *afaE-VIII*, *ireA*, *iroN* and *iss* were detected in the septicemic isolates. The results showed that the *E. coli* isolates from the septicemic outbreak had a clonal appearance, thus likely originating from a common source. The clone carried genes important for virulence, however, a significant explanation for the high pathogenicity was not revealed.

1. Introduction

The prevalence of the neonatal loss of lambs in Norwegian sheep husbandries were estimated to 3.3% in 2011 (Ringdal et al., 2011), which equals a loss of approximately 25,000 lambs. Infections caused by *Escherichia coli*, malnutrition, hypothermia and gastro intestinal diseases, are common causes of death in neonatal lambs.

E. coli are part of the intestinal microbiota in warm blooded animals and man and colonize the neonatal gut soon after birth. Although the majority of *E. coli* strains are commensals, some strains have the ability to cause disease. The categories of such pathogenic *E. coli* are divided into two major groups; the diarrhogenic *E. coli* (DEC) and the extraintestinal pathogenic *E. coli* (ExPEC). Septicemic *E. coli* within the ExPEC group are poorly defined regarding pathogenic traits.

A range of molecular and other typing methods are established for deciphering and recognizing pathogenic strains and for source attribution identification during outbreaks of *E. coli* infections. The resolution and applicability of the methods vary, and often a combination of typing methods is applied for a thorough investigation, serotyping, phylogenetic grouping, pulsed field gel electrophoresis (PFGE), and multi locus sequence typing (MLST).

Virulence factors in *E. coli* fall into five main categories; iron uptake systems, adhesins, capsular synthesis, invasins and toxins (Russo and Johnson, 2000). Several genes can encode a range of virulence factors within each category. The pangenome of *E. coli* consists of more than 17,000 unique genes, while individual *E. coli* genomes consist of about 5000 genes, of which 2000 are conserved and constitute the core genome (Touchon et al., 2009). This illustrates the genomic diversity of *E. coli* and allows for numerous combinations of virulence genes.

* Corresponding author. Tel.: +47 22 96 45 00.
E-mail addresses: trine.labee-lund@nvh.no, trine.labeelund@gmail.com (T.M. L'Abée-Lund).

0378-1135/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2013.05.004

In 2008, a sheep flock on the west coast of Norway experienced neonatal losses due to septicemia. The sheep flock had no previous history of such infections. The lambs underwent post mortem examination and *E. coli* isolates were grown from internal organs. Little is hitherto known about strains of *E. coli* causing septicemia in neonatal lambs and the current study intends to characterize the *E. coli* from an outbreak of septicemia in a Norwegian sheep flock.

2. Materials and methods

2.1. Outbreak description and isolation of bacteriological samples

In the spring of 2008, six neonatal lambs in one farm died of septicemia on the west coast of Norway. The sheep flock comprised of 230 ewes and had no known previous history of *E. coli* septicemia. Four of the septicemic lambs succumbed two to four days post-partum, and were observed paretic and moribund with opisthotonus prior to death. An additional two lambs died three weeks old (Supplementary data, Table S1). Bacterial samples were collected from viscera during post mortem examination.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2013.05.004.

Faecal swabs from five healthy lambs were collected at the same farm in the spring of 2009, to gather *E. coli* control isolates.

2.2. Bacteriological analyses

Bacterial isolates from internal organs of deceased lambs were identified as *E. coli* based on morphology, ability to ferment lactose, indole positivity and citrate negativity. A total of 19 isolates of *E. coli* were identified from the six lambs (Table S1).

Faecal swabs were streaked on bromo thymol blue lactose (BTB) plates, and the two quantitatively dominating lactose fermenting colonies from each BTB plate were selected as control isolates after being identified as *E. coli*. The controls were designated numbers 1 through 10 (Ctr1–Ctr10).

2.3. Genotyping

E. coli isolates from two internal organs of each of lambs 1 to 4 (Table S1) together with the ten *E. coli* control isolates were genotyped using Pulsed-Field Gel Electrophoresis (PFGE) after XbaI restriction as described by Gautom (Gautom, 1997). From the two remaining septicemic lambs, only *E. coli* from one internal organ from each lamb was available (Table S1), and these isolates were run on PFGE and compared with the *E. coli* isolates of the first four lambs. The interpretation of the DNA fragment polymorphism patterns was performed by visual comparison and each unique PFGE pattern was defined as a pulsotype (PT). Isolates belonging to the same PTs were considered indistinguishable, while isolates with PTs differing by more than five bands were considered heterologous.

2.4. DNA extraction and virotyping

Qiagen DNeasy blood and tissue kit was used to extract bacterial genomic DNA with the following modifications of the manufacturer's protocol (Qiagen, Hilden, Germany): the Luria-Bertani (LB) culture volume was increased to 2 mL and during the elution step the flow-through was run through the column twice.

The concentrations and quality of the DNA were measured by spectrophotometry on Nanodrop ND-1000 (Thermo Scientific, Wilmington, USA). The successful extraction of bacterial genomic DNA was confirmed by running a 16S r-DNA PCR prior to virulence gene detection. After DNA isolation, the Identibac ArrayTube protocol was applied (Identibac, Surrey, UK) on all the septicemic *E. coli* isolates from lambs 1–4 and the four *E. coli* controls (Ctr 1–Ctr 4).

Virulence genes that were not included on the Identibac Array Tube chip were detected by PCR. The genes and primers were selected on the basis of literature studies focusing on virulence factors in ExPEC (Table S2) (Ghanbarpour and Oswald, 2009; Chapman et al., 2006). PCRs were run on the septicemic isolate *E. coli* K46 (lamb 2) and the four *E. coli* controls. PCR was performed with Taq polymerase according to the recommendations of the manufacturer (Invitrogen, Carlsbad, USA).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2013.05.004.

2.5. Phylogenetic groups, serogrouping and MLST

A triplex PCR as described by Clermont and coworkers (Clermont et al., 2000) was used to assign the septicemic and the four control *E. coli* isolates to phylogenetic groups based on three DNA markers; the genes *chuA*, *yjaA* and the DNA fragment TSPE4C2 (Table S2).

The septicemic isolate *E coli* K46 from lamb 2, was serogrouped at the Norwegian Veterinary Institute (Oslo, Norway).

MLST was performed by Genoscreen (Lille, France) using the seven housekeeping genes *adk, fumC, gyrB, icd, mdh, purA* and *recA.*

3. Results

3.1. Genotyping

All eight *E. coli* isolates from lamb 1–4 and the two isolates from lambs 5 and 6 had identical PTs, designated PT1. The ten controls had eight heterologous PTs, all of which differed from PT1. Of these ten controls, four strains with different PTs were included as controls. The identical PTs of the septicemic *E. coli* isolates revealed that the *E. coli* isolates had a clonal appearance, and indicated that the outbreak had a common source of infection. This is further supported by antibiotic resistance and serum survival features, as the septicemic isolates from all six lambs were