

1 **Presence of extended spectrum β -lactamase producing *Escherichia coli* in**
2 **wild geese**

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28 Since 2000, extended-spectrum β -lactamase (ESBL) producing bacteria have increasingly
29 been detected in humans and animals. Their impact on human health has drawn much
30 attention worldwide. Many reports exist about the diversity of ESBLs among
31 Enterobacteriaceae from food-producing animals ¹. Also for companion animals, several
32 studies have been described ¹. Recently some surveys have suggested that European wild
33 birds may act as reservoirs of resistant bacteria ² and might have an epidemiological role in
34 the dissemination of resistance. Therefore, to gain more insight in the role of migratory birds
35 as reservoir in this, a large population of wild geese in Belgium was screened for the presence
36 of ceftiofur resistant *Escherichia coli* (*E. coli*).

37 For this purpose, cloacal swabs from 396 wild geese (354 *Branta canadensis* and 42 wild
38 *Anser anser domesticus*) originating from 6 wildlife areas in Belgium were collected and
39 within 4 hours inoculated on MacConkey agar plates (Oxoid LTD, Basingstoke, Hampshire,
40 England) supplemented with ceftiofur (8 mg/L). After overnight aerobic incubation at 37°C,
41 suspected *E. coli* colonies were purified on Columbia agar with 5% sheep blood (blood agar,
42 Oxoid) and phenotypically identified ³. To confirm resistance against the β -lactams, the
43 antimicrobial susceptibility of the *E. coli* isolates against ampicillin (10 μ g), ceftiofur (30 μ g)
44 and amoxicillin-clavulanic acid (20 + 10 μ g) (Neo-sensitabs, Rosco Diagnostica, Taastrup,
45 Denmark) was determined using the disk diffusion test according to the guidelines of the
46 Clinical Laboratory Standards Institute (CLSI, 2008) ⁴. The β -lactamases of the cultured *E.*
47 *coli* were characterized by performing polymerase chain reaction (PCR) for detection of genes
48 encoding TEM-, SHV-, CTX-M- and CMY- type enzymes as previously described ^{5,6}. To
49 establish the clonal relationship between the *E. coli* isolates, multilocus sequence analysis
50 (MLST) using seven conserved housekeeping genes (*adh*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and

51 *recA*) (<http://mlst.ucc.ie>) was performed ⁷. All PCR products were purified using a
52 Nucleospin Extract II kit (Marchery-Nagel GmbH & Co. KG, Düren) and sequenced using
53 GeneAmp PCR 9700 Applied Biosystems sequencer (Foster City, USA). For sequencing, the
54 PCR primers were used. The obtained nucleotide sequences were compared with those
55 previously described for *bla* genes (BLAST database: <http://www.ncbi.nlm.nih.gov/BLAST/>).
56 From the 396 faecal samples, 2 ceftiofur resistant *E. coli* isolates were obtained. The isolates
57 originated from geese of the same wildlife area (Donkmeer, Berlare). Characterisation and
58 sequencing of the genes encoding the β -lactamases showed that the first *E. coli* isolate,
59 originating from a Canada goose (*Branta canadensis*), carried a *bla*_{SHV} gene encoding ESBL
60 SHV-12. The sequence type of the *E. coli* isolate after MLST analysis corresponded to ST
61 1079. The second isolate, originating from a wild domestic goose (*Anser anser domesticus*),
62 was found to carry a *bla*_{TEM} gene encoding ESBL TEM-52. This isolate was assigned to ST
63 1844.

64 The population of wild domestic and Canada geese in Belgium is estimated at 10000 birds ⁸.
65 Since 396 wild geese were swabbed, approximately 4% of the total Belgian population was
66 included in the study. ESBL producing *E. coli* were only isolated out of 2 geese (0.5% of the
67 sampled animals). Analysis of the ESBL profile of the two ceftiofur resistant *E. coli* isolates
68 in this study resulted in the identification of the genes TEM-52 and SHV-12. These genes are
69 often present in ceftiofur resistant *E. coli* from poultry, cattle, pigs and humans ¹. The
70 sequence types of the two *E. coli* isolates already existed in the MLST Database
71 (<http://mlst.ucc.ie>). ST 1079 was previously isolated from a bovine that died because of extra-
72 intestinal pathogenic *E. coli* (ExPEC) septicaemia in the United Kingdom. ST 1844 was
73 isolated from a healthy human in France. This demonstrates that the MLST-types found in the
74 geese are not restricted to wild birds.

75 In conclusion, although the role of wild geese as a reservoir of bacteria carrying ESBL
76 encoding genes seems to be limited at present, the results of this study may indicate that these
77 resistance determinants have disseminated in the natural environment.

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83 **TRANSPARENCY DECLARATION**

84 None to declare.

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