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Analysis of Feces Samples Collected from a Wild-Bird Garden Feeding Station in Scotland for the Presence of Verocytotoxin-Producing *Escherichia coli* O157

Geoffrey Foster,[1]* Judith Evans,[2] Hazel I. Knight,[2,3] Alastair W. Smith,[2,3] George J. Gunn,[2] Lesley J. Allison,[4] Barti A. Synge,[1] and Tom W. Pennycott[5]

Scottish Agricultural College Veterinary Services, Drummondhill, Stratherrick Road, Inverness IV2 4JZ,[1] Scottish Agricultural College Epidemiology Research Unit, Animal Health Group, Scottish Agricultural College Research, Kings Buildings, Edinburgh EH9 3JG,[2] Centre for Tropical Veterinary Medicine, University of Edinburgh, Roslin, Midlothian EH25 9RG,[3] Scottish E. coli O157 Reference Laboratory, Western General Hospital, Crewe Road, Edinburgh EH4 2XU,[4] and Avian Health Unit, Scottish Agricultural College Veterinary Services, Auchincruive, Ayr KA6 5AE,[5] United Kingdom

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Composite wild bird feces collected at regular intervals from a garden feeding station in southwest Scotland over a 3-year period were examined for verocytotoxin-producing *Escherichia coli* O157. One sample was positive for *Escherichia coli* O157. The isolate belonged to phage type 21/28 and possessed vtx_2, *eaeA*, and enterohemorrhagic *E. coli hlyA* genes.

Verocytotoxin-producing *Escherichia coli* (VTEC) O157 is particularly prevalent in Scotland, where the majority of infections are sporadic (13). VTEC strains are characterized by the production of one or both of two toxins, Shiga toxin 1 or Shiga toxin 2, which are encoded by the genes vtx_1 and vtx_2. A subset of VTEC, designated enterohemorrhagic *E. coli* (EHEC), carries the additional virulence genes *eae*, associated with attachment and effacement of enterocytes in vitro, and *hly*, which encodes enterohemolysin (3).

Direct or indirect contact with animals and their products has been demonstrated to be important in transmission of VTEC O157 in human infections (5, 7, 10, 14). Cattle are regarded as the main animal reservoir for VTEC O157 (9, 15, 24), but the organism has also been recovered from a range of other domesticated animals, including sheep, goats, pigs, horses, and dogs (23); free-ranging deer (19, 20); zoo animals (4); rodents (2); and flies (2, 9). Among avian species, VTEC O157 has been reported to be present in broiler chickens (8), turkeys (11), and wild birds, including seagulls (25) and geese (22). Garden birds represent a group with which the Scottish public has increasing contact, but little is known about the potential impacts on human health that such interactions may cause. This paper is the first longitudinal study of the presence of VTEC O157 in the feces of wild birds visiting a garden feeding station in Scotland.

Two hundred thirty-one composite samples of wild-bird feces were collected over a 36-month period from a garden feeding station in a rural setting in Dumfries and Galloway, Scotland, as previously described (18). Specimens were collected twice weekly and the numbers and species of birds visiting the site recorded (18). The table (60 by 40 cm and 135 cm above the ground) was scraped clean daily to remove uneaten food, husks, and feces and was disinfected only if it was heavily soiled (18). Samples were transported to the laboratory and portions removed for *Salmonella* and *E. coli* O86 testing (18). The remainder was immediately frozen at −20°C and subsequently transferred to −80°C. Samples for testing were rapidly thawed in a water bath at 50°C before being tested for *E. coli* O157. Previous trials using spiked feces had demonstrated that *E. coli* O157 numbers were maintained when subjected to this rapid thaw process following freezing at −80°C (unpublished findings).

One-gram fecal samples were tested using a standard immunomagnetic separation methodology (6) followed by plating on sorbitol MacConkey agar (Oxoid, Basingstoke, United Kingdom) containing cefixime and tellurite (Mast Diagnostics, Bootle, United Kingdom) (CT-SMAC). Non-sorbitol-fermenting colonies on this medium were tested by agglutination with *E. coli* O157 latex reagent (Oxoid). The presence of vtx_1, vtx_2, *eaeA*, and EHEC *hlyA* genes was detected by PCR as described by Paton and Paton (16), except that assay 2, detecting rbf_{O111} and rbf_{O157}, was omitted. Isolates were phage typed according to the method of Ahmed et al. (1), and pulsed-field gel electrophoresis (PFGE) was carried out as described previously (26).

One sample out of 231 composite feces collected from a bird table in southwest Scotland over a 3-year period was positive for *E. coli* O157. It cannot be discounted, however, that initially viable *E. coli* O157 cells in some samples were not detected due to either the storage process, the sensitivity of the test method, or other factors. The positive sample was collected in June 2002, when birds seen visiting the table included blackbirds (*Turdus merula*), house sparrows (*Passer domesticus*), greenfinches (*Carduelis chloris*), and chaffinches (*Fringilla coelebs*). The positive isolate possessed vtx_2, *eaeA*, and EHEC *hlyA* genes and was phage type (PT) 21/28.

* Corresponding author. Mailing address: Scottish Agricultural College Veterinary Services, Drummondhill, Stratherrick Road, Inverness IV2 4JZ, United Kingdom. Phone: 44-1463-243030. Fax: 44-1463-711103. E-mail: geoffrey.foster@sac.co.uk.

This is the first report of the recovery of VTEC O157 from a garden feeding station. All the species recorded visiting the site prior to the positive sample being collected (blackbird, house sparrow, greenfinch, and chaffinch) can be found on farmland in the United Kingdom (12). Blackbirds, greenfinches, and chaffinches feed in hedges, woods, and near the edges of fields or in small paddocks, and rural house sparrows feed and nest mainly near farm buildings (12). Such species could therefore have been passively infected with VTEC O157 following direct or indirect contact with livestock. Alternatively, the source may have been another species whose presence had gone unnoticed.

Pawiak et al. (17) isolated one *E. coli* O157:K88 isolate among 200 isolates from nestling house sparrows and tree sparrows (*Passer montanus*), although the host sparrow species was not specified and specific tests for verocytotoxins not reported. It is of interest, however, that house sparrows were among the species observed during the period prior to the collection of the positive *E. coli* O157 isolate in this study. In a study of 12 feedlot and dairy farms in the United States, Hancock et al. (9) recovered *E. coli* O157 from one pooled bird feces sample, although those authors did not comment on the species of birds present.

PT 21/28 is the most frequently recovered phage type of *E. coli* O157 in the United Kingdom, in both humans (21) and cattle (15). In 2002, PT 21/28 was isolated from 65% of Scottish clinical cases of *E. coli* O157 infection; four of these patients resided in or had visited Dumfries and Galloway prior to the onset of infection. The PFGE profile of the bird isolate, however, did not match the PFGE profiles of the four Dumfries and Galloway clinical cases in 2002, nor has a matching pattern been observed among all the Scottish clinical isolates tested since. Most cases of human disease with *E. coli* O157, however, are caused by strains possessing vtx_2, *eae*, and EHEC *hly*, which were present in the bird sample.

Human VTEC O157 infections in Scotland often peak during the summer/autumn months, and most incidents are sporadic (13). While the strongest association for many of these cases is contact with farm animals, sources for some individual cases remain hard to identify (14). Garden feeding stations are common in the United Kingdom, and the isolation of VTEC O157 in the month of June indicates that they represent a potential source of human infection. In addition, small garden birds have acquired the habit of searching for food at picnic sites and outdoor restaurants, increasing the potential for transmission of VTEC O157 as well as other enteropathogens between birds and humans. It also is known that VTEC O157 appears and disappears from cattle herds over time (24), and it may be that local bird populations play a role in the spread of the organism between farms and possibly over larger distances due to migratory habits.

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