



Prevalence and diversity of Shiga toxin genes in Canada geese and water in western Lake Erie Region



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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) poses a major health risk by causing gastrointestinal illness and has been isolated from wild birds, including Canada geese (*Branta canadensis*). The major virulence factor for STEC infection is Shiga toxin. This study was designed to evaluate the occurrence and diversity of the Shiga toxin gene (*stx*) in Canada geese in the western Lake Erie region. Samples were collected from the Ottawa National Wildlife Refuge (ONWR) at Oak Harbor, Ohio from June to December, 2012, and occurrence of the *stx* gene was determined using polymerase chain reaction (PCR). Genetic diversity of *stx* variants among the fecal samples was examined using denaturing-gradient gel electrophoresis (DGGE). The Shiga toxin gene 2 (*stx2*) variant were detected in 20.8% ($n = 77$) of the geese fecal samples and 7% ($n = 71$) of the water samples. DGGE and clustering analysis showed a low *stx2* diversity and single genetic lineage of all the *stx2* fragments. All the *stx2* sequences from excised DGGE bands were similar to those from a toxin form of high potency (*stx2a*) and those from reported outbreak-causing serotypes (*E. coli* O157:H7, O165:H25, and O111:H–). Detection of *stx* from Canada geese suggested that viable Shiga toxin-producing *Escherichia coli* may be present. Further investigations, such as bacterial isolation, are suggested to better understand potential public health hazards in Lake Erie recreational areas, the role of Canada geese as a reservoir of Shiga toxin-producing *Escherichia coli*, and dissemination of these pathogenic bacteria.

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Introduction

In the United States, it is estimated that more than 110,000 cases of gastrointestinal illness are caused each year by Shiga toxin-producing *Escherichia coli* (STEC), with the majority of these cases attributed to *E. coli* O157:H7 (Mead et al., 1999). The major transmission route of *E. coli* O157:H7 is food (beef and produce); however, it can also be disseminated via other pathways, including animal contact and exposure via recreational and drinking water (Rangel et al., 2005). Shiga toxins are the main virulence factors of STEC, which are composed of two toxin types: Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). It has been shown that Stx2 has higher toxicity and genetic variance than Stx1, with the Stx2a and Stx2d having a higher potency than Stx2b and Stx2c (Fuller et al., 2011). Based on the diversity of the coding sequences, a subtyping method for *stx2* has been developed that targets partial sequences of the *stxAB₂* operon. It has been shown that Stx2 subtypes (Stx2a and Stx2c) are the primary cause of hemolytic uremic

syndrome (HUS) and bloody diarrhea among patients (Persson et al., 2007). Shiga toxin genes have been detected in various environments, including lake water (Smith et al., 2009), urban surface waters and sediments (Shelton et al., 2006; Tani et al., 2007).

Waterfowl may harbor human pathogens and facilitate their dissemination over long distance (Abulreesh et al., 2007). In fact, Shiga toxin genes have been found in seagulls (Makino et al., 2000), ducks (Wang et al., 2010) and mallards (Chandran and Mazumder, 2014). Among waterfowl species, Canada geese feces have been found to contain several human enteric pathogens, including various STEC serotypes (Kullas et al., 2002). Because Canada geese reside in urban, agricultural, and recreational areas, it is highly probable that Canada geese can spread geese-harbored pathogens. It has been reported that *stx2* has about a 10% prevalence in water samples collected from the eastern portion of Lake Erie (Smith et al., 2009); however, no previous study has been conducted on the western end of Lake Erie. The objectives of this study were (1) to evaluate the prevalence of *stx2* in water and fecal samples collected from Canada geese in the western Lake Erie area; and (2) to examine the genetic variance of *stx2* among different fecal samples from Canada geese. The findings of this study may help public health professionals and officials to better address potential zoonotic disease transmission risks from wildlife in recreational areas.

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Materials and methods

Site description and sample collection

The Ottawa National Wildlife Refuge (ONWR) provides a protected habitat for avian species, including Canada geese. Study sites were located in two areas, the ONWR and the swimming beach of the adjacent Magee Marsh Wildlife Area (MMWA), along the southwestern shore of Lake Erie at Oak Harbor, Ohio, USA (Fig. 1). The west and south

sides of the ONWR are surrounded by an agricultural area in northwestern Ohio. Water samples were collected at four locations along Crane Creek in the ONWR and at the MMWA swimming beach from May to December, 2012 (Fig. 1). A total of 71 water samples were collected, with 18 samples from Sites 1, 2, 3 and 17 samples from Site 4. Water samples were collected according to the USEPA's direct method of surface water sampling using four sterile 800 mL Whirl-Pak bags (Nasco, Fort Atkinson, WI) at each site. Using global positioning system (GPS) and onsite landmarks (e.g. sign posts, trees, etc.), we ensured that

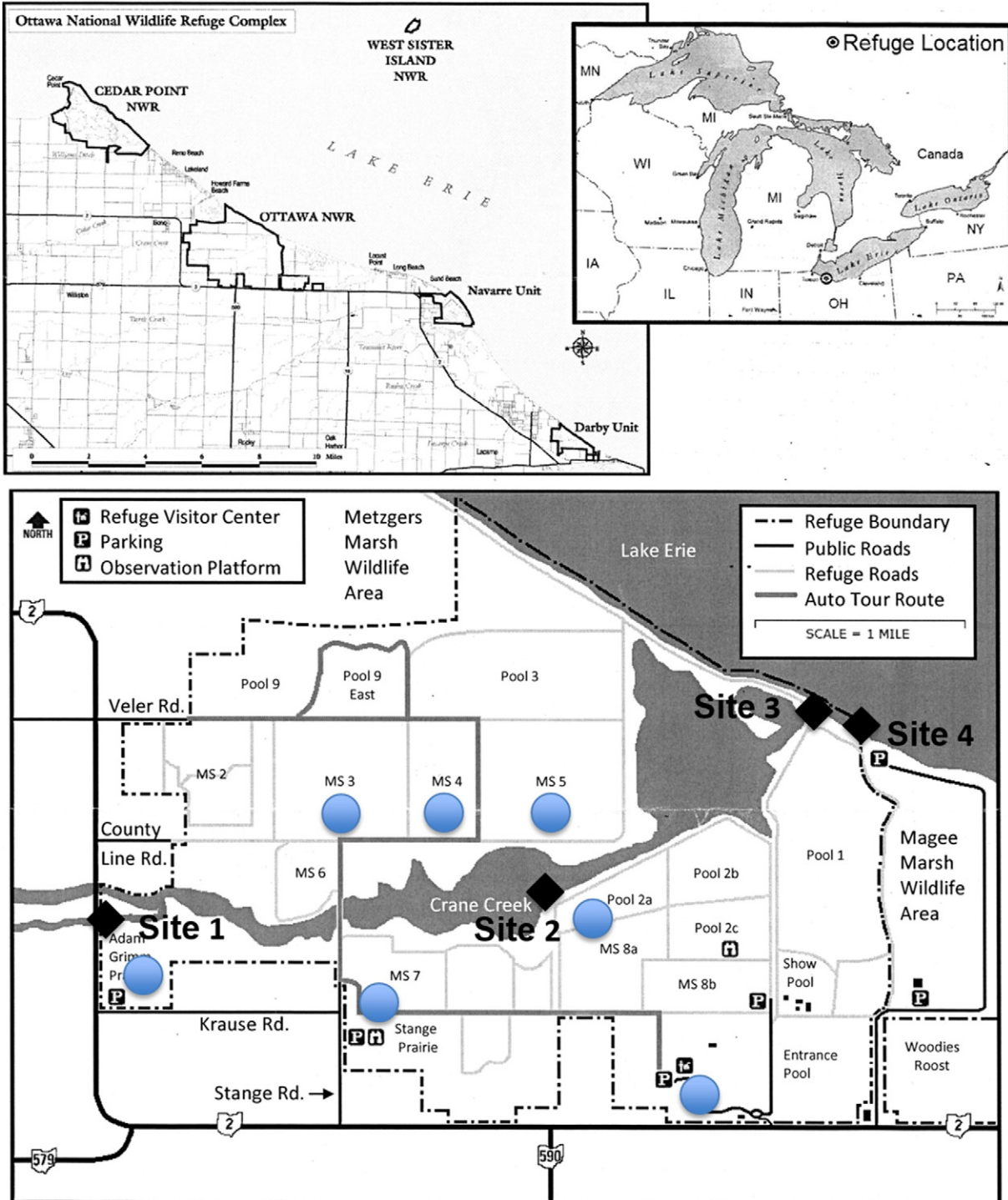


Fig. 1. Sampling sites of water and Canada geese feces at Ottawa National Wildlife Refuge. Diamonds show water sampling sites 1–4; circles denote the area where Canada geese feces were collected (modified from Rea et al., 2015).

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