Shiga Toxin Producing *Escherichia coli* Infections and Associated Haemolytic Uraemic Syndrome in New Zealand Children: Twenty Three Years of Epidemiology and Clinical Observations

William Wong, MB, ChB, FRACP*

Department of Paediatric Nephrology, Starship Children’s Hospital, New Zealand

*Corresponding author: William Wong, MB, ChB, FRACP, Department of Paediatric Nephrology, Starship Children’s Hospital, Auckland District Health Board, Private Bag 92024, Auckland, New Zealand, Tel: +64-9-3078900, Fax: +64-9-375-4371

Abstract

**Background:** The epidemiology of childhood haemolytic uraemic syndrome in New Zealand was on active surveillance from January 1, 1998 to December 31, 2020. The aim of this study is to describe the demographic, epidemiological and some clinical features of Shiga toxin producing *E. coli* infections (STEC) and its association with diarrhoea associated haemolytic uremic syndrome (D+HUS) over past 23 years.

**Methods:** The New Zealand Paediatric Surveillance Unit (NZPSU) sent out a monthly report card to all practising paediatricians with conditions under active surveillance. Those paediatricians who had cared for a child aged 0 to 15 years of age with D+HUS in the past month were requested to report their patient to the NZPSU. The reporting clinicians were then contacted by the principal investigator who sent out a questionnaire to the clinician requesting patient clinical and laboratory information. The group was divided into 2 cohorts; A: 1998-2008 and B: 2009-2020 to examine trends in STEC infections and childhood D+HUS.

**Results:** Two hundred and twenty six children aged 3 months up to 15 years (median 2.8 years (IQR 1.7-4.9) were diagnosed with D+HUS. *E. coli* O157: H7 was identified in 130 (57.1%) and 8 were due to serotypes 026 H11 and 0113. Over the 23 years, the mean annual incidence in children under age 5 between cohort A and cohort B was not statistically significant (2.2 per 100,000 vs. 2.85 per 100,000). The mean annual incidence was 1.2 per 100,000 in all children under age 15 for the whole study period. Disease severity as measured by the requirement for acute dialysis had not changed and was required in 128/226 (56.2%) of children for a median of 9 days (range 1-38). The overall mortality was 3/226 (1.3%), which was due exclusively to severe cerebral injury.

**Conclusion:** There has been an increase in STEC infections in childhood over the past 23 years, in part due improved methods of detection, however, the incidence children developing D+HUS has remained stable over the study period. *E. coli* serotypes other than O157:H7 have increased in prevalence over the past 6 years due to methodological changes in diagnostic laboratories, but with only a small number of infected children developing D+HUS. The mortality rate over the study period was low, consistent with other reported series.

**Keywords**

Shiga toxin producing *E. coli*, Haemolytic uraemic syndrome, Children, Demographics, Epidemiology

**Abbreviations**

AKI: Acute Kidney Injury; CI: Confidence Interval; D+HUS: Diarrhoea Associated Haemolytic Uraemic Syndrome; *E. coli*: *Escherichia coli*; ESR: Environmental Science and Research; HUS: Haemolytic Uraemic Syndrome; g/L: grams/litre; Hb: Haemoglobin; IQR: Interquartile Range; NZPSU: New Zealand Paediatric Surveillance Unit; OR: Odds Ratio; stx: Shigatoxin; SD: Standard Deviation

**Introduction**

Diarrhoea associated haemolytic uraemic syndrome (D+HUS) secondary to gastrointestinal infection is the most common cause of haemolytic uraemic syndrome...
Methods

Surveillance was conducted via a monthly report card sent to all practising paediatricians in New Zealand requesting information on any cases of HUS in which they have been involved in the previous month. Respondents who return an affirmative response were contacted by the principal investigator who then sent out a detailed questionnaire requesting demographic clinical and laboratory information. Positive STEC detections were notified to the regional public health authorities as suspected STEC infections invoked a mandatory report to public health authorities to a confirmed STEC infection and subsequent source identification. Response rates of paediatricians notifying the NZPSU have been excellent, varying from 94% between 2005-2009, 91.5% 2010-2014 and 90% from 2015-2020. Demographic, clinical and laboratory data were collated from completed questionnaires from paediatricians who had reported clinical care of a child with HUS (Supplementary File (Questionnaire)). Clinical features recorded included seizures, presence of hypertension, presence of anuria and its duration, requirement for and duration of acute dialysis. The prodrome period was defined as the time between onset of diarrhoea and clinical diagnosis of HUS, and the identification of a plausible bacterial agent was also included. Complications of HUS such as death, persistent hypertension, chronic kidney disease, cardiac complications, diabetes and pancreatitis were documented. The questionnaire was first developed in 1998 and has remained unchanged until 2008, when a question concerning any recent visit to a farm within two weeks of the presenting diarrhoeal illness was added.

The case definition of D+HUS was made on history of vomiting and or diarrhoea in any child less than 15 years of age with microangiopathic haemolytic anaemia (Haemoglobin < 100 g/L) with microscopic evidence of fragmented red blood cells, thrombocytopenia (Platelet count < 150,000 × 10⁹) and acute kidney injury (oliguria or anuria with elevated serum urea and creatinine for age and gender of child [19]. All children with other causes of HUS were excluded. Historically, STEC were detected by standard bacterial culture methods (plating stool on sorbitol MacConkey agar and chromogenic agar) and serological identification of the STEC, as confirmed by the Institute of Environmental Science and Research, Kenepuru, Porirua, New Zealand [20]. Chromogenic agar, CHROMagar STEC was introduced in 2011 to our hospital microbiology service but did not gain widespread usage through the rest of the country (Fort Richard Laboratories, New Zealand, www.fortrichard.com) Molecular testing for genes encoding Shiga-toxins was introduced in 2015 and phased into various hospital and community laboratories around the country over six years. This is a commercially available platform for the detection of a range of gastrointestinal infections [21].
Cases were assigned to specific districts health areas based on the domicile of the patient where the diarrhoeal illness had developed. The Auckland region has three health districts and the number of cases was combined into one value. The study population was divided into two cohorts; cohort A, January 1, 1998 to December 31, 2008 and cohort B, January 1, 2009 to December 31, 2020 to show trends in the epidemiology of STEC infections and HUS over the duration of the study. Census data obtained from Statistics New Zealand from 2001 through to 2018 were used to calculate regional incidence rates [https://www.stats.govt.nz].

Statistical analyses were performed using Graphpad Instat version 3.1 for Windows and Graphpad Prism 4.0. [22]. Continuous data are expressed as mean ± standard error of the mean (SEM) and compared using Student t-test. Categorical variables were represented as percentages of the total in each group and were compared using Chi-square or Fisher exact tests as appropriate. All P-values are two tailed and significance was defined as a p value < 0.05.

The surveillance study was approved by the University of Otago Medical School Research Ethics committee and the research was undertaken in accordance to the guidelines of the Declaration of Helsinki.

Results

Two hundred and twenty-six children aged 3 months to 15 years were reported to the NZPSU with the diagnosis of D+HUS during the study period. There were 127 females with 74.8% of the whole group being less than age five. There were 88 patients in cohort A, (1998-2008) and 138 in B (2009-2020). There was no significant difference in the age of diagnosis between males and females or between the two time cohorts. The mean age of presentation was 3.8 ± 0.2 years (SEM); median 2.8 years (interquartile range 1.7-4.9). Sixteen children (7%) under 12 months of age had diarrhoea associated HUS. One hundred and sixty-seven (73.4%) were referred to the paediatric nephrology centre in Auckland for further care, which is the only facility in the country that offers acute dialysis or kidney replacement therapy to children.

Geographical distribution

All regions of New Zealand have recorded children with HUS with a trend for increasing case rate in the Canterbury and Waikato; 70% (8 to 27 cases) and 59% increase (9 to 22 cases) respectively, whereas other regions have recorded a reduction (Figure 1). Increase in the in the Canterbury and Waikato childhood population over the study period was 5% and 8.3% respectively. In contrast in Auckland, the most populous region in the country, the annual incidence had remained unchanged. There had a been more than 4-fold increase in reported STEC infections amongst children in the past 23 years, with a trend towards higher numbers over during period B (Figure 2 and Figure 3). There has been a recent reduction in absolute HUS cases in the Waikato and Canterbury (Figure 4).

Bacteriology

In the 226 children with D+HUS, 138 (61%) cases had a Shiga toxin E. coli identified, of which 130 (94%) was E. coli O157:H7, and a further 8 children showing serotypes E. coli O113 and O26 H11. The distribution of stx1/stx2 positivity in New Zealand E. coli O157:H7
isolates over the study period was stx1- stx2+ (83%) and stx1+ stx2+ (17%), 99% of New Zealand E. coli O157:H7 express stx2 (personal communication). A potential source of infection was identified in 83/226 (36.7%) children, of which 85.5%, (71/83) had a history of a farm contact within 2 weeks of diarrhoea onset. Five children developed HUS following ingestion of unpasteurised or raw milk. Three children with HUS had Campylobacter jejuni detected in their stools, but were negative for E. coli O157:H7. A further 3 children had polymicrobial stool isolates containing Cryptosporidium and Aeromonas species.

Duration of prodromal illness and bloody diarrhoea (Table 1)

Two hundred twenty-two patients of the total cohort, (98.2%) had the duration of diarrhoea onset to HUS diagnosis recorded with a median of 6 days (IQR 4-7) from the onset of diarrhoea to diagnosis of HUS. There was a small but significant improvement in the mean time to HUS diagnosis by one day between cohort A and B. The history of bloody diarrhoea significantly increased the likelihood of isolation of STEC (OR 8.5, 95% CI 4.4 to 16.6).
cohorts did not show a statistically significant increase in incidence. Non \textit{E coli} \textit{O157:H7} serotypes have become more frequently isolated in the past ten years. Clinical features Pallor and bloody diarrhoea were common presenting symptoms (Table 1). Four patients had no history of diarrhoea but had \textit{E. coli} \textit{O157 H7} isolated from stool specimens. Seizures occurred in 31 (13.7\%) children with most having one or two brief ones but six children

### Trends over the past 2 decades
The incidence of children under 5 years of age developing STEC infections has gradually increased over the past two decades with a greater increase since 2012 which has been related to changes in methods of detection (Figure 2). The incidence of paediatric HUS cases over the past 23 years has fluctuated from 0.4 to 4.4 per 100,000 children with highest rates in the under five year olds, 2.85 per 100,000 from 2009 to 2020 (Figure 3), however, comparison between the two time

### Clinical features
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displayed multiple seizures with three developing early onset and repeated generalised seizures and coma; all showed diffuse cortical and subcortical neuronal injury which resulted in a fatal outcome in three and a fourth child was successfully treated with a course of Eculizumab. The overall mortality rate for the entire period was 3/226 (1.3%). One patient had progressed to end stage renal failure after more than 10 years of chronic kidney disease and has received a successful deceased donor kidney transplant. Fifty six percent (128/226) of the cohort required acute dialysis for acute kidney injury for a median of 9 days (range 1-35). There was no significant difference in dialysis rates between STEC positive and STEC negative children or between cohorts A and B. All children who required acute dialysis during acute phase of the illness recovered sufficient renal function to become independent of dialysis.

**Discussion**

Over the two decades of this study, trends of childhood STEC infections and HUS have been observed with increasing STEC infections from both O157:H7 and non O157 serotypes (Figure 5), although it has only been since 2008 that *E. coli* non O157:H7 and non-typeable strains have been identified on a regular basis due to improved detection methods.

The Canterbury and Waikato regions have shown a trend towards increasing number of HUS cases in the second period (2009-2020) of the study. The first child with STEC HUS from the South Island was reported in 2005, eight years after the surveillance study was commenced. Prior to this time, STEC HUS in the South Island was a rarely reported, and from 2010, case numbers have steadily increased. This increase may because of the intensification of farming in these two regions. In 2019-20 report from Dairy NZ, 71.4% of the dairy herds are in the North Island and 42% of dairy cows are in the South Island. In North Island, the Waikato region has the largest number of dairy cows (28.5%) followed by the Taranaki region (9.6%) In the South Island, the Canterbury region accounts almost 50% of all dairy herds [23]. Dairy farming in the South Island has seen the most rapid growth in the past 10 years, representing 43% of total milk production in New Zealand. The increase in the childhood population in the Canterbury region was only 5% during the study period, but the number of children with STEC-HUS had increased by 70%, suggesting a possible link between an increase in dairy and cattle farming activity and STEC infections. Of the 1.187 million people who live in the South Island (up to June 2020), just over one half of the people in the South Island live in the Canterbury region. The greatest risk of STEC infection in New Zealand comes from contact with the farm environment, animal contact, untreated water supplies.

The increase in reported STEC infections over the 20 year period can only be partially explained by improved diagnostic laboratory techniques such
as the introduction of more sensitive identification methods that utilised molecular techniques. As Figure 2 shows, the number of infections in children had been increasing from 2008, and the introduction of molecular based diagnostic assays from 2015 onwards resulted in a further rise in STEC infections isolated (https://www.sciencelearn.org.nz/resources/2095-new-zealand-dairy-farming-timeline). New Zealand has relatively high numbers of STEC cases, and contact with cattle faeces and living near cattle are risk factors for human infection. A study in 2018 assessed the national prevalence of STEC in young dairy cattle by randomly selecting 102 farms throughout New Zealand. The study used a molecular laboratory method showed STEC was found in 20% of calves on 75% of the farms studied, indicating widespread prevalence across the country [24].

During the study period, an increase in non O157:H7 infections amongst children have also been observed. Our dataset is limited in that prior to 2008, these serotypes were not routinely and accurately identified, however, since 2008, the number of non O157:H7 infections in children has progressively increased and has equalled O157:H7 infections (Figure 5), but the number of non O157:H7 associated HUS cases in children have remained very small, with only 8 children developing HUS being associated with O26 (n = 6), O179 (n = 1), and O113 (n = 1) serotypes. Shiga toxin 2 was identified in all of these cases, but eae gene was found only in the O26 isolates. The accuracy of this dataset has been verified by cross checking with information obtained from the national reference laboratory [personal communication, Institute of Environmental Scientific Research].

The association between the consumption of unpasteurised cows’ milk and STEC infections in 5 young children over the study period have been sporadic and requires further monitoring. A 2014 Risk Profile of children over the study period have been sporadic and unpasteurised cows’ milk and STEC infections in 5 young by cross checking with information obtained from the isolates. The accuracy of this dataset has been verified in the current study, a history of bloody diarrhoea increased the odds of an STEC infection by more than 31. In the current study, a history of bloody diarrhoea increased the odds of an STEC infection by more than eight-fold. As soon as bloody diarrhoea is present, primary healthcare practitioners should request a stool for rapid molecular diagnostics, a full blood count and record the symptom on the request form.

Campylobacter gastroenteritis which can also cause bloody diarrhoea is highly prevalent in our community and it may therefore be difficult to distinguish an enteric infection caused by Campylobacter or STEC [32,33]. Campylobacter jejuni was the only identified bacterial organism in 3 patients with HUS. There is debate as to whether Campylobacter can cause HUS as the organism does not produce Shiga-toxin. It has been reported that up to one third of STEC gastro-intestinal infections are co-infected with other bacterial or parasitic organism [33]. We did not consider that Campylobacter infection was the cause of the HUS as their clinical course did not appear any different to STEC-HUS.

The mean incidence of STEC-HUS in New Zealand in children under age 5 years appear is higher than many other developed countries, with it increasing from 2.2 to 2.85 per 100,000 over the two time periods. In a study from Belgium between 2009 and 2015, the annual incidence in children under 5 was 1.2 per 100,000 (range 0.6-2.1) [34,35]. A study from our nearest neighbour, Australia, (1994 to 1998) reported an incidence of 1.35 per 100,000/year, but a more recent study from the same country (2000 to 2010), showed the annual
incidence had fallen to 0.49 per 100,000 in children under age 5 [36,37]. An explanation for the difference between Australia and New Zealand may be attributed to increased environmental exposure due to the intensification of dairy farming in some areas of rural New Zealand and changes in methods of detection over time.

Seven percent (16/226) of the HUS children in our study were under the age 12 months’ of age. Infection with STEC in early infancy is thought to be rare. Our bacteriological data shows that that there has been significant increase in the cases of STEC infections over the period 2010 to 2020, with the largest increase in the under 12-months-old group (5 fold increase in the less than 12-month-olds compared with a 2.3 fold increase in the 1-4 age group (Supplementary Data). There are few reports of STEC-HUS incidence in infants under one year of age. In the Northern Italian HUS network study, the annual incidence in children less than 12 months of age was approximately 1.2 per 100,000 aged related populations [38]. Younger children with STEC-HUS are also thought to be a higher risk of a poor outcome. A population-based survey of 10 states in the United States conducted between 1997-2012, reported 168 infants under age 2 years and calculated that this age group had a 5.4% frequency of death compared with 2.8% in the 2-4-year age group [39]. In the current study, all infants less than 12 months of age at presentation with HUS survived with just over 50% needing short term dialysis. Our small numbers of very young children in the cohort makes meaningful comparisons difficult.

There are some limitations to the results observed in this study. As with many observational studies, a large number of observers were involved in collecting clinical data from affected patients, especially in the 25% that were managed in their local hospital facilities. This variability may have introduced some inconsistencies in the interpretation of clinical symptoms, and the duration of prodromal symptoms. One of main strengths of this study is that all the patients who were transferred to our centre for clinical care were reviewed either by the author or his immediate paediatric nephrology colleagues. The other major strength of this study is the clinical and laboratory data collected from each patient remained consistent throughout the 23-year period of the study thereby allowing for trends to be recognised more readily.

**Conclusion**

Shiga toxin *E. coli* infections resulting in HUS is a common cause of acute kidney injury in young children in New Zealand requiring acute dialysis. Trends documented in this report require further study as the prevalence of STEC infections in young children continue to rise each year, with the incidence of HUS in children less than age five appears to be increasing. The consequences of a STEC infection progressing to HUS can lead to devastating consequences with permanent physical and psychological disabilities and in some rare cases, death due to severe neurological injury. Although the short-term outcome appears to be excellent, the 56% of the group who required acute renal replacement therapy are at significant risk of chronic kidney disease. Public health authorities along with other stakeholders such as the veterinary community and managers of livestock could work to identify factors to reduce the increasing exposure to the risk of STEC infections in young children.

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**Declarations**

The principal author of this manuscript is the only author.

**Ethics approval**

Obtained from the University of Otago, Research Ethics Committee, Dunedin, New Zealand, 1997.

Consent to collect and publish clinical data was obtained from caregivers of patients.

The dataset from which this manuscript is based upon is the property of the author and the University of Otago, Dunedin, New Zealand. Under confidentiality and privacy rules of New Zealand, the dataset cannot be deposited in a public repository without the express consent of the study participants.

**Competing interest**

None.

**Funding**

None.

**References**


20. Personal communication. Institute of Environmental Science and Research.
NZPSU Study: Haemolytic Uraemic Syndrome (HUS) questionnaire

1. Reporting Details
   a. Date of report       ___/___/___
   b. Paediatrician Surname

2. Patient Details
   a. First name (first two letters only)                   ___ ___
   b. Surname (first two letters only)     ___ ___
   c. Date of Birth (day, month, year)                  ___/___/___
   d. Age at diagnosis of HUS (yrs/months)                  ______
   e. Date of diagnosis                    ___/___/___
   f. Sex                      __ M    __ F

3. Historical Data (please circle one - Y = yes, N = no, DK = don’t know)
   a. Diarrhoea prodrome                                  Y     N     DK
   b. If yes, time of onset in days prior to diagnosis                               ___________ days
   c. If yes, did the stool contain blood                  Y     N     DK
   d. Vomiting prodrome                    Y     N     DK
   e. Respiratory prodrome      Y     N     DK
   f. Family History of HUS                    Y     N     DK
   g. Previously/simultaneously affected sibling                 Y     N     DK
   h. Was HUS attributed to ingestion of food product?                 Y     N     DK
   i. If yes, please specify                                    ___________
   j. Has the child visited a farm in the past 2 weeks                              Y     N     DK

4. Presenting or subsequent signs/symptoms (please circle)
   a. Anaemia        Y     N     DK
   b. Jaundice        Y     N     DK
   c. Fever                      Y     N     DK
   d. Oliguria        Y     N     DK
   e. Anuria        Y     N     DK
   f. If yes, duration       ________days
   g. Hypertension                     Y     N     DK
   h. Seizures        Y     N     DK
   i. If yes, number                      ________
   j. If yes, nature eg: generalised, focal                                ______________
   k. Intracranial haemorrhage                   Y     N     DK
   l. Other (specify)                                                                                                     ______________
5. Laboratory Findings

a. Minimum Haemoglobin       _________ g/dL
b. Minimum Platelets                    _________ × 10⁹/L
c. Minimum Serum sodium                   _________ mmol/L
d. Maximum Creatinine                    _________ µmol/L
e. Maximum Urea                    _________ mmol/L
f. Neutrophil count at presentation                  _________ × 10⁹/L
g. Abnormal RBC morphology (including fragmented or burr cells, spherocytes, polychromasia) Y     N     DK
h. Stool pathogen isolated                    Y     N     DK
i. If yes, specify                                                                                                       ______________
j. Verotoxin-producing E. coli (serotype 0157:H7) isolated               Y     N     DK
k. Has a stool sample been sent to ESR?                               Y     N     DK

6. Treatment

a. Dialysis        Y     N     DK
b. If yes, specify type                                                                                            ______________
c. Duration        ________ days
d. Other/specify
  eg: supportive (hypertensives, anticonvulsants)                                     ______________
  eg: therapeutic (fresh frozen plasma, plasmapheresis)                           ________________
e. Duration of hospital care (days)                                                                     _____________days

7. Complications/Outcome (please circle)

a. Cardiomyopathy                    Y     N     DK
b. Diabetes mellitus       Y     N     DK
c. Persistent hypertension        Y     N     DK
d. Persistent renal impairment      Y     N     DK
e. End-stage renal failure        Y     N     DK
f. Pancreatitis                   Y     N     DK
g. Death                         Y     N     DK

Thank you for completing this questionnaire. A short follow-up questionnaire will be sent in 12 months seeking information on long-term outcome.

Please retain a copy of this form for records

Please return to: Dr William Wong