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Association of age and gender with *Torque teno virus* detection in stools from diarrheic and non-diarrheic people

Julie Brassard [a,*], Marie-Josée Gagné [a], Danielle Leblanc [a], Élyse Poitras [a], Alain Houde [a], Valerie F. Boras [b], G. Douglas Inglis [c,**]

[a] Agriculture and Agri-Food Canada, Food Research and Development Centre, 3600 Casavant Blvd. West, St. Hyacinthe, Quebec J2S 8E3, Canada
[b] Department of Laboratory Medicine, Chinook Regional Hospital, 960-19th Street S, Lethbridge, Alberta T1J 1W5, Canada
[c] Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403-1st Avenue S, Lethbridge, Alberta T1J 4B1, Canada

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ABSTRACT

Background: Torque teno virus (TTV) is a small virus belongs to Anelloviridea family. TTV is a disease orphan virus but it has often been associated with a variety of pathologies and co-infections. TTV was recently identified as an infectious agent that could potentially be involved in cases of acute enteritis.
Objectives: To ascertain the presence of TTV in stools from diarrheic and not diarrheic people, and to investigate an association between infection, and patient age and gender.
Study design: Stool samples from people exhibiting signs of enteritis (954) and from non-diarrheic individuals (76) were collected in the former Chinook Health Region (CHR) in Southwestern Alberta, Canada from May 2008 to April 2009. Viral genetic material was extracted, and detection and quantification of TTV were carried out by real-time PCR. The presence of other viral and bacterial enteric pathogens was also investigated.
Results: More ($P < 0.001$) diarrheic people (38.8%) tested positive for TTV DNA than non-diarrheic individuals (18.4%). Furthermore, viral load was greater ($P < 0.001$) in stools from diarrheic (2.0×10^7 copies/g) than non-diarrheic (2.0×10^3 copies/g) people. TTV DNA was detected most often in diarrheic individuals that were 0–5 (57.3%) and greater than 81 (59.0%) years of age. Combined across age, the prevalence of TTV was higher among men than women ($P = 0.003$). Co-infections with other enteric pathogens were observed.
Conclusions: This study revealed a significant association between TTV prevalence and viral load, and enteritis. Also, TTV prevalence was significantly higher in the very young and elderly suggesting that immunological status is important.

1. Background

The *Torque teno virus* (TTV) is a small non-enveloped virus with a circular single-stranded negative-sense DNA genome of 3.8 kb and recently assigned to the family Anelloviridae, and genus *Alphatorquevirus* [1]. It was first reported in 1997 in a Japanese patient with hepatitis of unknown etiology [2]. Since then, the virus has often been associated with a variety of pathologies and co-infections, including viral hepatitis, asthma, idiopathic pulmonary fibrosis and autoimmune rheumatic disorders, and in some cases, the viral load was implicated as a determining factor in the severity of the infection without necessarily being directly related to a clinical manifestation [3–7]. Viral replication of TTV is not fully understood, and evidence that the virus possesses cell tropism is lacking as TTV replication has been reported in several organs/tissues including liver, bone marrow, lymph nodes, spleen, lung, and peripheral blood mononuclear cells [8–10]. Detection rates of TTV in the blood of non-diarrheic people ranges from 5% in Brazil to 90% in Russia, which suggests that the virus causes an asymptomatic chronic infection [11,12]. TTV shares characteristics with other enteric viruses including size, structural similarities, and a high prevalence of occurrence in surface waters, wastewater, water treatment plants, and on hospital fomites [13–16]. Recently, TTV was implicated as an incitant of acute enteritis [17,18]. Enteric infections are one of the main causes of morbidity and mortality in the world affecting all age groups, and the majority of cases of

* Corresponding author. Fax: +1 450 768 7851.
** Co-corresponding author. Fax: +1 403 382 3156.
E-mail addresses: julie.brassard@agr.gc.ca, juliebrassard@hotmail.com (J. Brassard), douglas.inglis@agr.gc.ca (G.D. Inglis).

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enteritis are of unknown etiology. It has been suggested that TTV might be transmitted through the fecal-oral route [19]. The high prevalence of TTV in the environment suggests that environmental TTV may be an important source of infectious virions. It is therefore important to evaluate the prevalence of TTV in diarrheal disease in various populations of human beings in order to shed light on the epidemiology of disease incited by TTV and develop effective mitigation strategies.

2. Objectives

To comparatively assess the prevalence and loads of *Torque teno virus* in stools from diarrheic and non-diarrheic people, and to ascertain the degree to which age and gender affect prevalence/virus loads in diarrheic individuals.

3. Study design

3.1. Samples

A total of 954 stool samples from diarrheic people and 76 samples from non-diarrheic individuals (healthy volunteers) as controls were collected in the former Chinook Health Region (CHR) in Southwestern Alberta, Canada from May 2008 to April 2009. Within 2–3 days of submission, and after culture-based detection of bacterial pathogens was completed, subsamples of stools were stored at −20 °C until processed for molecular-based detection/quantification of bacterial and viral pathogens. Information provided with the samples included collection date, gender, age, and place of habitation (i.e. postal code) of the submitting individual. For each month, the distribution of diarrheic samples was ≈20 samples from individuals between 0 and 16 years of age, ≈40 samples from individuals between 17 and 60 years of age, and ≈20 samples from individuals over 60 years of age. The presence of other viral and bacterial enteric pathogens known to cause acute enteritis was determined in all samples at the diagnostic facility of the CHR, and at the Agriculture and Agri-Food Canada (AAFC) Research Centres at Lethbridge, Alberta and Saint-Hyacinthe, Québec as described previously by Inglis et al. [20].

3.2. Viral nucleic acids isolation

Stool samples were diluted 1:5 (w/v) in sterile PBS, pH 7.2 (Invitrogen, Burlington, ON, Canada) before centrifugation for 20 min at 4000 × *g*. The stool suspensions were adjusted to reach 1% sodium dodecyl sulphate (Sigma–Aldrich, Oakville ON, Canada) and 100 μg/mL of Proteinase K (Qiagen, Mississauga, ON, Canada). The mixtures were incubated at 37 °C for 1 h. Viral genetic material from 140 μl of the stool suspension was extracted with QIAamp Viral RNA Mini (Qiagen) protocols adapted for the QIAcube robotic workstation (Qiagen) using QIAamp Viral RNA body fluid: manual lysis protocol. In order to protect the extracted nucleic acid material from exogenous RNases, RNase inhibitor (RNaseOUT, Invitrogen) was added to the final AVE elution buffer. Viral RNA and DNA were extracted simultaneously by QIAamp Viral RNA Mini and the genetic material solutions were stored at −80 °C until use.

3.3. Detection of TTV DNA

The TaqMan assay for the detection of TTV was carried out in 25 μl of a reaction mixture with 2.5 μl of extracted DNA and 22.5 μl of master mix. Brilliant I QPCR core reagent kit (Stratagene, La Jolla, CA, USA) and contained 5.0 mM of $MgCl_2$, 150 nM HumanF forward primers, 300 nM of HumanR reverse primer, and 250 nM of Human TaqMan probe [21]. PCR amplifications were performed with a Mx3005P qPCR system (Agilent Technologies, Santa Clara, CA) in a 96-well format under the following conditions: 95 °C for 10 min for initial denaturation, followed by 40 cycles of amplification with denaturation at 95 °C for 10 s, and annealing and extension at 60 °C for 1 min.

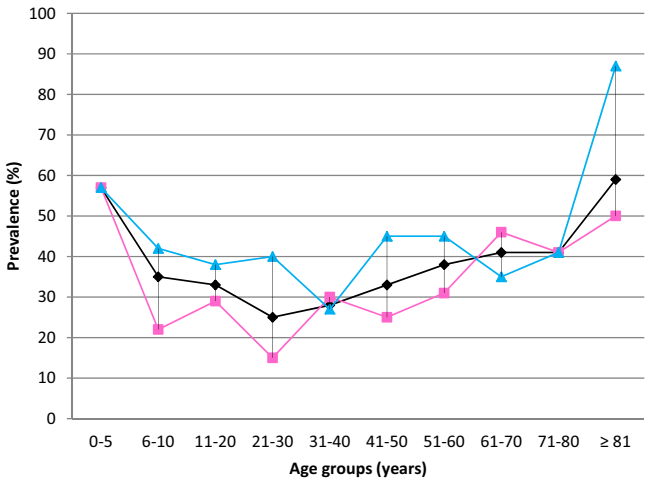

Fig. 1. Prevalence of *Torque teno virus* DNA in stool samples in different age groups and gender of diarrheic individuals. Blue line (-▲-) represents prevalence of TTV in male diarrheic individuals; pink line (-■-) represents prevalence of TTV in female diarrheic individuals; black line (-♦-) represents prevalence of TTV in all diarrheic individuals in the present study (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

3.4. Statistical analysis

Statistical analyses were performed using GraphPad Prism v6 (GraphPad Software Inc, San Diego, CA, USA). A Fisher's exact test (viral prevalence, distribution across age groups and prevalence of other enteric viruses) and an unpaired Mann–Whitney test (viral load) were applied to ascertain differences between the diarrheic and non-diarrheic groups, and *P* values ≤0.05 were considered significant.

4. Results

TTV was detected in stools from diarrheic and non-diarrheic individuals living in Southwestern Alberta from May 2008 to April 2009. From the 1030 stool samples examined, TTV prevalence was higher ($P < 0.001$) in diarrheic individuals (38.8%) compared with non-diarrheic individuals (18.4%) (Table 1). Furthermore, the viral load of TTV in the stools as measured by real-time PCR was also higher ($P < 0.001$) in diarrheic people (Table 1).

The prevalence of TTV in stools from diarrheic people as a function of age was also evaluated. Ten age groups were established, and the prevalence of TTV was not distributed equally ($P < 0.001$) across the age groups (Fig. 1). TTV was more common in younger (0–5 years; $P = 0.01$) and older (≥81 years; $P = 0.02$) individuals (Fig. 1; Table S2). TTV was detected least often in the 21–30 years of age group; the lowest viral loads were also observed in stools for individuals of this age group (Table S2).

The association between the prevalence of TTV in stools from diarrheic individuals as a function of gender, and of gender relative to age were also examined. Across age groups, the prevalence of TTV was higher ($P = 0.003$) among male individuals (43.8%) than female individuals (34.7%) (Table 1). Variations were observed between the different age groups associated with the gender, but, generally, the prevalence of TTV was much higher in men (Fig. 1 and Table