18

The Yukon Old Crow *Helicobacter pylori* Infection Project

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Background: *Helicobacter pylori* infection has been a growing community health concern in northern Canada. Both *H. pylori* prevalence and stomach cancer rates are elevated in Arctic Aboriginal populations. This project arose as part of the Canadian North *Helicobacter pylori* (CANHelp) Working Group addressing health concerns raised by residents of Old Crow, Yukon (population=250, ~90% Aboriginal). It was approved by the Vuntut Gwitchin General Assembly. This project was designed and conducted in collaboration with a local planning committee. Purpose: to investigate the disease burden related to *H. pylori* infection and identify strategies for reducing health-related risks in Old Crow. Methods: During 2010 and 2011, all 250 residents of Old Crow were invited to be screened for *H. pylori* infection by UBT and interviewed using structured questionnaires. All residents of Old Crow, who gave informed consent, were eligible. In 2012, all residents were invited to undergo endoscopy with gastric biopsy, in temporary endoscopy units in the Old Crow Health Centre. Participants ≥15 years of age could also enrol in a treatment trial comparing two 10 day *H. pylori* therapies: sequential therapy consisted of a proton pump inhibitor and amoxicillin for days 1-5, followed by a proton pump inhibitor, clarithromycin and metronidazole for days 6-10; quadruple therapy consisted of a proton pump inhibitor with bismuth, metronidazole, and tetracycline for days 1-10. Follow-up UBT was used to determine the success of therapy Results: From November 2010 to August 2012, 199 residents consented to participate, ages ranged from 1-88 years. Of the 199 participants, 145 completed questionnaire-based interviews, 192 underwent a UBT (UBT positivity=68%), 65 consented to upper gastrointestinal endoscopy, 63 had biopsies collected for culture and histopathology, 86 consented to treatment, and 70 enrolled in the treatment trial. For histology results see Table. Antibiotic resistance frequencies from 53 participants with successful culture were as follows: 42% for metronidazole, 25% for clarithromycin, 8% for ciprofloxacin, 2% (borderline) for tetracycline, and 0 for amoxicillin, nitrofurantoin, and rifampicin. Treatment trial preliminary results: sequential therapy, treatment success 60% (12/20; 95% confidence interval 36%-81%), quadruple therapy 85% (17/20; 95% confidence interval 62%-97%). This difference suggests superiority of quadruple therapy. Discussion: Old Crow residents have a high prevalence of *H. pylori* infection, and gastritis is severe. Quadruple therapy is better than sequential therapy. The success of the Old Crow *H. pylori* Project, as demonstrated by the high level of participation, is a result of close partnership with the local planning committee and ongoing community engagement.
Results histology gastric biopsies

Inflammation	All participants (n=63)	All H. pylori positive participants (n=57)
Mild (%)	5	3
Moderate (%)	29	32
Severe (%)	59	65
Atrophy (%)	67	74
Intestinal metaplasia (%)	33	35

19

Does Drinking and Environmental Water Play a Role As a Reservoir in *Helicobacter pylori* Transmission? Evidence From the South Region of Poland

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The main transmission pathway of *Helicobacter pylori* (Hp) has not been so far identified but recent evidence suggests that the waterborne aspect of Hp transmission. We conducted the innovative project in Poland aimed to detect the presence of Hp in drinking water samples in Cracow and surroundings, because there has not been any research conducted in polish population concerning such topic. One hundred twenty nine water samples from different municipal water distribution system, rivers, drinking water tanks and wells were collected and analyzed between June and October of 2012. Samples of 1000 mL of water were concentrated by centrifugation. Obtained pellet was resuspended in 1 ml of PBS used for the Hp culture and remaining portion was stored at -20°C for DNA extraction and subsequent gastric colonization of Mongolian gerbils. Water samples were subjected to PCR for the presence of Hp using primer pair: Cluster2: GGCGTTATCAACAGAATGGC and B1J99: CTCAGTTCGGATTGTAGGCTGC targeting the hypervariable region flanking the 16S rRNA gene in Hp. All samples were negative for Hp culture but twelve out of hundred twenty nine samples collected from the Cracow municipal water distribution system were Hp DNA positive. These Hp positive samples unsuccessfully colonized the stomach of Mongolian gerbils. Among twelve samples which were tested twice (June and October), eight were positive only in June, but not in October. We conclude that 1) Hp DNA but not viable Hp could be detectable in municipal drinking water samples; 2) water bacteria became non-culturable by traditional methods from envinromental source such as water and it also failed to colonize animal stomach possibly due to bug transition from spiral to its coccoid and U-shaped forms in aqueous envinroment, and 3) water Hp DNA detection could be influenced by the variation of temperature at different seasons (work supported by grant No 2011/01/B/NZ/01539 to M.K.).

20

The First Report on the Prevalence and Epidemiology of *Helicobacter pylori* in Bhutan

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Background: The epidemiology of *Helicobacter pylori* infection and risk factors associated with in Bhutan are not previously studied. The World Health Organization reported the incidence of stomach cancer to be very high in Bhutan. Bhutan is small mountainous country bordering India and China and consists of four geographical regions, west, east, central, and south. Aim: We conducted a cross-sectional study to determine the seroepidemiologic pattern of *H. pylori* among Bhutanese from the four regions with emphasis on water source and household sanitation. Methods: Between June and November 2012, blood sample from patients with complaints of dyspepsia and blood donors were collected after an informed consent. Demographic information, occupation, family size living in the same household, consumption of betel nut, and aspects household environment including type of latrines, source of drinking water were collected. All serum samples were tested for *H. pylori* Immunoglobin G (IgG) by Enzyme Linked Immuno-sorbent Assay (ELISA) using MAGIWELL ELISA kit from United Biotec, USA. Results: Two hundred forty four patients between the ages of 17 and 75 years participated in the study; 102 males, and the mean age was 38 (+ 14.2) years. The overall prevalence of H.pylori among patients was 86% with no difference between males and females (90% vs. 83%, respectively, p=0.12). The prevalence was almost identical among all age groups; 81% at age 17-20, 84% (20-29), 93% (30-39), 82% (40-49), 87% (50-59) and 82% at >60 years (p=0.51). *H. pylori* prevalence was lower in the south region of Bhutan (78%) compared to the central region (97%) (OR=8.6; 95%CI=1.1-55; p=0.02), Eastern region (91%) (OR=2.7; 95%CI=1.1-7.2, p=0.004) or the western region (83%) (OR= 1.4, 95% CI=0.8-3.1, p=0.07). Crowding showed no significant effect on *H. pylori* prevalence as well as source of drinking water, type of occupation, type of latrines or consumption of betel nut. When logistic regression analysis was applied with all the variables in the model, the residing region was the only variable was emerged to be significant. The prevalence among blood donors was less than the prevalence among the studied patients (76% vs. 86%, OR=1.9; 95%CI=1.2-3.2; p=0.01). However, that prevalence was almost identical across all age groups. Conclusions: The high prevalence of antibodies to *H. pylori* among patients and volunteers in all groups could contribute to the high incident rate of gastric cancer in Bhutan. The lowest prevalence in southern part of the country could be due to its tropical whether or due to the difference in the ethnicity as most of its population are of Indian and Nepal origin. Further data regarding *H. pylori* infection in Bhutan are critical to understanding the epidemiology of the infection and to developing surveillance and prevention strategies for gastric cancer.

21

Caspase 8 Maintains Epithelial Cell Adhesion and Intestinal Homeostasis In Vivo Through Regulation of Clathrin-Dependent Endocytosis and Autophagy

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Background: Caspase 8 is a key mediator of ligand-activated apoptosis, but has other functions, including regulation of endocytosis in epithelial cells. The physiological importance of these different activities is poorly understood. The aim of this study was to study the physiological functions of caspase 8 in intestinal epithelial cells (IECs). Methods: We generated mice specifically lacking Casp8 in IECs (Casp8 ΔIEC) by crossing Casp8fl/fl mice to Tg(Vil-cre) mice, and examined them for cell death and endocytosis under constitutive and challenge conditions. The role of TNF-dependent signaling and autophagy was tested by additional ablation of Tnfr1 (Casp8ΔIEC/Tnfr1-/-) or Atg7 in IECs (Casp8ΔIEC/Atg7ΔIEC). Results: At 6 weeks Casp8ΔIEC mice did not present any macroscopic or microscopic intestinal abnormalities compared with Casp8fl/fl mice. However, IEC from Casp8 ΔIEC mice lacked the normal constitutive degradation of proteins important for clathrin-dependent endocytosis. To examine the consequences of this disturbance in endocytosis, we infected mice with the murine attaching/effacing pathogen, C. rodentium. Casp8 ΔIEC mice displayed significantly more weight loss and mortality than Casp8fl/fl mice after infection. Microscopically, Casp8ΔIEC mice presented a complete destruction of small intestinal villi and ileitis. Furthermore, stimulation of Casp8ΔIEC mice with LPS for 4 hours caused marked detachment of small intestine IECs and led to villus destruction and mucosal inflammation. Immunofluorescence analysis revealed disappearance of basal β1-integrin in IECs of Casp8ΔIEC mice after LPS stimulation, suggesting active endocytosis of adhesion molecules. Consistent with this, LPS-induced IEC detachment was prevented in Casp8 ΔIEC mice treated with the endocytosis inhibitor chlorpromazine. LPS-induced IEC detachment was prevented in Casp8ΔIEC/Tnfr1-/- mice, demonstrating a role of TNF α in these events. Furthermore, Casp8ΔIEC mice treated with LPS showed abnormal autophagic activation in IECs compared to Casp8fl/fl mice, and IEC detachment was absent in Casp8 ΔIEC/Atg7ΔIEC mice. Casp8ΔIEC/Atg7ΔIEC mice were also protected from intestinal inflammation induced by C. rodentium. Conclusions: Caspase 8 controls IECs adhesion and maintains intestinal barrier integrity in response to infectious stimuli by regulating clathrin-dependent endocytosis and autophagy. This work demonstrates a major physiologic role of caspase 8 in maintaining intestinal homeostasis and controlling intestinal inflammation.

22

Goblet Cells Deliver Luminal Retinoids and Imprint CD103+ Lamina Propria Dendritic Cells

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The small intestine lamina propria (LP) underlies the absorptive villous epithelia and contains a significant population of CD103+ myeloid (CD11b+, CD11c+, MHCII+) DCs. Through the generation of the biologically active vitamin A metabolite all-trans retinoic acid (ATRA),