Research Article

Norovirus Infections And Association With Animal Exposure In Sarawak, Malaysia

Sarah E. Philo [1], Siaw-Jing Ha [2,3], Wei-Honn Lim [2], Benjamin D. Anderson [1,4], Gregory C. Gray [1,4,5], Teck-Hock Toh [2,3]

Author's Affiliation:
1- Division of Infectious Disease, School of Medicine and Global Health Institute, Duke University, Durham, North Carolina, USA.
2- Clinical Research Center, Sibu Hospital, Ministry of Health Malaysia, Sibu, Sarawak, Malaysia.
3- Faculty of Medicine, SEGi University, Kota Damansara, Selangor, Malaysia.
4- Duke Kunshan University, Kunshan, China.
5- Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

Correspondence:
Teck-Hock Toh, Email: tohth@moh.gov.my

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Background: Diarrheal diseases continue to be one of the most significant killers of children worldwide, and many diarrheal diseases are zoonotic. In Sarawak, Malaysia, not much is known about the viruses causing disease in humans and animals, and there is little evidence describing the role noroviruses play in diarrheal disease.

Aims: This study aimed to estimate the prevalence of noroviruses in children admitted with acute diarrhea and examined the risk factors of animal exposure for the development of diarrheal illness.

Methods: Stool samples were collected from children (age one month to 12 years) admitted to the hospital for acute gastroenteritis. Laboratory-confirmed acute gastroenteritis other than norovirus was excluded. Stool samples were tested for norovirus genogroups I and II/IV with real-time reverse transcriptase-polymerase chain reaction. At the time of sample collection, information was collected about prior animal exposure and medical history, including the previous hospitalization for diarrhea.

Results: A total of 70 participants enrolled in the study, and three tested positive for norovirus GII/IV. Logistic regression was performed and none of the animal exposure variables was statistically associated with increased odds of the previous hospitalization for diarrhea. Still, prior playing with cat non-significantly increased the odds of the previous hospitalization by 3.78 (95% CI: 0.89, 16.11).

Conclusion: Although norovirus was not highly prevalent in children, diarrheal disease causes a significant disease burden in the study population. Future work should aim to elucidate risk factors for severe diarrhea and determine the prevalence of other disease-causing pathogens.

Keywords: infectious diarrheal disease, norovirus, zoonoses

INTRODUCTION

Norovirus was first discovered in 1972 following an outbreak of nonbacterial gastroenteritis in 1968 in Norwalk, Ohio.[1] Noroviruses are classified into five genogroups (GI-GV), with genogroups I, II, and IV commonly causing disease in humans and genotypes of genogroups I and II causing disease in pigs.[2-5] It is estimated that noroviruses are responsible for 18% of all cases of acute gastroenteritis in children and adults, including both diarrheal and vomiting, worldwide.[6]

Although norovirus has the potential to infect both humans and animals, little research has been done to identify norovirus zoonotic events. Human noroviruses have been detected in pigs, wild birds, and rats that did not present disease signs.[7,8] Additionally, estimates suggest up to 63% of pigs are positive for human norovirus genogroup I.[9] Because human and porcine norovirus GII are closely genetically related, there is concern that species-specific viruses could recombine to create viruses with unpredictable phenotypes and disease risks.[10]

In Malaysia, the diarrheal disease was responsible for an estimated 9,952.2 disability-adjusted life years in children under five years in 2015.[11] As the burden of norovirus in Sibu Hospital is currently unknown, this study sought to estimate the prevalence of norovirus in children admitted to Sibu Hospital with diarrheal disease and examined the risk factors in terms of animal exposure for the development of diarrheal illness.

METHODS

This was a cross-sectional study seeking to estimate the prevalence of norovirus among children admitted to the hospital with diarrheal disease. This study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-17-378-34495), and the Duke Medicine Institutional Review Board, United States (Pro00081683). All methods were performed per ethical standards in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

At the time of admission for diarrheal disease, pediatric patients were screened for inclusion and exclusion criteria and briefed on the study in their primary language of either Mandarin, Malay, or English. Children with diarrhea at the time of enrollment and older than one month to 12 years old were included. Exclusion criteria were a confirmed laboratory diagnosis for a pathogen causing acute gastroenteritis other than norovirus or comorbid conditions requiring the patient to take laxatives. After the guardians provided written informed consent to participate, a fresh stool sample was collected using Fecal Swab collection kits (Copan Diagnostics, Inc., Murrieta, California, United States). After collection, samples were stored at 4°C for up to 24 hours before being moved to a -80°C freezer for long-term storage and sample processing. At the time of enrollment, Medical Officers helped the patients and their guardians complete a brief survey. The survey collected demographic information as well as previous animal exposure and medical history. The animals we were interested in were pigs, cows, rodents, ducks, goats, dogs, cats, and others that the family could provide.

Stool samples were shipped on dry ice to Duke University in Durham, North Carolina, for molecular analysis, where samples were stored at -80°C. Viral RNA was extracted from ten-fold diluted stool samples using the spin protocol of the QIAamp Viral RNA Mini Kit (QIAGEN, USA). Stool samples were analyzed for norovirus types I, II, and IV using real-time reverse transcriptase PCR.[12-14] ATCC quantitative synthetic RNA positive controls for norovirus genogroups I and II were used for all reactions (ATCC, Manassas, Virginia, United States).

Result databases were maintained in REDCap and Microsoft Excel (REDCap, Nashville, Tennessee, United States; Microsoft Corp., Redmond, Washington, United States). Statistical analyses were carried out using STATA version 14.0 (StataCorp LLC, College Station, Texas, United States). Descriptive statistics were generated for demographic variables and potential risk factors, including animal exposure(playing with animals, handling animals, caring for animals), cat exposure, dog exposure, and age category. Animal exposure frequencies were stratified by the previous hospitalization due to acute diarrhea because it was assumed animal exposure remains constant over time. Logistic regression was performed to obtain unadjusted odds ratios for potential risk factors with the primary outcome of the previous hospitalization from diarrheal disease.

RESULTS

Seventy children were enrolled in the study from June 13 to August 1, 2017, ranging from one month to 10 years old (mean age = 30.8 months). Of the participants enrolled, 43 (61.4%) were male (**Error! Reference source not found.**). A majority of patients enrolled were Iban, followed by Chinese, and Melanau/Malay (**Error! Reference source not found.**), representative of the ethnicity in Sarawak. Of the participants who identified as being of 'other' ethnicity, two were Kadazan, and two were Kenyah, one each of Dusun, Punan, and Bidayuh.

Table 1. Demographic information of individuals enrolled in the study.

Variables		Ntot = 70 (%)
Age	1-12 months	24 (35.8)
	12-24 months	17 (25.4)
	2-5 years	13 (19.4)
	5 years & older	13 (19.4)
Ethnicity	Iban	43 (62.3)
	Chinese	11 (15.9)
	Melanau/Malay	8 (11.6)
	Others	7 (10.1)
Sex	Male	43 (61.4)
	Female	27 (38.6)
Household size	3-5	35 (50.0)
	6-9	30 (42.9)
	>10	5 (7.1)
Previous hospitalization	Yes	13 (18.57)
	No	57 (81.43)
Norovirus II/IV infection	Yes	3 (4.29)
	No	67 (95.71)
Norovirus I infection	Yes	-
	No	-

Missing data for age (n=3) and ethnicity (n=1) variables

Data regarding animal exposure and type over the course of the norovirus incubation period of 48 hours were collected for all individuals enrolled in the study (**Error! Reference source not found.**). Participants most commonly played with animals during the 48 hours before hospital admission, namely the cat (n=10), dot (n=4) and rabbit (n=1) (**Error! Reference source not found.**). The numbers of children who cared for and handled the animals are shown in **Error! Reference source not found.**.. Thirteen children had been previously hospitalized for acute diarrhea. None of the children had any sort of exposure to pigs, cows, rodents, ducks, goats, and chickens.

Table 2. Unadjusted odds ratios for the relationship between animal exposure and previous hospitalization for acute diarrhea.

Variables		Previous Hospitalization		No hospitalization		Unadjusted OR (95% CI)
		N = 13	%	N = 57	%	
Handled animals	No	12	18.8	52	81.2	Ref.
	Yes	1	16.7	5	83.3	0.9 (0.1, 8.1)
Played with animals	No	9	15.8	48	84.2	Ref.
	Yes	4	30.8	9	69.2	2.4 (0.6, 9.4)
Cared for animals	No	13	18.8	56	81.2	Ref.
	Yes	0	0	1	100.0	-
Played with cat	No	9	15.0	51	85.0	Ref.
	Yes	4	40.0	6	60.0	3.8 (0.9, 16.1)
Played with dog	No	12	18.2	54	81.8	Ref.
	Yes	1	25.0	3	75.0	1.5 (0.1, 15.7)

Viral RNA amplification for norovirus genogroup I was unsuccessful for human stool samples, as the assay could not be validated using the genogroup I ATCC quantitative synthetic RNA positive control. No fluorescence signal was observed in the positive control despite altering cycling conditions and obtaining new primers and probes. Three children tested

positive for norovirus genogroups II or IV, resulting in a prevalence of 4.3%. Because there were not enough patients positive for norovirus to conduct a risk analysis, logistic regression was performed using the primary outcome of the previous hospitalization for acute diarrhea (**Error! Reference source not found.**). None of the potential risk factors (including household size) were significantly associated with increased odds of the previous hospitalization for diarrheal disease. 'Played with animals in the past 48 hours', were non-significantly associated with the previous hospitalization for diarrhea with unadjusted odds ratios (OR) each of 2.4 (95% CI: 0.6, 9.4) (**Error! Reference source not found.**). Prior playing with cat was trending on significance with an unadjusted OR of 3.8 (95% CI: 0.9, 16.1), while playing with dog was not with an unadjusted OR of 1.5 (95% CI: 0.1, 15.7) (**Error! Reference source not found.**). None of the age categories led to a significantly different OR for previous hospitalization (**Error! Reference source not found.**).

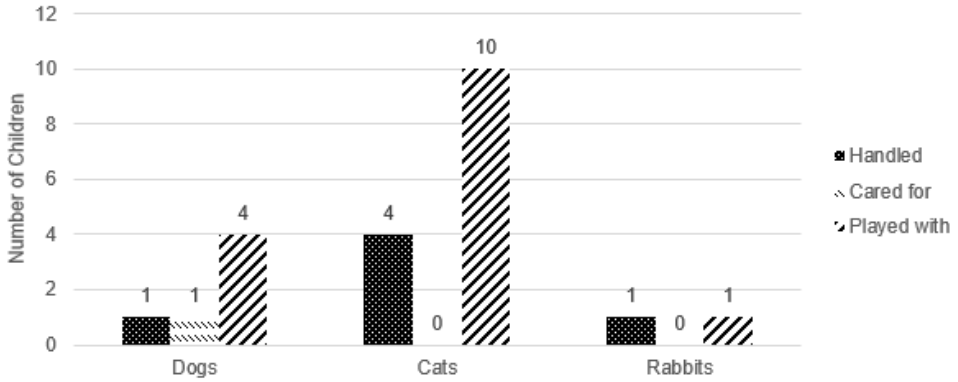

Fig. 1: Self-reported animal exposure of individuals enrolled in the study during the 48 hours prior to enrollment.

DISCUSSION

We enrolled 70 children admitted to Sibu Hospital for acute diarrhea, three of whom tested positive for norovirus GII/IV with a prevalence rate of 4.3%. Because we did not have a large norovirus-positive population in our study group and could not validate norovirus GI molecular assays successfully, we decided to look at associations between animal exposure and the previous hospitalization for acute diarrhea. Thirteen children reported previous hospitalization to diarrhea, but we could not conclusively link prior animal exposure and previous hospitalizations for diarrheal disease. Despite this, systematic reviews show significant positive associations between domestic animal exposure and diarrhea, with the strongest associations occurring when the specific pathogen, such as *Campylobacter* species, enterohemorrhagic *Escherichia coli* / Shiga toxin-producing *Escherichia coli*, *Cryptosporidium* species, and *Giardia* spoecies, is identified.[15,16]

The strongest association we were able to show between animal exposure and diarrhea was the relationship between playing with cats and previous hospitalizations for diarrhea. It is well understood that dogs and cats may transmit zoonotic diarrheal diseases to humans.[17,18] While there was no significant association between cat exposure and hospitalization for diarrhea in this study, it seems plausible that playing with, handling, or caring for cats could lead to an increased risk of diarrhea among children in Sarawak, Malaysia.

Of the study group, only three patients tested positive for a norovirus infection. Therefore, norovirus represents a small proportion of the overall diarrheal disease burden in Sibu, with a prevalence of 4.3%. With this low prevalence, there does not seem to be a compelling need for health care facilities and Sarawak to embrace

norovirus diagnostics widely. However, larger and longer assessments for norovirus should be performed before completely ruling out these viruses as important pathogens. Norovirus is more prevalent in the cold winter months at higher latitudes, and there is some evidence that suggests norovirus is more prevalent during the rainy season in tropical regions.[19,20] Because this study was carried out during the dry season in Sarawak, testing for norovirus during the rainy season in Malaysia will help establish the seasonal variation in transmission in tropical regions.

CONCLUSION

While this study was limited by the sample size, its brief duration, the failure of assays against genogroup I, and the relatively small number of confirmed norovirus infections, it was the first of its kind in Sarawak, Malaysia, and results are valuable to medical providers. However, before norovirus can be ruled out as a common cause of diarrhea and molecular diagnostics adopted at the hospital, larger and year-round assessments should be made due to the potential seasonality of the virus in tropical settings.

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