AWARD NUMBER: W81XWH-18-1-0074

TITLE: Host Epithelial Responses in Pathophysiology of *Campylobacter jejuni* Post infection Irritable Bowel Syndrome

PRINCIPAL INVESTIGATOR: Madhusudan Grover

CONTRACTING ORGANIZATION: Mayo Clinic

REPORT DATE: October 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2019	Annual Report	01SEP2018 - 31AUG2019

4. TITLE AND SUBTITLE
Host epithelial responses in pathophysiology of *Campylobacter jejuni* post infection irritable bowel syndrome

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0074

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Madhusudan Grover, M.B.B.S.

E-Mail: grover.madhusudan@mayo.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Mayo Clinic
200 1st Street SW
Rochester, MN 55905

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland, 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Intestinal infections, including with *C. jejuni* are common during military deployment. Epidemiological studies from military and civilian populations have shown that up to 1 in 6 suffering from a gastrointestinal infection may develop post-infection IBS (PI-IBS). This risk is even higher if there is comorbid psychological stress. This proposal integrates the use of colonoids derived from human volunteers who can be considered demographically at high-risk for PI-IBS development and the use of clinical *C. jejuni* isolates that have been known to cause PI-IBS. Over the last year, we have recruited 20 healthy volunteers (12 females, 8 males) between the ages of 18-35 years without or with the presence of chronic psychological stress. Sigmoid colonic biopsies were obtained to isolate intestinal crypts which were used to develop colonoids. These were infected with a set of clinical *C. jejuni* isolates and *in vitro* infectivity was studied. Interim analysis suggests that all colonoids get infected with *C. jejuni* but demonstrate differential virulence patterns (adhesion, invasion and barrier disruption). This suggests human colonoids can serve as a unique model for studying host interactions with *C. jejuni*. Transcriptomic and proteomic studies will be performed next on the infected colonoids to determine molecular changes induced by the infection. These will be compared between colonoids obtained from males and females and those without and with psychological stress. Overall, these studies will identify the influence of host characteristics upon interaction with the microbe and the epithelial responses elicited. These will help identify the role of acute infection in subsequent development of post-infection irritable bowel syndrome.

15. SUBJECT TERMS: Post-infection irritable bowel syndrome; gastroenteritis; bacterial; microbiota; barrier function; organoids

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	14	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The Centers for Disease Control and Prevention estimates that every year 1 in 6 Americans suffers from an episode of gastrointestinal infection. Approximately 14% of those may subsequently develop post-infection irritable bowel syndrome (PI-IBS), a 4-fold greater risk than unexposed individuals studied over the same time period. *Campylobacter*, a leading cause of bacterial enterocolitis in the U.S. and around the world has been associated with PI-IBS development. The military population, especially those deployed to Southeast Asia, is particularly at risk for *Campylobacter* infection and for subsequent PI-IBS development. The overarching hypothesis of this proposal is that acute injury involving unique host and pathogen characteristics results in molecular changes that are relevant for *C. jejuni* PI-IBS development. We plan to develop colonoids from volunteers that can be considered high-risk and low-risk patients for PI-IBS development and determine molecular and physiological response to acute *C. jejuni* infection with PI-IBS causing strains.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Post-infection irritable bowel syndrome; gastroenteritis; bacterial; microbiota; barrier function; organoids

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: To determine host epithelial responses with PI-IBS causing acute *C. jejuni* infection	**Timeline**	**Site 1** Mayo Clinic	**Status**
Major Task 1: Human biospecimen collection and cultures	Months		
Subtask 1: Local IRB Approval	0-2	Dr. M Grover	Completed
Subtask 2: Human volunteer recruitment (screening visit)	3-6	Dr. M Grover	Completed
Subtask 3: Sigmoidoscopy & biopsies (20 human subjects)	3-6	Dr. M Grover	Completed
Subtask 4: Colonoid cultures	3-7	Dr. M Grover	Completed
Milestone(s) Achieved	0-7		100%
Milestone Achieved: HRPO/ACURO Approval	-	-	Completed

Major Task 2: Infection and molecular studies			
Subtask 1: Colonoid- *C. jejuni* functional studies	3-8	Dr. M Grover	Completed
Subtask 2: Colonoid- *C. jejuni* molecular studies	8-13	Dr. M Grover	- *In vitro* completed on 15/20 subjects -Transcriptomics and proteomics pending
Subtask 3: Analysis, compilation of results & generation of manuscript	13-18	Dr. M Grover	Pending
Milestone(s) Achieved:	3-18		50%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

The overall project goal is to determine the host epithelial responses with PI-IBS causing acute *C. jejuni* infection. As proposed, colonoids have been derived from healthy females/males, 18-35 years-old, with or without chronic psychological stress and infected with PI-IBS causing *C. jejuni* strains. In an interim analysis, all *C. jejuni* strains tested attached and invaded colonoid monolayers derived from all of the volunteers. Two strains caused greater adhesion and invasion in all three colonoids compared to the third strain (Adhesion mean (SEM): 4.32×10^{-2} (2.33×10^{-3}), 9.36×10^{-3} (4.82×10^{-4}) vs 7.79×10^{-3} (3.51×10^{-3}); Invasion mean (SEM) 3.47×10^{-4} (2.13×10^{-5}), 2.33×10^{-4} (1.36×10^{-4}) vs 4.57×10^{-5} (1.73×10^{-5}) (ANOVA, $p<0.05$, n=3/group for both analyses). Additionally, a greater drop in transepithelial resistance (TER) was seen in colonoid monolayers exposed to the two strains with higher adhesion and invasion (Mean % of baseline TER at 30 hrs post-infection 2.58% (1.21), 14.14% (3.39) vs 42.29% (18.49), $p<0.01$). These results correlated with virulence of these strains in conventional T84 monolayers. Thus, the interim and preliminary analysis suggests that human colonoids are useful to assess the role of the host in infectivity and epithelial barrier responses to *C. jejuni* infection. Transcriptomic and proteomic characterization of the *C. jejuni* infection in these colonoids is in progress. Overall this data provides a unique human model for studying bacterial virulence which can be extended to other enteric and non-enteric pathogens.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

1. The project allowed post-doctoral fellow (Dr. Adam Edwinson) to gain proficiency in development of colonoids from human colonic biopsies and perform infection experiments with *C. jejuni* as well as assessments of infectivity, barrier function, RNA and protein extraction. As the project moves into molecular assessment (transcriptomics and proteomics), the fellow will get substantial opportunities for integration of data and assessment of pathways involved in interactions between psychological stress and infection in epithelial responses. He will be engaged in presenting the work and writing the manuscript.
2. An abstract on preliminary findings from *in vitro* studies has been submitted for the ***Experimental Biology 2020*** conference. This will allow us to disseminate the work to a broader research community. Once the molecular studies are completed, we will plan on presenting the findings at MHSRS 2020.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The following specific tasks will be completed in next reporting period:

1. Finish *in vitro* studies on the ongoing 5 colonoids.
2. RNA and protein extraction from all infected colonoids followed by transcriptomic and proteomics experiments.
3. Bioinformatics combining the infectivity data with molecular (RNA and protein) changes.
4. Statistical analysis comparing changes in sex (male/female) and psychological stress (low/high) states.
5. Presentation of results and submission of manuscript to a high impact journal.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The project has provided feasibility that colonoids can be reproducibly generated from human colon crypt cells and they get infected with *C. jejuni*, the most common cause of bacterial gastroenteritis (food poisoning) in the U.S. with estimated 1.3 million cases annually. Thus, we have provided a novel model for studying human host interactions with *C. jejuni.* This model can be used by others in the field. Additionally, this project will lay foundation for the use of studying bacterial interactions with intestinal epithelium derived from hosts with varying genetic and demographic backgrounds. This can be adopted for studying other intestinal as well as non-intestinal infections.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

This project has an impact on the broader discipline of infectious diseases. Animal models and immortalized cell lines do not capture the heterogeneity of human hosts. The model provides a paradigm where *in vitro* models can be used that recapitulate the heterogeneity, uniqueness and complex nature of the human host. The broader principle can be extrapolated and developed for other infections both in military and civilian populations.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

The project will raise awareness about a common human bacterial pathogen and inform if males/females or people without/with psychological stress exhibit unique patterns of molecular intestinal injury with *C. jejuni.* This may help triage patients who are at high risk for serious intestinal injury and downstream complications like post-infection irritable bowel syndrome.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change *Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The proteomics experiments were originally proposed to be conducted at the Washington University proteomics core as fee for service. That core is discontinuing the tissue proteomics service in Jan 2020. Hence, we will perform the proteomics at University of Minnesota Center for Mass Spectrometry and Proteomics. To avoid freeze-thaw and have homogeneity during processing, we will also perform the RNAseq at University of Minnesota Genomics Center instead of Mayo Clinic. Mayo Clinic and University of Minnesota PIs and programs often use complimentary services at the two sites.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*

- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Madhusudan Grover
Project role: PI
Research identifier (ORCID ID): 0000-0001-5092-0831
Nearest person month worked: 0.12
Contribution to Project: Dr. Grover is responsible for the oversight of all research activities. He screened study participants, performed endoscopic procedures and obtained colonic biopsies. He supervises the postdoctoral fellow on all experiments and analysis. He coordinates different molecular studies and will be engaged in summarizing the findings and drafting the manuscript.
Funding: Dr. Grover is also supported by NIH K23 DK103911.

Name: Adam Edwinson
Project Role: Post-doctoral student
Researcher Identifier (ORCID ID): 0000-0002-1538-6765
Nearest person month worked: 3.6
Contribution to Project: Dr. Edwinson has developed colonoids from the biopsies, performed infectivity experiments and collected material for molecular studies. He will be engaged in the transcriptomics and proteomics experiments and subsequently for analysis and drafting the manuscript. He will present preliminary findings at upcoming meetings.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

ENDED

P01 DK68055-12 (Farrugia)	07/15/2015-06/30/2020	0.6 calendar
NIH/NIDDK	$1,249,947	
Pathobiology of the Enteric System		
Dr. Grover discontinued support from this grant.		
Dong-A ST Research Institute	1/14/2015 - 12/05/2019	0.12 calendar
	$602,177	
Double-Blind, Placebo-Controlled, Randomized, 4-Week Phase II Clinical Trial for Assessment of Efficacy and Safety of Motilitone in Patients with Functional Dyspepsia		
Grover		

NEW

R03DK 120745 (Grover)	05/01/2019 – 03/31/2021	0.12 calendar
NIDDK	$75,000	
Mechanisms of Barrier Dysfunction in Post-infection Irritable Bowel Syndrome		

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*

- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

- Nothing to report

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

- Attached

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

- Addendum with technical progress
- Grover (PI) PCPS

Technical Progress Report Addendum

Campylobacter jejuni invasion in colonoids was compared between the three clinical isolates tested. In colonoids developed from the low stress healthy volunteers, two isolates (ID 9, 13) demonstrated a greater invasion (0.0005 and 0.0002) as compared to the third isolate (8.2e-005, ID 26, **Figure 1a**). This corresponded to the invasion differences observed with these isolates on T84 monolayers where isolate 9 and 13 had significantly greater invasion than isolate 26. However, when the same isolates were compared on colonoids from high stress volunteers, no differences were obtained between the three isolates (**Figure 1b**). Similarly, the highly invasive isolates resulted in a significantly greater barrier disruption of colonoid monolayers as compared to the less invasive isolate (86%-97% drop vs 67% drop in transepithelial resistance) in colonoids from low stress but not high stress volunteers (**Figure 2a and b**). This suggests that upon infection in epithelia derived from high stress volunteers, the isolates may demonstrate differential invasiveness and barrier disruption. Additional colonoid infections are in progress and will be combined with these to develop final output. Subsequently, transcriptional and proteomic profiling will determine if the colonoids demonstrate variable responses based on stress levels of the host or the bacterial invasiveness. A minority of colonoids monolayers (4) failed to continuously grow to allow the invasion and barrier experiments to be completed. Future experiments on the starting material (biopsies) might allow determination of biological reasons for failure of robust proliferation in this subset of colonoids.

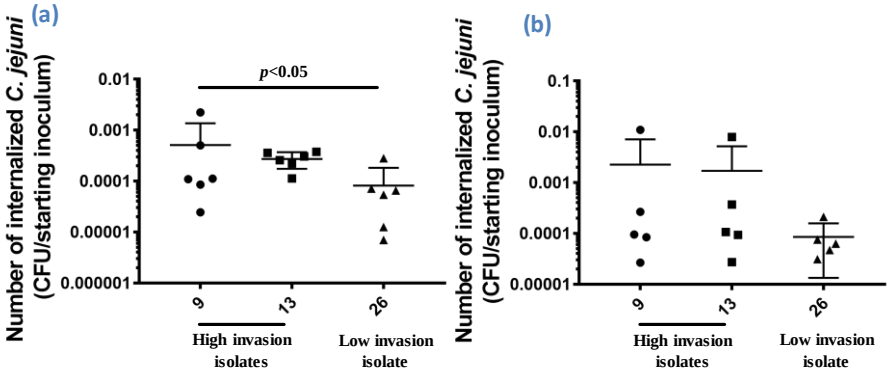

Fig 1. Comparison of *C. jejuni* invasion **(a)** low stress volunteers and **(b)** high stress volunteers. 2-way ANOVA (Kruskal-Wallis) with post-hoc comparisons.

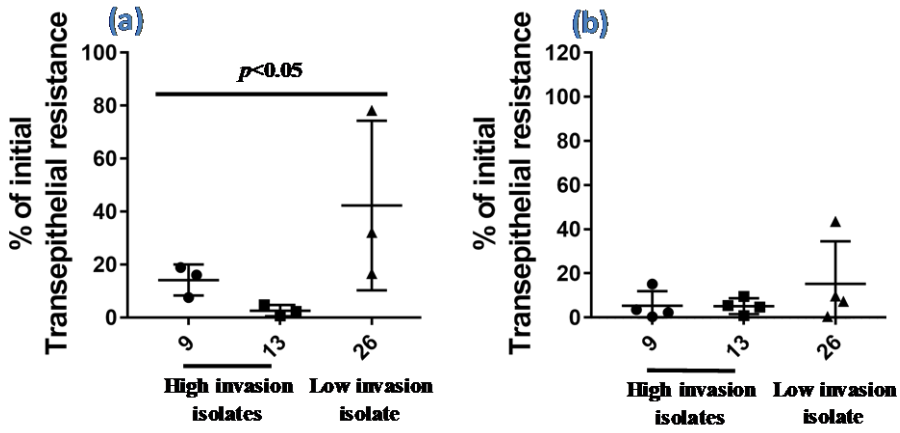

Fig 2. Comparison of *C. jejuni* infection induced drop in transepithelial resistance **(a)** low stress volunteers and **(b)** high stress volunteers. 2-way ANOVA (Kruskal-Wallis) with post-hoc comparisons.

PREVIOUS-CURRENT-PENDING SUPPORT FOR DOD

Grover, Madhusudan

PREVIOUS/COMPLETED (completed within the last 5 years**)**

Grant Title/Main PI's Last Name/Grant Number:	Performance of 13C mannitol for in vivo measurement for small intestinal permeability Grover Grant ID N/A
Effort (Calendar Months):	0.12 calendar
Funding Agency:	Mayo Clinic
Grants Officer Name & the Address of Funding Agency:	Janice S. Grace Mayo Clinic 200 First Street SW Rochester, MN 55905
Project Dates:	04/28/2015 – 04/27/2016
Funding Amount:	$100,000
Project Goals:	The goal of this project was to determine the performance of 13C mannitol for measurement of intestinal permeability
Specific Aims:	^{13}C mannitol is superior than ^{12}C (regular) mannitol for measurement of intestinal permeability
Overlap:	None

Grant Title/Main PI's Last Name/Grant Number:	AGA Rome Foundation Award Grover AGA-Rome 2015
Effort (Calendar Months):	0.12 calendar
Funding Agency:	AGA/Rome Foundation
Grants Officer Name & the Address of Funding Agency:	Wykenna S. C. Vailor American Gastroenterological Association 4930 Del Ray Avenue Bethesda, MD 20814
Project Dates:	07/01/2015 – 06/30/2016
Funding Amount:	$50,000
Project Goals:	The goal of this project was to determine the role of epithelial barrier function in the development of *C. jejuni* PI-IBS and to determine the role of *C. jejuni* virulence in the development of PI-IBS.
Specific Aims:	To assess the structure and function of the colonic epithelial barrier in acute *C. jejuni* enteritis patients and follow these patients at 6 months to ascertain PI-IBS development. To determine differences virulence gene

	expression of PI-IBS and control *C. jejuni* strains.
Overlap:	None

Grant Title/Main PI's Last Name/Grant Number:	Biomarkers for intestinal permeability in patients with functional lower gastrointestinal disorders associated with constipation Grover Study No. LUB-IIT-0005
Effort (Calendar Months):	0.12
Funding Agency:	Takeda Pharmaceuticals America
Grants Officer Name & the Address of Funding Agency:	Karen Klahn Manager, Global Medical Affairs, External Research One Takeda Parkway, B2,3170 Deerfield, IL 60015
Project Dates:	4/7/2014 – 12/9/2019
Funding Amount:	$283,920
Project Goals:	The goal of this study is to determine *in vivo* and *ex vivo* barrier function changes in constipation predominant IBS.
Specific Aims:	To determine barrier and secretory function in constipation predominant IBS.
Overlap:	None

Grant Title/Main PI's Last Name/Grant Number:	Double-Blind, Placebo-Controlled, Randomized, 4-Week Phase II Clinical Trial for Assessment of Efficacy and Safety of Motilitone in Patients with Functional Dyspepsia Grover N/A
Effort (Calendar Months):	0.12
Funding Agency:	Dong-A ST Research Institute
Grants Officer Name & the Address of Funding Agency:	Du Xiaofei, PhD Phytomedicine Research Team 21 Geumhwa-ro 105beon-gil Giheung-gu, Yongin-si Gyeonggi-do 446-905 South Korea
Project Dates:	1/14/2015 - 12/5/2019
Funding Amount:	$602,177
Project Goals:	The major goal of this project is to study safety and efficacy of Motilitone in functional dyspepsia
Specific Aims:	1) To evaluate efficacy of the investigational drug Motilitone at 90 mg in patients with FD as measured by change in maximum tolerated volume and aggregate symptom score on the nutrient drink test; 2) to assess the safety and tolerability of Motilitone at 90 mg in subjects with FD; and 3) to assess efficacy of Motilitone on gastric emptying at 4 hours following test meal.
Overlap:	None

CURRENT/ACTIVE

Grant Title/Main PI's Last Name/Grant Number:	Gastroparesis Clinical Research Consortium - Data Coordinating Farrugia U24DK 74008-11
Effort (Calendar Months):	0.12
Funding Agency:	National Institute of Diabetes and Digestive and Kidney Diseases
Grants Officer Name & the Address of Funding Agency:	Bob Pike Grants Management Branch, NIDDK 9000 Rockville Pike Bethesda, MD 20892
Project Dates:	12/1/2016 - 11/30/2021
Funding Amount:	$237,469
Project Goals:	The study will serve to better understand the pathological basis of gastroparesis. The ultimate aim of the study is to use the knowledge gained to design effective therapeutic approaches to gastroparesis.
Specific Aims:	To determine cellular and molecular changes in human gastroparesis.
Overlap:	None

Grant Title/Main PI's Last Name/Grant Number:	Barrier function alterations in post-infectious irritable bowel syndrome Grover K23DK 103911-2
Effort (Calendar Months):	9
Funding Agency:	National Institute of Diabetes and Digestive and Kidney Diseases
Grants Officer Name & the Address of Funding Agency:	Bob Pike Grants Management Branch, NIDDK 9000 Rockville Pike Bethesda, MD 20892
Project Dates:	8/3/2015 - 6/30/2020
Funding Amount:	$139,089
Project Goals:	The major goals of this project are to determine colonic barrier function and microbiota in patients with *C. jejuni* PI-IBS
Specific Aims:	Aim 1: Determine the role of colonic epithelial barrier function in the development of *C. jejuni* PI-IBS development. Aim 2: Determine the role of *C. jejuni* virulence in the development of PI-IBS Aim 3: Determine the role of host microbiota in the development of *C. jejuni* PI-IBS
Overlap:	None

Grant Title/Main PI's Last Name/Grant Number:	Gut permeability, sensitivity and symptomatology: Is there a link and explanation for exacerbation of symptoms post meal. Grover N/A
Effort (Calendar Months):	0.12
Funding Agency:	Takeda Pharmaceuticals America
Grants Officer Name & the Address of Funding Agency:	Karen Klahn Manager, Global Medical Affairs, External Research One Takeda Parkway, B2,3170 Deerfield, IL 60015
Project Dates:	4/1/2014 – 3/31/2020
Funding Amount:	$182,466
Project Goals:	The major goal of this project is to determine the role of pCLE in measurement of small intestinal barrier function.
Specific Aims:	To determine whether small bowel barrier function as measured by pCLE correlates with visceral sensitivity and symptomatology under fasting conditions, as has previously been shown using the traditional technique of urine excretion of probe molecules
Overlap:	None

Grant Title/Main PI's Last Name/Grant Number:	Host epithelial responses in pathophysiology of Campylobacter jejuni post infection irritable bowel syndrome. Grover W81XWH-18-0074
Effort (Calendar Months):	0.12
Funding Agency:	Department of Defense
Grants Officer Name & the Address of Funding Agency:	Susan Dellinger Department of the Army US Army Medical Acquisition Activity 820 Chandler Street Fort Detrick, MD 21702-5014
Project Dates:	9/1/2018 – 2/29/2020
Funding Amount:	$108,148
Project Goals:	The underlying hypothesis is that human colonoids modeling high-risk patients for PI-IBS (without and with psychological distress) have a characteristic molecular and physiological response during acute *C. jejuni* infection with PI-IBS causing strains. This was awarded under the "Discovery Award" program, a small-scale pilot grant mechanism.
Specific Aims:	To determine host epithelial responses with PI-IBS causing acute *C. jejuni* infection.
Overlap:	None

Grant Title/Main PI's Last	Mechanisms of Barrier Dysfunction in Post-infection Irritable Bowel Syndrome

Name/Grant Number:	Grover R03DK 120745
Effort (Calendar Months):	0.12
Funding Agency:	National Institute of Diabetes and Digestive and Kidney Diseases
Grants Officer Name & the Address of Funding Agency:	David Saslowsky BG 2DEM RM 6017 6707 DEMOCRACY BLVD BETHESDA MD 20817
Project Dates:	5/2/2019 – 3/31/2021
Funding Amount:	$75,000
Project Goals:	The overarching goal is to determine the role of serine proteases in barrier disruption and their regulation in the gastrointestinal tract.
Specific Aims:	1. Determine the mechanism by which serine proteases disrupt barrier function in PI-IBS. 2. Determine the proteases and protease inhibitors responsible for elevated fecal proteolytic activity in PI-IBS.
Overlap:	None

PENDING

Grant Title/Main PI's Last Name/Grant Number:	Pathophysiology of post-infection irritable bowel syndrome Grover R01DK 124200
Effort (Calendar Months):	4.8
Funding Agency:	National Institutes of Health
Grants Officer Name & the Address of Funding Agency:	TBD
Project Dates:	4/1/2020 – 3/31/2025
Funding Amount:	$250,000
Project Goals:	The goal is to determine microbial regulation of serine proteases and barrier disruption induced by serine proteases
Specific Aims:	1. To determine the mechanism by which alteration in commensal gut microbiota drives proteolytic activity in PI-IBS 2. To determine the mechanism by which host serine proteases disrupt barrier function in PI-IBS
Overlap:	None