Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 52. The Gastrointestinal Microbiome in Parasitic Infections - Towards Testing the "Hygiene Hypothesis"

7. **Start and End Date of Research Project:** 3/12/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Isabella M Cattadori, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 356,370

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Cattadori, Isabella	PI	11%-6 mo	24,791
Grove, Deb	Collaborator, genetic sequencing	39%-1 mo	5,744
Mitchell, Susan	Technician	100%-3mo	16,425
Adams, Dan	Research assistant /Technician	100%-2 mo	4,616
Pathak, Ash	Research associate	50%-2 mo 100%-2mo	22,563

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Kapur, Vivek	Co-PI	5
Praul, Craig	Collaborator, genetic sequencing	5
Albert, Istvan	Collaborator, Bioinformatics	5
Sebastian, Aswathy	Post Doc	20
Schneebele, Rob	Research assistant	100
Zaghtiti, Nabeel	Research assistant	100

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Infinity digital camera	Parasitological work (helminth staging, sexing measuring and egg counting)	$2,000
Cam-recorder	Rabbit behavior (monitoring of coprophagic behavior)	$550

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X _____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

It is our intention to continue this study by refining our questions on the role of the host gastrointestinal microbiome on chronic helminth infections. Based on the conclusive findings of the current study we will test new hypotheses through further internal and/or external funding.

12. Future of Research Project. What are the future plans for this research project?

The immediate plan is to complete the analytical work and publish the results. This is critical for guiding us to the future research plan.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X _____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	3			1
Female	1			1
Unknown				
Total	**4**			**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	4			2
Unknown				
Total	**4**			**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White	4			
Black				
Asian				2 (Indian)
Other				
Unknown				
Total	**4**			**2**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X _____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Our study contributes to advancing our understanding of the microbiome-helminth

interaction in the gastrointestinal tract. The strong interdisciplinary aspect of the research has provided many opportunities for strengthening interactions among collaborators/facilities at Penn State Main Campus and for promoting new collaborations. Moreover, the involvement of undergraduate and graduate students in research experience has led to many opportunities for training students in a comprehensive and rigorous way of thinking, driven by fundamental questions on the biology of parasitism, host-parasite and dynamics of chronic infections.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X _____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X _____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Objectives and specific goals

The broad objective of this study is to investigate the impact of parasitic infections on the dynamics and composition of the commensal microbiome of the gastrointestinal (GI) tract. We will leverage ongoing studies using the well-established model of helminth infections (*Trichostrongylus retortaeformis*) of the rabbit (*Oryctolagus cuniculus*) by performing experimental animal manipulation and high-throughput DNA sequencing of the host GI bacterial community. Bioinformatic analysis will be applied to the genomic data to characterize the microbiome assembly and statistical analysis will be used to examine the relationship between microflora community and helminth dynamics.

This host-parasite system has many similarities with the helminth infections of humans, and findings can be used to identify general rules driving parasitic-commensal interactions and to seek alternative methods for controlling infections. Importantly, this system is unique in that host's coprophagy allows individuals to naturally boost themselves with their own microbiome and potentially modulate the severity of GI helminth infections. The project has two specific goals.

Goal 1. To determine the dynamical association between intensity of infection and changes in commensal microbiome in the GI tract. By sampling rabbits over the course of the infection we anticipate to clarify how changes in helminth load influence microbiome assemblage and composition.

Goal 2. To prevent host's coprophagy and examine the consequences on microbiome assemblage and infection severity. We propose that coprophagy is a form of 'self-medication' from gastrointestinal infections and expect to identify differences in the microbiota by manipulating this

rabbit behavior.

By comparing temporal changes in the helminth-microbiome association during normal and restrained settings and over the course of the infection this study will strengthening fundamental theory on host-parasite-microbiome interaction and provide a fertile ground for developing new hypotheses on the role of the gastrointestinal microbiome in infectious diseases.

Approach

Animal experiments

A four treatment design experiment was applied: Control (C, untouched animals), Infected (I, helminth doses animals), Control with Collar (CC, untouched but with Elizabethan collar) and Infected with Collar (IC, helminth doses and with Elizabethan collar), out-bred New Zealand White male rabbits (60 days old; Harlan, US) were used. I and IC individuals were trickle dosed every week by gavage with infective third stage larvae of *T. retortaeformis* (1000 L3), C and CC received water. Groups of 4 individuals were sacrificed at days 15, 30 and 60 days post infection, 4 rabbits were also sacrificed at day 0 (baseline cases). These sampling points were selected to provide information on how host microbioma in the small intestine and caecum and parasite abundance change over the course of the experiment where infection proportionally accumulates (trickle doses) and coprophagy is constantly prevented in collared individuals.

For every sampled rabbit tissue samples were collected from the small intestine and caecum for microbiome and metabolomics analysis. Peripheral blood, mucus and tissue from the small intestine were also collected for immunological analysis. Helminths were collected from the small intestine and parasitological work performed following our established protocols (1-4). Rabbit's feces were sampled every week to estimate egg shedding rate.

Microbiome (DNA extraction and genomics)

Using a sterile scissor, ~ 5 cm piece of small intestine-section 1 (SI-1) was cut. Using a disposable sterile forceps (TWD catalog # DF8988P-S) to hold, the ingesta were gently washed with PBS, and each piece was cut open longitudinally. The DNA extraction was performed following the protocol from the Powersoil isolation kit (Mo Bio catalog # 12888-100) with minor modifications. Using a sterile swab applicator (Puritan Medical Products catalog # 25-806) the mucosa lining the inside of the small intestine was gently collected. The cotton tip of the applicator swab was cut and placed directly into the tubes containing beads and were gently vortex to mix and allow the sample to be dispersed into the solution. Next, heated lysis buffer (60°C) was added to the tubes and the tubes were vortex briefly, and were placed in a bead beater for 10 minutes at maximum speed for a rapid thorough homogenization. Following the protocol, sterile DNA-Free PCR Grade Water (Teknova catalog # W3335) was used for DNA elution. The concentration of the extracted DNA was evaluated using a NanoDrop Spectrophotometer and a *Qubit* 2.0 Fluorometer (Life Technologies). The procedure was performed in duplicate tissue sections from each experimental animal.

The sequence conservation of 16S rRNA genes allows for phylogenetic analysis and taxa identification among different species of bacteria. Our preliminary 16S rRNA sequencing results on small intestine samples revealed that both regions V1V2 and V3V5 were good candidates for

identifying organisms at the species level. Further preliminary sequencing results and comparison bioinformatics analyses confirmed that V3V5 region assigned higher percentage of sequences at the species level. To characterize the microbial communities, 347F (GGAGGCAGCAGTRRGGAAT) and 803R (CTACCRGGGTATCTAATCC) primers were chosen to a highly conserved V3V5 region in the 16S rRNA gene (5-7). The PCR products (1000 bps) were purified using the AgencourtAMPure technology (Beckman Coulter, Brea, CA) as described in 454 Technical Bulletin #2011-002, Short Fragment Removal Procedure. After clean-up, the products were quantified by both *Qubit* (LifeTechnologies, Carlsbad, CA) and qPCR using the KAPA Biosystems Library Quantification Kit (KapaBiosystems, Woburn, MA). Products were pooled based on molar amounts, run on a 1% agarose gel and extracted. After clean-up with a QIAquick PCR Purification kit (Qiagen,Valencia, CA) quality and quantity were assessed using a DNA 7500LabChip on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and *Qubit* quantification.

The sequencing was performed using a quarter PTP plate on a 454 Life Sciences Genome Sequencer FLX+ (Roche Diagnostics, Indianapolis, IN) per 454 specifications. One-way read amplicons (Lib-L) were prepared using bar-coded fusion primers Forward: CCATCTCATCCCTGCGTGTCTCCGACTCAG-MID- Forward Specific Primer and Reverse: CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-Reverse Specific Primer. PCR reactions (25 ul) for the 16s amplicons contained 5 pmoles of forward and reverse primers, ds DNA (10 to 40 ngs), 5 nmoles of dNTP, 0.25 uls of TAQ (Fast Start High Fidelity PCR system, Roche, Indianapolis, IN), and 2.5 ul of 10X buffer supplied with the enzyme. Samples were denatured at 94°C for 3 min, then cycled (Gene AMP PCR System 9700; Life Technologies, Carlsbad, CA) 27 to 35 cycles of 94°C for 15 sec, 55°C for 45 sec and 72°Cfor 60 sec with a final extension at 72°C for 8 min. Genome work was performed at the Genomics Core Facility at Penn State. Samples with less than 20,000 read were repeated aiming for an average of 50,000 read/sample.

Immunology

Quantification of systemic and local immune responses follows our established protocols (2-3). Cytokine gene expression in the small intestine duodenum was performed using Q-RT-PCR and antibodies IgA and IgG in the mucus and serum were quantified using ELISAs.

Parasitology

The small intestine was divided in 3 sections and helminths were collected in each section using our standardized procedures (2-3). Specimens of both sexes (40 males and 40 females) were stored in formalin and then used to quantify body length and width and eggs in female uterus using our digitalized system approach (1, 4).

Metabolomics

Lipids were extracted by the Folch method (8), dried under nitrogen and then stored under argon in glass vials to minimize oxidation and contamination from plasticizers. Lipid extracts were separated by reverse phase UHPLC on a Charged Surface Hybrid column and analyzed using an AB Sciex 5600 quadrupole time-of-flight mass spectrometer. In addition to the survey scan, up to twenty MS/MS product ion scans per duty cycle were acquired to aid in structural elucidation. Tools such as: MarkerView, LipidBLAST and LipidView were implemented to identify lipids and assess lipids that varied between the groups.

Bioinformatics and statistical analysis

The data set was analyzed using two different approaches. In the first approach, we have looked into the taxonomic classification using Bayesian classifier. In the second approach, similarity search approach was used to identify the taxonomies. We have used the Bayesian classifier implemented in MOTHUR package for the taxonomic classification. Fasta sequences, quality information and flow data was extracted from the binary files using MOTHUR's sffinfo command. Primer and adapter sequences were removed and the entire data set was subjected to quality trimming. Quality trimming was performed by checking for an average base quality of 35 within window size of 50 base pairs. The high quality filtered sequence reads were aligned with SILVA-compatible database reference alignment provided by MOTHUR. SILVA alignment is 50,000 columns long and hence further screening was done to remove columns in the alignment that don't contain data. Screen.seqs and filter.seqs commands were used to filter the alignment so that all our sequences overlap in the same region. The aligned sequences were then classified using the Bayesian classifier implemented in MOTHUR.

The high quality sequence reads were subjected to another round of analysis using BLAST (Basic Local Alignment Search Tool). These sequences where searched against a database of 16S microbial sequences using nucleotide-blast (blastn). The blast results were then analyzed with MEGAN (METaGenome Analyzer). MEGAN calculates the taxonomic content of blast hits using NCBI taxonomy. The program uses Lowest Common Ancestor (LCA) algorithm to assign each read to the lowest common ancestor of the set of taxa that it hits using a set of parameters. In the taxonomic classification by MEGAN, each node is labeled by a taxon. The size of the node corresponds to the number of assigned reads. Comparative visualization of the taxonomic classification of the samples was obtained using MEGAN.

Cytokine data were visually presented following the comparative $2^{-\Delta\Delta Ct}$ method (9) where Ct values of infected rabbits at every sampling point (DPI) were scaled over the average Ct of the whole controls and the mean and standard error of the scaled Cts from the infected hosts calculated at each sampling point (2-3). The antibody optical density values, background corrected, were transformed and standardized into optical density indexes (OD index) (2). Generalized linear models (GLM) or linear mixed effect models (with restricted maximum likelihood, LME-REML) are used to analyze the immunological and parasitological data.

Results and Discussion

Microbiome

The analysis of this complex dataset is still in progress. As previously reported we found that few bacteria taxa are more common in some treatments than others and this pattern is consistent across taxa levels (Fig. 1). For example, Proteobacteria, Firmicutes and Actinobacteria are well represented across all the treatment and time points while Cyanobacteria_Chloroplast and Fusobacteria are less represented, especially at time point 30 and 60. Bacteroidetes and Synergistetes showed the tendency to be more common in control and control+collar although sampling time appear to affect these patterns. We also found that there is a temporal variation in the occurrence of the taxa, some are more common at the beginning of the trial while other taxa become more abundant later on. The caecum samples are in the pipeline and will be processed using the same approach.

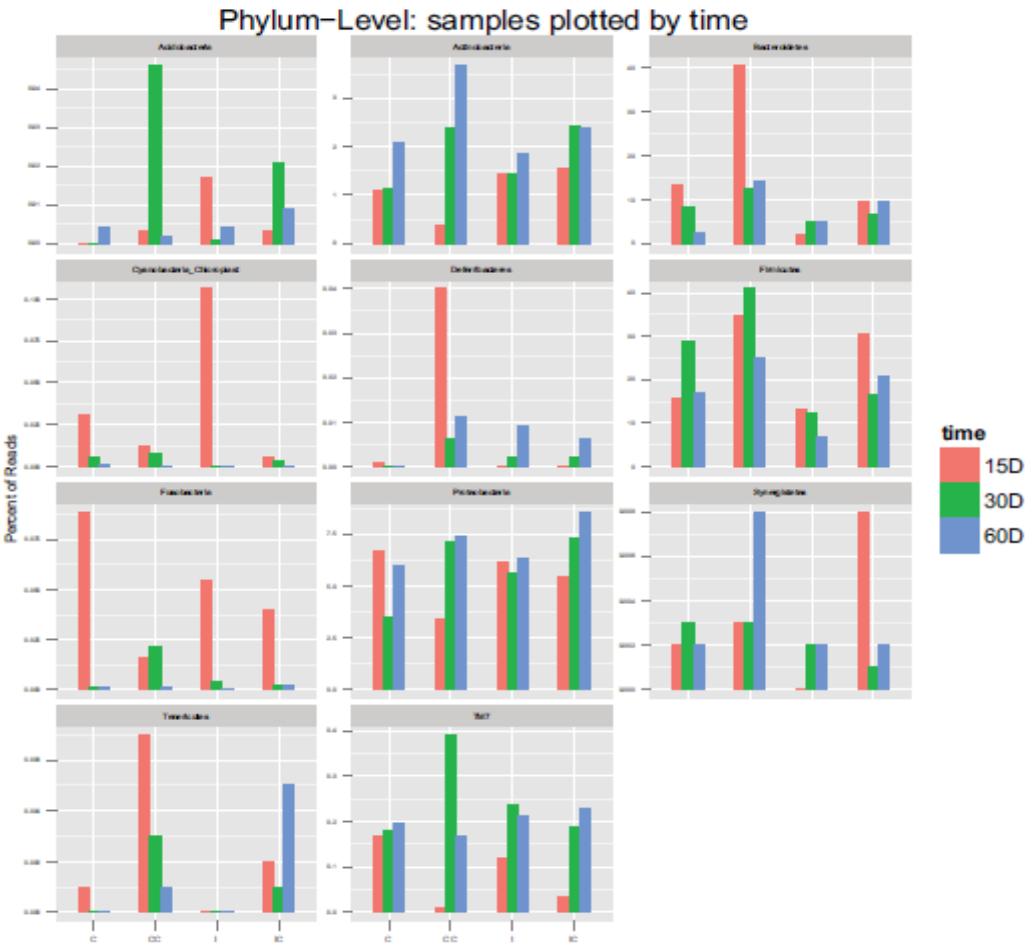

Fig. 1 Percentage of reads (y-axis) at day 15, 30 and 60 days into the trial at phylum level (different plots) by treatment (x-axis, from left to right: controls, controls+ collars, infected and infected+collars).

As part of the routine analysis, and here reported for illustrative purposes, phylogenetic microflora comparisons were carried out among samples and among time points (Fig 2-3). As previously reported in other studies and confirmed in our work, there is a relatively large variation in microflora composition between individuals, which can affect the detection of clear differences between treatments.

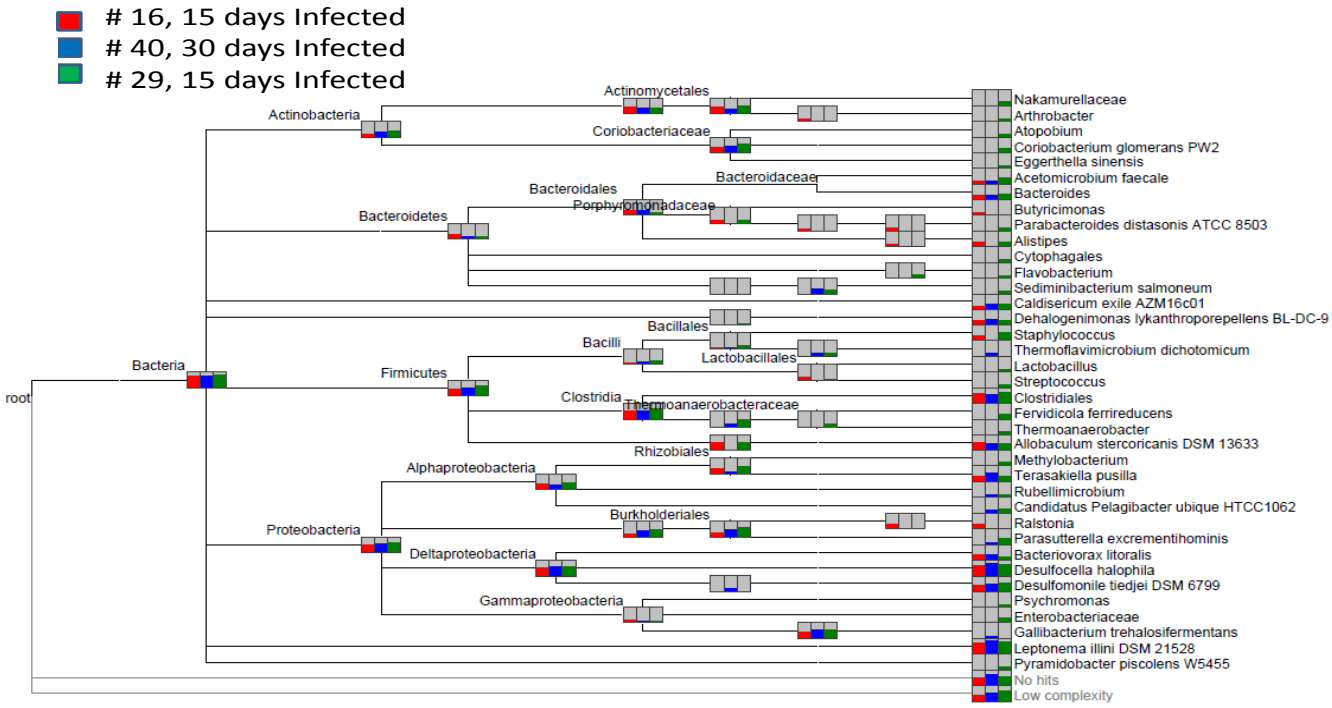

Fig 2. Small intestine duodenum microbiota community comparison based on 3 infected samples at 3 different time points.

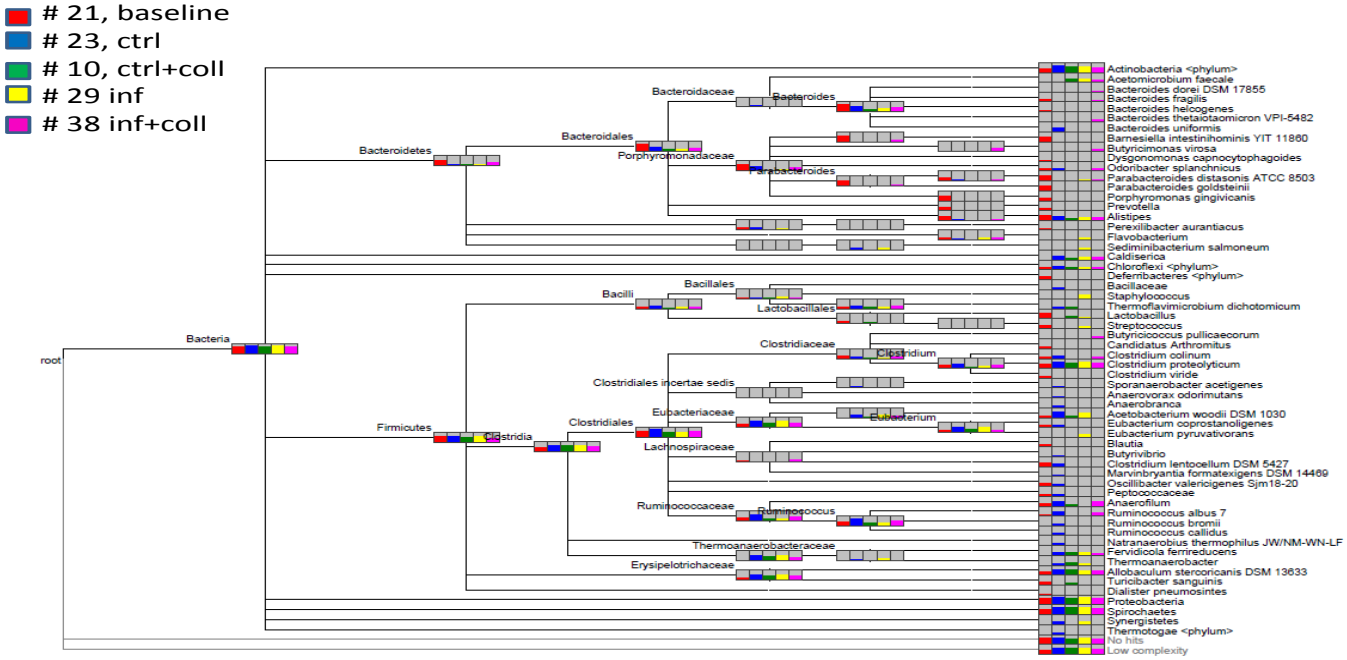

Fig. 3 Small intestine duodenum microbiota community comparison based on infected samples at 60 days into the trial along with one baseline sample. Red: baseline (sample 21), blue: control (sample 23), Green: control-collar (sample 10), yellow: infected (sample 29), magenta: infected-collar (sample 36)

Immune response in the duodenum

Very strong Muc2 and Muc5 gene expression was observed in all the 3 treatments -infected, infected+collars and controls+collars (Fig. 4), and the response was much stronger in infected than control+collar individuals (mean $2^{-\Delta\Delta Ct}$ range: 525-4036). Much lower values but second in gene expression, particularly in the infected groups, were IFNg, IL13 and IL4. Control+collar groups showed a general peak in cytokine expression at 30 DPI while for the two infected groups a stronger signal was found around 30 DPI in infected+collar and later on at 60DPI in Infected only rabbits. The remaining cytokines showed baseline or close-to baseline values for the three treatments. This cytokine trend was consistent with our previous work using rabbits infected with *T. retortaeformis* only as well as with this and a second helminth in the stomach (Murphy et al. 2011, 2013). It is interesting to note the closer pattern between infected+collar and control+collar than between infected+collar and Infected cases. Further analyses are in progress to tease apart the intricacy of these patterns.

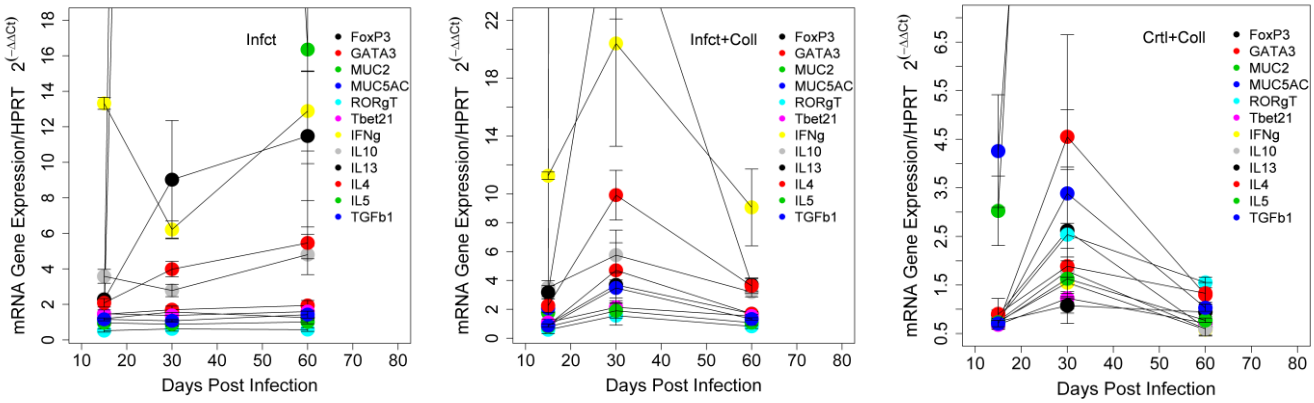

Fig. 4 Cytokine expression (Mean ± S.E.) over the course of the infection in the small intestine duodenum and by treatment (Infected –top left, Infected+Collars –top right and Controls+Collars -bottom). Data are presented as $2^{-\Delta\Delta Ct}$ (i.e. normalized Ct values of hosts scaled over the Control-only hosts) housekeeping gene: HPRT.

A somatic IgA and IgG response to the worm infection was found in the mucus and serum (Fig. 5). In the mucus both IgG and IgA increased with time and values were significantly higher in infected than control individuals.

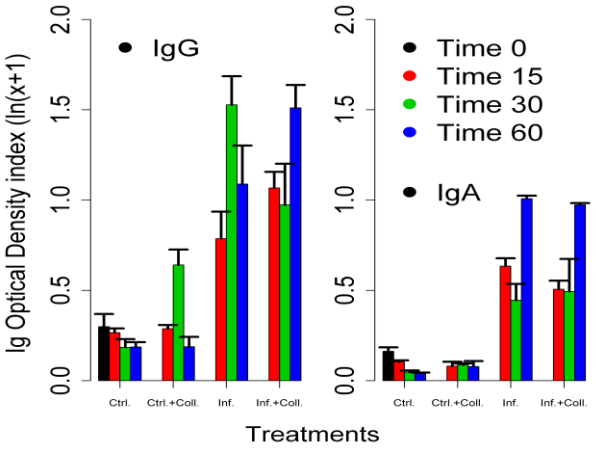

Fig. 5 Mean optical density index for IgG and IgA in the small intestine mucus of rabbits by experimental treatment and experimental time.

Parasitology

Helminth abundance was comparable between infected and infected+collar individuals (mean±s.e.: 283.89±64.69 *vs* 249.22±67.14) (Fig. 6 left side), abundance was consistently higher in the first segment of the small intestine and lower in the third segment, in agreement with our previous findings using this system.

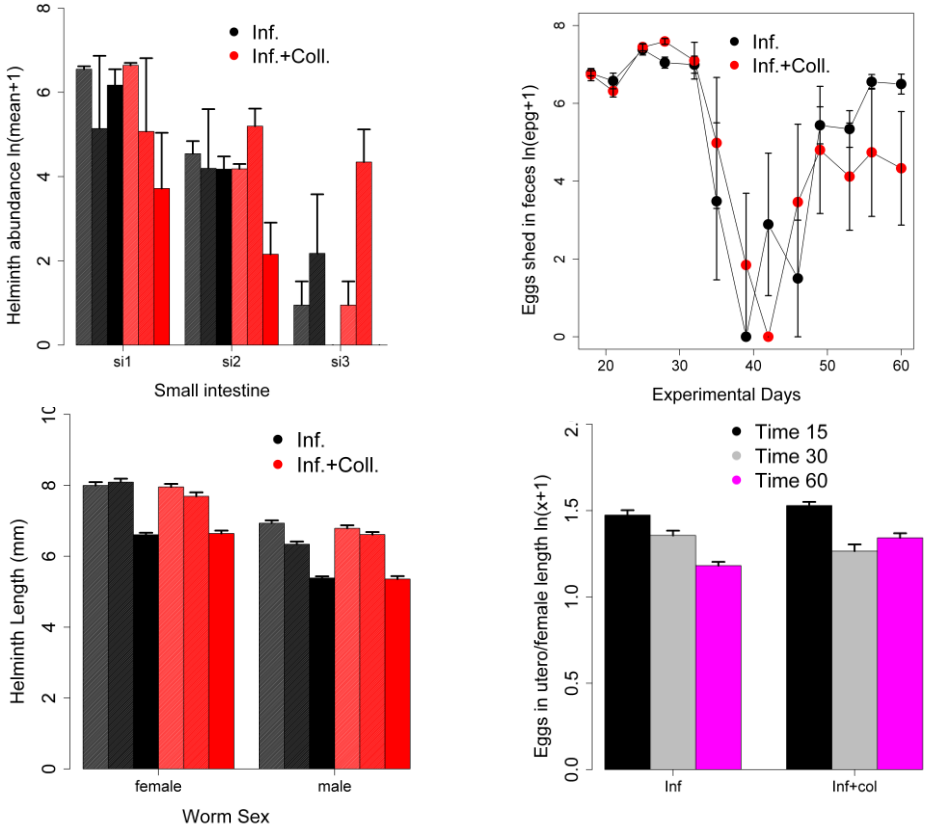

Fig. 6 Left: Mean helminth abundance by small intestine segment (SI1 to SI3) in infected and infected+collared individuals and by sampling time (color gradient from high to low 15, 30, 60 day of sampling, respectively). Right: Mean eggs shed per gram of host feces over the
Beyond the know sex bias in worm length -i.e., females are longer than males- both male and female body length was significantly different between infected and infected+collar individuals (male mean± SE= I: 6.2±0.05, IC: 7.05±0.05, $p<0.001$; female mean± SE= I: 7.50±0.06, IC: 7.05±0.05, $p<0.001$), the shorter length was recorded at 60DPI for both sexes (Fig. 6).

Helminth fecundity –i.e. eggs in utero/worm length- was significantly different between infected and infected+collar individuals (mean± SE= I: 3.00±0.06, IC: 3.26±0.05, $p<0.01$) (Fig. 6). Eggs in utero were at the highest number at 15DPI and at the lowest at 60DPI in Infected and 30DPI in infected+collar hosts (Fig. 6).

The amount of parasite eggs shed in rabbit feces was comparable between infected and infected+collar individuals (Fig. 6). For both treatments, shedding consistently dropped at around 40 days post infection but increased again by 60 days post infection, where infected rabbits showed a tendency to shed more than infected+collar hosts.

Together, these results support the hypothesis of a possible effect of the collars on parasite dynamics and traits, despite some variability in the data. This could have affected the gut microflora-worm interaction directly and/or through the host immune response. Careful analyses are in progress to clarify such relationship and the possible mechanisms driving this pattern.

Metabolomics

Preliminary principal component analysis (PCA) of lipids in the small intestine duodenum identified two main clusters, the first combined control and control+collar individuals while the second included infected and infected+collar hosts. However, the two clusters partially overlapped due to the large between-individual variability, partly driven by sampling rabbits at different time points during the course of the experiment. This pattern was also apparent for the lipid metabolomics in the caecum. Data are still under careful analysis to identify more clear patterns as well as key lipids, or group of lipids, representative of each treatment and how they varied between the groups.

References

1. Chylinski C., Boag B., Stear M. & Cattadori I.M. 2009. Effects of host characteristics and parasite intensity on growth and fecundity of Trichostrongylus retortaeformis infections in rabbits. Parasitology, 136: 117-123.
2. Murphy L, Nalpas N., Stear M. & Cattadori I.M. 2011. Explaining patterns of infection in free living populations using laboratory immune experiments. Paras. Immunol., 33: 287-302.
3. Murphy L., Pathak A.K. & Cattadori I.M. 2013. A co-infection with two gastrointestinal nematodes alters host immune responses and only partially parasite dynamics. Paras. Immunol., 35: 421-432.
4. Cattadori I.M., Wagner B.R., Wodzinski L.A., Pathak A.K., Poole A. & Boag B. 2014. Infections do not predict shedding in co-infections with two helminths from a natural system. Ecology, dx.doi.org/10.1890/13-1538.1.
5. Ahn, J., et al., Oral microbiome profiles: 16S rRNA pyrosequencing and microarray assay comparison. PLoS One, 2011. 6(7): p. e22788.
6. Klindworth, A., et al., Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, 2013. 41(1): p. e1.
7. Nossa, C.W., et al., Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J Gastroenterol, 2010. 16(33): p. 4135-44.
8. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. The Journal of biological chemistry. 1957; 226:497-509.
9. Livak KJ & Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. Methods 2001; 25:402-408.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No
19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X _____ No_____

If yes, please describe your plans:

Data analysis is still very much in progress, based on the final results we will aim for a substantial paper combining the microbiome and parasitological work. The immunological and metabolomic results could be included in this paper or be part of a second or even a third independent paper. The final plan depends very much on the quality and strength of the results.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The animal experiment followed smoothly our original design with no unexpected outcomes except for the collared animals. Towards the end of the experiment we found that collared rabbits could still engage in coprophagy by eating soft feces dropped on the cage floor. While the amount eaten was less than non-collared individuals, it might have affected some of our final results. We are aware of this issue during our data analysis and interpretation.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Cattadori, Isabella, M.	Assistant Professor in Biology, Eberly College of Science
eRA COMMONS USER NAME (credential, e.g., agency login) Isabella Cattadori	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Milan (Italy)	B.Sc	1990	
Stirling University (UK)	PhD	1999	Biology
Stirling University (UK)	Post Doc fellow	2000-2002	Biology
Sheffield University (UK)	Post Doc	2003-2006	Biology
The Pennsylvania State University	Post Doc/ Resear. Assoc.	2003-2008	Biology
University of Glasgow (UK)	Senor lect./Res. fellow	2006-2008	Biology

A. Personal Statement

I have a background in population biology and over the last 10 years I have been working on parasite community dynamics and host-parasite interaction in mammals. Most recently, my research has focused on the immuno-epidemiology of co-infection using the rabbit and its common parasites/pathogens as a study system. I am interested in understanding the molecular mechanisms of host-parasite interaction, how these mechanisms change during infections and, ultimately, how within-host processes affect disease transmission and persistence at population level. I use field and lab work and an interdisciplinary approach that combines immunology, parasitology, epidemiology and analytical and modeling tools to describe the results. Recently, I have worked in disentangling how the systemic and local immune profile is affected by single, dual and triple infections with two gastrointestinal nematodes (*T. reteortaeformis* and *G. strigosum*) and the respiratory a bacterium (*B. bronchiseptica*), and how these immune mediated processes affect shedding rate. The current grant application fits well into my long term personal interest on processes of host-parasite interaction.

B. Positions and Honors

2009- Assistant Professor, Dept. Biology, Penn State University, PA
2009- Visiting Fellow, University of Glasgow (UK)
2006/08 Senior Lecture/Royal Society University Research Fellow, University of Glasgow (UK) & Research Associate, Penn State University, USA
2000/02 European Union Marie Curie Individual Fellowship, University of Stirling (UK)
1994/96 Graduate Research Fellowship, Centro di Ecologia Alpina, CEA (Italy)
1992/94 Graduate Research Fellowship, Istituto Nazionale Fauna Selvatica, INFS (Italy)

Professional Memberships

1997- British Ecological Society
2004- Ecological Society of America

C. Selected Peer-reviewed Publications

Relevant to the current application

10. Takar J., Pathak A.K., Murphy L., Albert R. & Cattadori I.M. 2012. Network model of immune responses reveals key effectors to single and co-infection kinetics by a respiratory bacterium and a gastrointestinal helminth. *PLoS Comp. Biol.*, *8(1): e1002345.*
11. Murphy L., Pathak A.K. & Cattadori I.M. 2013. A co-infection with two gastrointestinal nematodes alters host immune responses and only partially parasite dynamics. *Paras. Immunol.*, *35: 421-432.*
12. Murphy L., Pathak A.K. & Cattadori I.M. 2013. A co-infection with two gastrointestinal nematodes alters host immune responses and only partially parasite dynamics. *Paras. Immunol., 35: 421-432.*
13. Pathak A.K., Pelenski C, Boag B. & Cattadori I.M. 2012. Immuno-epidemiology of chronic bacteria-helminth co-infections: observations from the field and evidences from the laboratory. *Int. J. Parasit., 42: 647-655*
14. Cattadori I.M., Wagner B.R., Wodzinski L.A., Pathak A.K., Poole A. & Boag B. 2014. Infections do not predict shedding in co-infections with two helminths from a natural system. *Ecology, in press.*

Additional recent publications

1. Pathak A.K., Creppage K., Werner J. & Cattadori I.M. 2010. Immune regulation of a chronic bacteria infection and consequences for pathogen transmission: the case of *Bordetella bronchiseptica* in rabbits. *BMC microbiology*, **10:** 226.
2. Graham A. Cattadori I.M., Lloyd-Smith J., Ferrari M., Bjornstad O.N. 2007. Transmission consequences of co-infection: cytokines writ large? *Trends in Parasitology, 6: 284-291*.
3. Stear M.J., Boag B., Cattadori I.M. & Murphy L. 2009. Variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep – from heritabilities to gene identification. *Parasite Immunology, 31: 274-82*.
4. Van Kuren A. Boag B., Hrubar E. & Cattadori I.M. 2013. Variability in the intensity of nematode larvae from gastrointestinal tissues of a natural herbivore*, Parasitology, 140: 632-640.*
5. Cattadori I.M., Boag B., Bjørnstad O.N., Cornell S. & Hudson P.J., 2005. Immuno-epidemiology and peak shift in a seasonal host-nematode system. *Proc. R. Soc. B, 272, 1163-1169.*

D. Research Support

NSF-LTREB Cattadori IM (PI) 2007/12
Effects of environmental changes on the long term dynamics of parasites in an age structured host population.
The goal of this project is to quantify the effect of climate change of transmission of parasitic gastrointestinal helminthes in natural rabbit populations and the consequences on host-parasite infections.

NSF Cattadori IM (PI) 20012/15
Host tolerance and resistance: applying ecological concepts to the dynamics of parasite infections.
The goal of this study is to understand how the processes of gastrointestinal helminth infection within the host can explain population level parasite dynamics.

BIOGRAPHICAL SKETCH

NAME Vivek Kapur	POSITION TITLE Professor and Associate Director
eRA COMMONS USER NAME (credential, e.g., agency login) vkapur	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Univ. Agricultural Sci., Bangalore, India	BVSc	1981-86	Veterinary Medicine
Univ. Agricultural Sci., Bangalore, India	MSc	1986-89	Poultry Science
Pennsylvania State University, Univ. Park, PA	PhD	1988-91	Veterinary Science
Baylor College of Medicine, Houston, TX	Post-doc	1992-95	Microbial Pathogenesis

A. Personal Statement

My background includes clinical training in veterinary medicine, doctoral training in the microbiology, population genetics and pathogenesis, and postdoctoral training in the molecular evolution and mechanisms of pathogenesis of a wide variety of bacterial pathogens, including *Escherichia coli*, *Streptococcus, Staphylococcus, and Mycobacterium* spp. As an early adopter in the field of microbial genomics, my research group has been responsible for the complete genome sequencing of many of the most important pathogens that cause diseases in animals and humans, and our findings have led to important insights on the evolution, physiology, and mechanisms of pathogenesis of these organisms, as well as the identification of numerous targets for diagnostic test development and vaccines. My group has also had a long-standing interest in the development of diagnostic assays and vaccines, I am listed as an inventor on several issued and licensed US and international patents relating to diagnostics and vaccines and co-founder of several start-up companies in these areas.

B. Positions and Honors

Positions and Employment

2011-present Associate Director, Huck Institutes of the Life Sciences, Penn State, University Park, PA
2007-2011 Head, Department of Vet. & Biomed. Sci, Penn State, University Park, PA.
2007-present Professor of Microbiology and Infectious Diseases, Penn State, Univ. Park, PA
2003-2007 Professor, Department of Microbiology, University of Minnesota, St. Paul, Minnesota.
2003-2007 Director, Biomedical Genomics Center, University of Minnesota, Minneapolis, Minnesota
1995-2003 Assistant and Associate Professor, Departments of Microbiology and Veterinary Pathobiology, University of Minnesota, St. Paul, Minnesota.
1995-1999 Assistant Professor, Department of Veterinary Pathobiology, University of Minnesota, St. Paul, Minnesota.
1991-1995 Postdoctoral Research Associate and Research Instructor, Department of Pathology, Baylor College of Medicine, Houston, Texas

Honors and Other Experience (past 5 years and other significant)

2013 – Chair, NIH Study Sections, IDM-10, IDM-M90 and IDM-M30; **2012** - Chair, Study Section, IDM-10; **2011** Chair IDM-M02 Study Section; Excellence in Innovation Award, Univ. MN; **2010** –
College of CSR Reviewers; Faculty 1000; **2009** - Chair, NIH IDM-Q (50) Microbiomes Study Section; co-Chair, IDM-M12 Study Section; Chair, Scientific Advisory Board PI2 program, Genome Canada; **2008** - Pasteur Veterinary Award, International Society for Genomics and Evolutionary Microbiology; Elected, ISOGEM Distinguished Fellow; Chair, NIH IDM- R Study Section; **2004** - co-founder Syntiron, LLC; **2003 -** co-Founder, ANDX Inc.; **2002 -** Schofield Medal, University of Guelph; **2000**: Pfizer Award for Research Excellence; Outstanding Achievement Award, University of Minnesota; **1998** - Collegiality Award, University of Minnesota; co-Founder, GeneMining, LLC; **1997** - CVM Award for Graduate Education; Merck Award for Creativity in

Teaching; co-Founder, Integrative Genomics, LLC; **1990** - B. Shivappa gold medal for overall academic achievement, and, University gold medal for overall academic achievement, Univ. Agric. Sci., India

Issued US Patents: (1) "*Escherichia coli* strain 364 and use of said strain as a vaccine" (U.S. Patent 5,641,491, June 24, 1997); (2) "Methods and composition for identifying Group A *Streptococcus*" (U.S. Patent 6,030,835, February 29, 2000); (3) "Pathogenicity and protective attributes of major clones of *Escherichia coli* recovered from chickens during processing" (U.S. Patent 6,096,322, August 1, 2000); (4) "Recombinant Sef14 fimbrial protein from *Salmonella*" (U.S. Patent 6,495,334 December 17, 2002); (5) "Mycobacterial diagnostics" (U.S. Patent 7,074,559 July 11, 2006); (6) "Sample collection device and method" (U.S. Patent 7,141,033 November 28, 2006); (7) "Mycobacterial diagnostics" (U.S. Patent 7,867,704 January 11, 2011)

C. Selected Peer-reviewed Publications with relevance to Genomics and Diagnostics (from more than 150 peer-reviewed publications; H-index = 51; i10 index = 133; > 11,000 citations)

1. Abrahamsen, M. S., T. J. Templeton, S. Enomoto, J. E. Abrahante, G. Zhu, C. A. Lancto, M. Deng, C. Liu, G. Widmer, S. Tzipori, G. A. Buck, P. Xu, A. T. Bankier, P. H. Dear, B. A. Konfortov, H. F. Spriggs, L. Iyer, V. Anantharaman, L. Aravind, and V. Kapur. 2004. Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. **Science** 304:441-5.
2. Baechler, E. C., F. M. Batliwalla, G. Karypis, P. M. Gaffney, W. A. Ortmann, K. J. Espe, K. B. Shark, W. J. Grande, K. M. Hughes, V. Kapur, P. K. Gregersen, and T. W. Behrens. 2003. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. **Proc Natl Acad Sci U S A** 100:2610-5.
3. Herron-Olson, L., J. R. Fitzgerald, J. M. Musser, and V. Kapur. 2007. Molecular correlates of host specialization in *Staphylococcus aureus*. **PLoS One** 2:e1120.
4. Kapur, V., D. G. White, R. A. Wilson, and T. S. Whittam. 1992. Outer membrane protein patterns mark clones of *Escherichia coli* O2 and O78 strains that cause avian septicemia. **Infect Immun** 60:1687-91.
5. Li, L., J. P. Bannantine, Q. Zhang, A. Amonsin, B. J. May, D. Alt, N. Banerji, S. Kanjilal, and V. Kapur. 2005. The complete genome sequence of *Mycobacterium avium* subspecies *paratuberculosis*. **Proc Natl Acad Sci U S A** 102:12344-9.
6. Li, L., S. Munir, J. P. Bannantine, S. Sreevatsan, S. Kanjilal, and V. Kapur. 2007. Rapid expression of *Mycobacterium avium* subsp. *paratuberculosis* recombinant proteins for antigen discovery. **Clin Vaccine Immunol** 14:102-5.
7. May, B. J., Q. Zhang, L. L. Li, M. L. Paustian, T. S. Whittam, and V. Kapur. 2001. Complete genomic sequence of *Pasteurella multocida*, Pm70. **Proc Natl Acad Sci U S A** 98:3460-5.
8. Paustian, M. L., A. Amonsin, V. Kapur, and J. P. Bannantine. 2004. Characterization of Novel Coding Sequences Specific to *Mycobacterium avium* subsp. *paratuberculosis*: Implications for Diagnosis of Johne's Disease. **J Clin Microbiol** 42:2675-81.
9. Smoot, J. C., K. D. Barbian, J. J. Van Gompel, L. M. Smoot, M. S. Chaussee, G. L. Sylva, D. E. Sturdevant, S. M. Ricklefs, S. F. Porcella, L. D. Parkins, S. B. Beres, D. S. Campbell, T. M. Smith, Q. Zhang, V. Kapur, J. A. Daly, L. G. Veasy, and J. M. Musser. 2002. Genome sequence and comparative microarray analysis of serotype M18 group A *Streptococcus* strains associated with acute rheumatic fever outbreaks. **Proc Natl Acad Sci U S A** 99:4668-73.
10. Xu, P., G. Widmer, Y. Wang, L. S. Ozaki, J. M. Alves, M. G. Serrano, D. Puiu, P. Manque, D. Akiyoshi, A. J. Mackey, W. R. Pearson, P. H. Dear, A. T. Bankier, D. L. Peterson, M. S. Abrahamsen, V. Kapur, S. Tzipori, and G. A. Buck. 2004. The genome of *Cryptosporidium hominis*. **Nature** 431:1107-12.

D. Research Support (Cumulative extramural grant awards as PI or co-PI exceed $50m)

There is no overlap of the proposed project with any of the current or pending proposals

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Albert, Istvan	Associate Professor
eRA COMMONS USER NAME IALBERT	

EDUCATION/TRAINING

Babes-Bolyai University, Cluj Napoca, Romania	B. Sc.	06/95	Physics
Babes-Bolyai University, Cluj Napoca, Romania	M. Sc.	06/96	Physics
University of Notre Dame, Notre Dame, IN, USA SUN Microsystems, USA IBM Corporation, USA	Ph. D JAVA Programmer XML Developer	05/01 03/02 04/03	Physics Programming Data Management
Stanford University, Stanford, CA	Bioinformatics Certificate	05/04	Bioinformatics

A. Personal Statement

As the director of the Bioinformatics Consulting Center (BCC) at Penn State I am ready to provide the computational expertise necessary to process and analyze the sequencing data produce by the proposal titled "*The Gastrointestinal Microbiome in Parasitic Infections - Towards Testing the "Hygiene Hypothesis"*" We have access to the hardware and software infrastructure necessary to move the data efficiently through the various data analysis protocols. We have experience using established meta-genomics software packages such as **MEGAN**, **QIIME** and **mothur** and we can perform the appropriate data analysis procedures to identify and differentiate between the community memberships, abundance distributions and structures present in different bacterial communities. In the final stages my group can provide assistance in guiding all collaborators through various types of functional analyses, from ontology and enrichment studies to network inferences. Our established data sharing and management platform and our access to high performance computing resources allows us to take on the data processing challenges that have been proposed.

B. Positions and Honors

2010-present Associate Professor, Department of Biochemistry and Molecular Biology, Pennsylvania State University, PA

2006-2010 Assistant Professor, Department of Biochemistry and Molecular Biology, Pennsylvania State University, PA

2003-2006 Research Associate, Bioinformatics Consulting Center, Huck Institutes for the Life Sciences, Pennsylvania State University, PA

2001-2003 Lab Manager, GroupLens Research Group, Department of Computer Science, University of Minnesota, MN

1996-2001 Research Assistant, Department of Physics, University of Notre Dame, IN

C. Selected Peer-Reviewed Publications (Selected from 29 peer-reviewed publications)

Most relevant to the current application

1. *Microbiome remodeling leads to inhibition of intestinal farnesoid X receptor signaling and decreased obesity. Fei Li, Changtao Jiang,Kristopher W. Krausz, Yunfei Li, Istvan Albert, Haiping Hao, Kristin M. Fabre,James B. Mitchell, Andrew D. Patterson & Frank J. Gonzalez (2013),* ***Nature Communications, 4*** *(2013)*
2. *Spatial Distribution of Bacterial Communities on Volumetric and Planar Anodes in Single-Chamber Air-Cathode Microbial Fuel Cells by Vargas IT, Albert IU, Regan JM. in* ***Biotechnol Bioeng****. Apr 25, (2013)*
3. *BioStar: An Online Question & Answer Resource for the Bioinformatics Community Laurence D Parnell, Pierre Lindenbaum, Khader Shameer, Giovanni Marco Dall'Olio, Daniel C Swan, Lars Juhl Jensen, Simon J Cockell, Brent S Pedersen, Mary E Mangan, Christopher A Miller, Istvan Albert (2011),* ***PLoS Computational Biology*** *7(10): e1002216. doi:10.1371/journal.pcbi.1002216*
4. *Travis N. Mavrich, Ilya P. Ioshikhes, Bryan J. Venters, Cizhong Jiang, Lynn P. Tomsho, Ji Qi, Stephan C. Schuster, Istvan Albert, and B. Franklin Pugh* (2008), A barrier nucleosome model for statistical positioning of nucleosomes throughout the yeast genome, **Genome Research** 12
5. *Travis N. Mavrich,, Cizhong Jiang, Ilya P. Ioshikhes, Xiaoyong Li, Bryan J. Venters, Sara J. Zanton, Lynn P. Tomsho, Ji Qi, Robert Glaser, Stephan C. Schuster, David S. Gilmour, Istvan Albert, and B. Franklin Pugh* (2008), Nucleosome organization in the Drosophila genome, **Nature** 453, 358-362
6. *Istvan Albert, Travis N. Mavrich, Lynn P. Tomsho, Ji Qi, Sara J. Zanton , Stephan C. Schuster, and B. Franklin Pugh* (2007), Translational and rotational settings of H2A.Z nucleosomes across the Saccharomyces cerevisiae genome **Nature 446, 572-576.**
7. *Daniel Blankerberg, James Taylor, Ian Schenk, Jianbin He, Yi Zhang, Matthew Ghent, Narayan Veeraraghavan, Istvan Albert, Webb Miller, Kateryna Makova, Ross C. Hardison, and Anton Nekrutenko* (2007), A framework for collaborative analysis of ENCODE data: Making large-scale analyses biologist-friendly, **Genome Research 17:960-964**

Additional recent publications of importance to the field (in chronological order)

1. Saadatpour A, Wang R-S, Liao A, Liu X, Loughran TP, et al. (2011) Dynamical and Structural Analysis of a T Cell Survival Network Identifies Novel Candidate Therapeutic Targets for Large Granular Lymphocyte Leukemia. **PLoS Computational Biology** 7(11): e1002267. doi:10.1371/journal.pcbi.1002267 (2011)
2. Assieh Saadatpour, Istvan Albert, Reka Albert (2010) Attractor analysis of asynchronous Boolean models of signal transduction networks **Journal of Theoretical Biology** 266, 641-656.
3. Istvan Albert, Juilee Thakar, Song Li, Ranran Zhang , Reka Albert (2008), Boolean network simulations for life scientist **Source Code for Biology and Medicine** 3:16.

D. Research Support

Current Support

Project Title: Damage mitigation in signal transduction networks
Sponsor: National Science Foundation, PHY 1205840
PI: Reka Albert Total Amount: $653,496
Total Award Period: 05/01/2012 - 04/30/2016
Effort (person months): 0.75 Summer Role: Co-PI, 0.5 calendar month

Project Title: MRI: Acquisition of an Illumina HiSeq2000 as a core sequencing instrument for genomics and gene expression research
Sponsor: National Science Foundation
PI: Michael Axtell Total budget requested: $798,876
Role: Co-PI (0 person months)

Project title: Summer Course on Analyzing Next-Generation Sequencing Data,
Sponsor: National Institutes of Health, 1 R25 HG006243-01
PI: Titus Brown
Total Award Period: 06/01/2012/06/01/2014 Total Budget Requested: $50,000
The goal of the project is to introduce attendees with a strong biology background to the practice of analyzing short-read sequencing data. Role: Instructor, 0.5 month

Project title: Sex-specific gene expression in malaria parasite Plasmodium falciparum
Sponsor: National Institutes of Health,
PI: Liwang Cui
Total Award Period: 12/01/2011 to 11/30/2013 Total Budget Requested: $405,357
The goal of the project is to study the molecular difference between male and female gametocytes during the sexual differentiation of the parasite. Role Co-PI, 0.5 months

Project title: Southeast Asia Malaria Research Center Supplement
Sponsor: National Institutes of Health,
PI: Liwang Cui
Total Award Period: 12/01/2011 to 11/30/2013 Total Budget Requested: $189,143
The goal of the project is to genetic diversity and artemisin resistance of the malaria parasites.
Role Co-PI, 0.5 months

Recently Completed Research Support

1 UL1 RR033184-01 Sinoway (PI) 06/01/11-06/01/16
Penn State Clinical and Translational Science Institute
The goals of the institute are to speed the translation of laboratory discoveries into treatments for patients, to engage communities in clinical research efforts, and to train a new generation of clinical and translational researchers
Role: Co-PI, Leader of Biomedical Informatics

DBI-0543285 Nekrutenko (PI) 07/01/06-07/01/09
Tailoring Genomic Data to the Needs of Experimental Biologists and Educators
The goal of the proposed project is the development of a simple and compact system for the integration of genome-based information as well as tools for genome analysis. The proposed system will include a number of innovative computational solutions that interpret simple input and guide the user during the search process and allow the scientific workflow to be described in a simple, but sufficiently powerful meta language.
Role: Co-PI