Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

Cassidy Rist, DVM

Date

Human Health in Ranomafana National Park, Madagascar: Nutritional Indicators and

Self-Reported Diarrheal Symptoms

By

Cassidy Rist, DVM MPH

Global Epidemiology

Carla Winston, PhD Committee Chair

Paul Weiss, MS Committee Member

Sarah Zohdy, PhD Field Advisor

Human Health in Ranomafana National Park, Madagascar: Nutritional Indicators and Self-Reported Diarrheal Symptoms

By Cassidy Rist

Doctor of Veterinary Medicine University of Florida 2005

Thesis Committee Chair: Carla Winston, PhD

An abstract of

A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University in partial fulfillment of the requirements for the degree of Master of Public Health in Global Epidemiology

2014

ABSTRACT

Purpose: This study describes the human nutritional status and prevalence of self-reported diarrhea in six villages located within the 5 km buffer zone of the Ranomafana National Park, Madagascar, and discusses components of the human-animal interface that have potential to contribute to food security and zoonotic disease transmission.

Methods: A cross-sectional cluster sample survey was performed over an eight-week period from June 14th to August 9th, 2013. Sixty-two households within six villages bordering the Ranomafana National Park (RNP) were randomly selected for the study. Collection methods included in-person surveys, as well as physical assessments to acquire anthropometric data. A total of 303 individual surveys were completed and 257 individual participants had anthropometric data available for analysis. Livestock ownership and frequency of animal protein intake were evaluated for association with malnutrition. Livestock husbandry practices were evaluated for association with self-reporting of diarrheal disease. Statistical analysis was conducted with SAS-callable SUDAAN 10.0.

Results: Thirty-six percent of children < 5 were underweight and 20% were stunted. Eleven percent of the adult population had a low BMI (< 18.5). Diarrhea was selfreported in 16% of the population. In multivariable regression, children who ate animal protein more than once a week had significantly lower odds [adjusted odds ratio (aOR) 0.05; 95% confidence interval (CI) 0.01, 0.26] of being underweight, and adults living in households with livestock had significantly lower odds of having a low BMI (aOR 0.32; 95% CI 0.10, 0.98). Individuals who lived in poultry-owning households and kept their poultry inside the house at night had 2.46 times the odds of reporting diarrhea (95% CI 1.09, 5.57). Individuals over 5 years of age who reported handling feces at least once a month had 2.61 times the odds of reporting diarrhea (95% CI 1.03, 6.62).

Conclusions: Investigation of the human-animal interface in this community can help generate further hypotheses on how improvements in livestock management can be directed to improve food security and decrease the risk of zoonotic disease transmission.

Human Health in Ranomafana National Park, Madagascar: Nutritional Indicators and Self-Reported Diarrheal Symptoms

By

Cassidy Rist

Doctor of Veterinary Medicine University of Florida 2005

Faculty Committee Chair: Carla Winston, PhD

A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University in partial fulfillment of the requirements for the degree of Master of Public Health in Global Epidemiology 2014

TABLE OF CONTENTS

I.	IntroductionPage 1
II.	BackgroundPage 2
III.	Methods Page 8
IV.	ResultsPage 18
V.	DiscussionPage 23
VI.	Strengths and WeaknessesPage 27
VII.	Future Directions
VIII.	References Page 31
IX.	TablesPage 36
	a. Table 1: Demographic and household characteristics
	b. Table 2: Nutritional indicators and self-reported diarrhea
	c. Table 3: Characteristics of the human-animal interface, as defined by
	livestock ownership, frequency of animal protein intake and animal
	husbandry practices
	d. Table 4: Associations of frequency of animal protein intake, livestock
	ownership, and covariates with four nutritional indicators
	e. Table 5: Associations animal husbandry practices and covariates with self-
	reported diarrhea within the past 6 months
	f. Table 6: Associations of protein frequency intake and livestock ownership
	with four nutritional indicators
	g. Table 7: Associations of animal husbandry practices and self-reported
	diarrhea

Х.	Figure 1: The Ranomafana National Park boundary line Pa	ige 42
XI.	Appendix A: Household Survey Pa	ige 43
XII.	Appendix B: Individual Survey Pa	ige 47

I. INTRODUCTION

The World Bank ranks Madagascar 132^{nd} out of 190 countries for Gross Domestic Product (1). Over 80% of the rural population lives on less than \$1.25 USD a day (2), and less than a third of rural Malagasy have access to clean water or adequate sanitation (3). Under-five mortality is at 6.2% (4), with over half of the deaths attributable to diarrheal diseases, malaria and pneumonia (3). In 2013, the Global Hunger Index placed Madagascar in the bottom quarter of all countries, reflecting the 36% of children under 5 who are underweight and the 33% of the total population who are undernourished (4). With such significant health and nutritional problems, it is important to identify the factors that contribute to the cycles of illness and food insecurity. Identification of these influential factors on a local level can inform targeted public health interventions that are community specific and potentially regionally scalable.

This study describes the human nutritional status and prevalence of self-reported diarrhea in six villages located within the 5 km buffer zone of the Ranomafana National Park (RNP), Madagascar. Livestock ownership and frequency of animal protein intake are evaluated for association with indicators of malnutrition. Livestock husbandry practices are evaluated for association with self-reporting of diarrheal disease. These analyses provide insight into the role of livestock in the health of rural communities within the RNP region.

II. BACKGROUND

Study Site

Ranomafana National Park is located in central southeastern Madagascar, and is one of six protected areas in the region embedded within a matrix of mixed human land use and natural land cover. The park boundaries were established in 1991 as a result of the combined efforts of the Malagasy government, the local villages bordering the proposed park area, and a primate biologist, Dr. Patricia Wright, who advocated for the park's establishment due to the discovery of a lemur species new to science (*Hapalemur aureus*), and the rediscovery of a lemur species (*Prolemur simus*) once thought to be extinct (*5*).

There are 127 villages that lie within a 5 km buffer zone around the RNP (*Figure 1*). The villages have access to healthcare via a series of centrally located community health clinics and hospitals located along the main road that runs through the park. Some of the villages are located along the main road, while others are between a 30-minute and 16-hour hike from road access. In Madagascar, medical care is provided free of charge to children under 5 years of age, but for all others, it is a fee-for-service system.

Malnutrition and Nutritional Indicators

Malnutrition includes both over- and under- nutrition, however within the context of most developing countries like Madagascar, under-nutrition is of greatest concern (6). The World Food Program defines malnutrition as "a state in which the physical function of an individual is impaired to the point where he or she can no longer maintain adequate bodily performance processes such as growth, pregnancy, lactation, physical work and

resisting and recovering from disease." For rural populations who rely primarily on subsistence agriculture to meet their nutritional needs, maintaining appropriate caloric intake to perform the physical labor associated with agricultural work is essential to providing food for the family. The additional challenges associated with rural living, including poor sanitation, limited access to safe water, and close contact with livestock, potentially increase the risk of exposure to pathogens that can cause severe illness in malnourished populations.

Malnutrition occurs when there is a failure to meet nutrient requirements due to lack of macronutrients (i.e. carbohydrates, protein, fat) and/or micronutrients (i.e. vitamins and minerals), or due to alterations in the digestion and/or absorption of these nutrients (7). Protein requirements for the average population are estimated to be 0.83 grams/kg body weight per day, however for many developing countries protein intake falls well below this value (8). Protein energy malnutrition (PEM) is the most common cause of immunosuppression worldwide, and it is likely through the mechanism of decreased immunity that PEM and prevalence of infectious disease are correlated (7).

Growth in children under 5 is an internationally recognized indicator of the nutritional status and health of a population (6,9). Adults and older children have a greater ability to access reserves of energy compared to young children, therefore physical manifestations of malnutrition may not be as apparent in the older population (6). Anthropometric data can be used to generate nutritional indicators for malnutrition in a population by comparing individual measures of age, sex, weight and height (or length if < 2 years old)

to the median of a reference population. Anthropometric indices in children are expressed as the number of standard deviations (i.e. z-scores) from the median of the reference population; internationally accepted reference values were developed by the Centers for Disease Control and Prevention, National Center for Health Statistics and the World Health Organization (*6*). Z-scores are calculated using the following formula:

(Measured value – Median value of reference population) SD of reference population

Underweight is defined as a weight more than 2 standard deviations (SD) below the population reference weight-for-age (i.e. z-score < -2) and is generally associated with lack of caloric intake (9). Childhood underweight is the number one risk factor for causes of death in low-income countries, causing an estimated 2 million deaths each year (10). Stunting is defined as a height more than 2 SD below the population reference height-for-age (i.e. z-score < -2) and occurs as a result of long-term nutritional deprivation or chronic intestinal disease causing a decrease in nutrient uptake. Stunting has been associated with delayed mental development, poor school performance and reduced mental capacity (6). Stunting also affects girls as they reach sexual maturity, causing a smaller pelvic size and increase in obstetrical complications (6).

Indicators developed for children (e.g. underweight, stunting) are not used to assess the nutritional status of an adult (\geq 18 years). Instead, the most useful measure of malnutrition in adults is Body Mass Index (BMI), which is calculated as the weight in kilograms divided by the square of the height in meters (kg/m²)(6). Underweight in adults is graded based on severity: Grade 1 = BMI 17 to <18.5; Grade 2 = BMI 16 to <17; and

Grade 3 = BMI < 16. A BMI < 17 has been clearly linked to increases in illness; however a BMI < 18.5 is more consistently used as the cut-off point to assess the prevalence of adult underweight within a population (6). The percent of the adult population who fall below this value may serve as an indicator for the potential impact that intermittent periods of food insecurity can have on a population. In seasonal times of decreased food availability, or if unexpected interruptions in food supplies occur, adults who are between a BMI of 17 and 18.5 are at-risk for dropping below a score of 17, the documented cutoff associated with increased risk of illness. Impact may be more severe in situations where there are minimal local or country resources available to address issues of food availability, or if food security is not a government priority (*6*). Approximately 3-5% of a normal, healthy population will have a BMI < 18.5 (*6*).

Measures of BMI are also used in the 5-17 year-old population, however, to account for changing growth rates a BMI-for-age index is used. Cut-offs for underweight are based on z-scores similar to indices used for stunting and underweight in the < 5 year-old population (*11*), and have been shown to correlate well with the grades of underweight used in adults (12). For example, those with a BMI-for-age z-score of < -1 are equivalent to adults with a BMI score of < 18.5.

Diarrheal Disease

Diarrheal disease is the leading cause of death of children in developing countries, with more than 1 billion annual cases in children under 5, causing 2 to 2.5 million deaths each year (*13*). Enteric pathogens affect intestinal absorption and cause diarrhea, both of which impair nutritional status, and have a lasting impact on the cognitive and physical

development of a child (14,15). Risk factors for severe childhood diarrhea include lack of access to clean water, inadequate sewage disposal, exposure to livestock, low standards in food handling and hygiene, and decreased access to medical care (16).

Of the 10 bacterial and parasitic pathogens most commonly associated with acute diarrhea in children, four have zoonotic transmission from livestock to humans either through direct contact or from contaminated food or water (*13*). Zoonotic pathogens such as *Salmonella enteritidis*, *Campylobacter spp.*, *Escherichia coli* (verotoxin- or shigatoxin- producing strains), and *Crypotosporidium parvum*, have the potential to cause life-threatening illness through severe complications such as hemolytic uremic syndrome (*E. coli*) or chronic diarrhea and malnutrition (*C. parvum*, *Campylobacter spp.*) (*12*). Non-typhoidal *Salmonella* alone has been estimated to cause 93.8 million cases of diarrhea each year with a conservative estimate of 155,000 deaths (*17*).

Fieldwork

The fieldwork conducted for this study was performed in collaboration with Centre ValBio (CVB), a research station founded by Dr. Patricia Wright and Stony Brook University, and located within the peripheral zone of the RNP. The CVB currently employs over 50 local Malagasy to work as research assistants and forest guides for the large number of national and international researchers who use the Centre as a home base for research activities in the RNP area.

This project represents collaboration between Emory University, the Centers for Disease Control and Prevention, CVB, and the University of Antananarivo. The Emory Global Health Institute (EGHI) provided funding for student participation through the Multidisciplinary Team Field Scholars Award. The objective of the overarching project is to study the ecology of infectious disease in communities surrounding the RNP, with a focus on diarrheal disease and malaria. A team of four EGHI students and two students from the Emory Master's in Development Program designed and implemented the 2013 summer field study with the assistance of Drs. Tom Gillespie and Sarah Zohdy of Emory's Departments of Environmental Sciences and Environmental Health. The data presented in this thesis document represent a portion of the overall study.

III. METHODS

Study Questions

Study questions for this thesis are:

- What is the effect of livestock ownership and animal protein consumption, measured by frequency of intake, on nutritional indicators in children under 5 (stunting and underweight), children ages 5-17 (BMI-for-age), and adults ≥18 years (BMI)?
- Are there animal husbandry practices that are associated with self-reporting of diarrheal symptoms? Animal husbandry practices to be evaluated include:
 - Keeping poultry inside the house
 - Participating in the slaughter of livestock
 - Cooking/preparing meat for consumption
 - Handling livestock feces

Study population

Six villages bordering the RNP were selected for inclusion in the study using the following criteria:

- The CVB Health and Hygiene Team has made at least one prior visit to the community and has an up-to-date list (within the past year) of village households and household members
- Intact forest, as part of the protected Ranomafana National Park (RNP), is within 3 km of the village

• Villages are located along the main route through the RNP or within reasonable walking distance (3 hours or less) from the main route.

Eligible households were those that included at least one member under the age of 18 years, and eligible individuals included all members of selected households. Household lists provided by the CVB Health and Hygiene Team were used to enumerate all households within the six selected villages. The enumerated households were randomly ordered and the first 10 on the list whose household included a child under the age of 18 were asked to participate in the study. If a household declined to participate, it was replaced with the next house on the randomly generated list that met the study criteria. Once the head-of-household (HOH) agreed to participate in the study, a household survey was administered (Appendix A). Additionally, all household members, including the HOH, were asked to participate in an individual survey and physical assessment (Appendix B). Individuals who participated in the survey could choose to decline the physical assessment portion of the study.

Both verbal and written consent were obtained for all participants 18 years and older. Verbal and written assent were obtained for those 10-17 years of age, and parents of children under the age of 10 acted as proxies for their children for the individual surveys. Although initially reviewed for approval by the Emory University Institutional Review Board, this work was subsequently determined by Emory University to be public health practice and therefore did not require a human subjects review. Data

The data used in this project were collected over an eight-week period from June 14th to August 9th, 2013. Collection methods included cross-sectional in-person surveys at the household and individual level, as well as individual physical assessments. Data were collected with the assistance of three members of the CVB Health and Hygiene Team who spoke fluent Malagasy and English, and were trained by Emory team members on paper survey administration techniques. An Emory student in the Nurse Practitioner's program performed the physical assessments with language translation assistance from one member of the CVB Health and Hygiene Team.

To ensure accurate data quality, results of the household surveys, individual surveys, and physical assessments were double entered in Excel by two separate individuals. The data were imported into SAS and the Compare procedure was used to find inconsistent entries. When inconsistencies were found, the original data collection instruments were reviewed to determine the correct value for the entry.

Outcome measures

Nutritional indicators were created using anthropometric data collected during the physical assessment. For children < 5, age was recorded in 1-month increments up to 6 months of age and then in 6-month increments thereafter. For children \geq 5, age was reported in 1-year increments. Height (for those \geq 2 years), length (for children < 2 years), and weight measurements were taken using standard techniques (*18*). For adults \geq 18 years, BMI was calculated as kg/m² and was categorized as low if < 18.5. Children

age 5-17 years old, were categorized as having a low BMI if the BMI-for-age z-score was more than 1 SD below the median value (z-score > -1). Children < 5 were categorized as stunted if the z-score was more than 3 SD below the median value of height-for-age (zscore < -3); Children < 5 were categorized as underweight if the z-score was more than 3 SD below the median weight-for-age (z-score < -3). For both stunting and underweight in children < 5, the z-score cut-off of < -3 rather than < -2 was chosen due to the potential measurement bias introduced by recording age at six-month intervals. By making the cutoff value lower, there is greater confidence that those categorized as underweight or stunted is an accurate classification.

Self-reporting of diarrhea was defined as affirmative if the individual answered 'yes' to having diarrhea, with or without blood, at the time of the survey or anytime within the previous six months. Diarrhea was defined as having three or more loose or watery stools in a period of 24 hours.

Exposure variables

The frequency of animal protein intake was self-reported on the individual survey. Questions of frequency were asked about eggs, poultry, pork and beef. Frequency is reported as never/less than once a month, 1-4 times a month (including once a week), and more than once a week. Frequency data were combined for the different protein sources to create two dichotomous variables for total animal protein intake: low protein intake was defined as eating animal protein less than once a month (including never); and high protein intake was defined as eating any animal protein source more than once a week. Only individuals who reported eating at least one animal protein more than once a week were included in the high protein intake category; those who reported eating multiple animal products 1-4 times a month were not included, as it could not be determined if they fell on the lower or upper end of this category. Frequency intakes were reported for individuals \geq 1 year of age.

Livestock husbandry practices were determined using both the household and individual surveys. The HOH was asked if the family owned any zebu, pigs, or poultry (i.e. chickens, ducks, geese, or turkeys). If the answer to poultry was 'yes,' the HOH was asked if they were kept inside the house at night. Participation in the slaughter of livestock, cooking/preparing meat for consumption, and handling of animal feces were determined using the individual survey. Participation in slaughter and cooking/preparing meat were affirmative if an individual reported participating in any of the activities with zebu, pigs or poultry at least once in the past 4 weeks. Handling of animal feces was affirmative if an individual reported handling the feces of zebu, pigs or poultry at least once a month.

Additional covariates of interest to this study as potential socioeconomic indicators include education, household income, owning livestock (zebu, pigs, poultry), owning a latrine, and quality of housing materials (roof/floor). Education was specified as an adult (≥18 years) having no education/incomplete primary education, having completed primary education, or having some secondary/higher education. Household income, latrine ownership, and livestock ownership (zebu, pigs, poultry) were determined by answers recorded on the household survey. Ownership of poultry included chickens, ducks, geese, and/or turkeys. The survey administrator recorded materials used for roof and floor construction of the home. Houses with metal roofs were considered to have highest-quality roof materials; roofs made of bamboo, barrel or thatching were grouped together as poor-quality roofing materials. Houses with floors made of cement, finished wood boards and/or tile were considered to have highest-quality floor materials; floors made of mud or mud and unfinished wood were grouped together as poor-quality flooring materials.

Analysis

Analyses were performed using SAS 9.3 (Cary, NC) and SAS-callable SUDAAN. Data were examined for missing or implausible values using univariate procedures for continuous variables, and frequency procedures for categorical variables. Missing values were assumed to be missing at random. Single imputation was performed for missing values at the household level using the household mean stratified on village, and for missing values at the individual level using individual means stratified on village, age, and gender. The following values were imputed: household income (n=1 household); touching animal feces (n=3); and education level (n=1).

All analyses were performed using a total weight calculated to adjust for the cluster design at the household level and for individual non-response. Data regarding age status of all household members in the village were available for three of the six villages. For these villages, the household weights were calculated using the following formula: Household weight = all eligible households/number of selected households

For the villages where age data on household members were not available, the number of eligible households was estimated as:

Estimate of eligible households = (hv/hs)*ht where:

hv = number of households visited before 10 eligible households were found hs = number of households selected

ht = total number of households in the village

The estimated number of eligible households was then used in place of the actual number of eligible households to determine the household weight.

An individual weight of 1 was given to all individuals in a household if all members elected to participate in the study. For individuals in households where not all members participated, adjustment for non-response was used to determine the individual weights for each participant:

Age < 5 years:	# of < 5 year-olds in household/ $#$ responding
Age 5 -17 years:	# of 5-17 year-olds in household/# responding
Age \geq 18 years:	# of \geq 18 year-olds in household/# responding

The individual total weight was calculated as:

Total weight = Household weight *Individual weight

Descriptive statistics for outcome variables, exposures and covariates were calculated using the entire study population. For purposes of regression analysis, any independent variables with > 2 categorical answers were re-coded to a dichotomous format. The household income cut-off point was chosen as 85,000 airary as it is the value that marks the 75th percentile for income. Education level was defined as those who never went to school or had incomplete primary education vs. a complete primary education or higher. Additionally, due to the small sample size, some variables were combined for analysis. Households owning zebu and/or pigs were grouped together, and a variable was created that grouped households into those owning any livestock vs. those owning none. Poor-quality roof and flooring materials were combined into a variable defined as households with both a poor-quality roof and floor (poor-quality housing materials) vs. households with at least one high-quality housing material.

Bivariate associations between nutritional indicators (underweight, stunting, low BMI), exposures and potential covariates were evaluated using the Wald chi-square test, and using the SUBPOPN statement in SUDAAN to limit the analysis to the appropriate age category (e.g. underweight and stunting limited to < 5 years of age group). The Wald chisquare test was also used to assess the bivariate associations between self-reported diarrhea, exposures, and potential covariates.

Twelve multiple regression models were developed to assess the individual associations of high-protein intake, low-protein intake, and ownership of livestock (zebu, pigs, and/or

poultry) with the four nutritional indicator outcomes (underweight, stunting, low BMI in \geq 5 year-olds, and low BMI in \geq 18 year-olds). Four multiple regression models were developed to assess the individual associations of keeping poultry in the house at night, participating in livestock slaughter, participating in the cooking or preparing of meat for consumption, and handling animal feces with the outcome of self-reported diarrhea.

Regression models were assessed for issues of collinearity using a SAS Macro. Covariates or their interaction terms were removed from the model sequentially if the highest Condition Index (CI) was > 30 and more than one model term had a variance decomposition proportion (VDP) > 0.5. Models with acceptable CIs and VDPs were then used as the gold standard model to further assess confounding.

Models for nutritional indicators contained the exposure of interest and the covariates of household income, and poor-quality housing materials. Education level was additionally included in the models for low BMI in the \geq 18 year-old population. Covariates were included to adjust for the potential socioeconomic effects on protein intake, livestock ownership, and nutritional status. Latrine ownership was not included as it was significantly correlated with household income (p < 0.01), suggesting the inclusion of both may be redundant or cause problems with collinearity. Household income was retained in all models regardless of its significance, however poor-quality housing materials and education were assessed for inclusion using backwards elimination based on a p-value > 0.1. Final models were then compared to the starting model and kept only

if the OR changed < 10% and the CI showed improved precision when compared to the full model.

Models for the associations of participating in livestock slaughter, cooking/preparing meat for consumption, and handling animal feces were developed for the \geq 5 year-old population and included the covariates age, sex, household income, livestock ownership, latrine ownership, and poor-quality housing materials. Age, sex, and household income were kept in the model regardless of their significance; the remaining covariates were assessed for inclusion using the backward elimination method described for the nutritional indicator models. The model of the association of keeping poultry in the house at night was developed for all ages, but limited to those living in households that owned poultry. Covariates included age, sex, household income, latrine ownership and poor-quality housing materials. As with the other models, age, sex and household income were included in the model regardless of their significance, and the other covariates were assessed using the previously described method. Unlike the models for nutritional indicators, latrine ownership was included in all of the initial models for self-reported diarrhea because of its potential direct effect on the reporting of diarrhea, separate from its potential to be a socioeconomic indicator.

IV. RESULTS

A total of 62 household surveys and 303 individual surveys were completed. One household declined inclusion in the study and the next eligible household on the randomly enumerated list was selected. Twenty-three individuals in selected households declined; the majority were adult males (n=14, 61%). A total of 257 of the 303 individual participants completed the physical assessment and had anthropometric data available for analysis.

Descriptive Statistics

Demographic and household characteristics for the population of the six villages are reported in *Table 1*. Approximately 47% (SE 2.15%) of the population was above 18 years of age, and 57% (SE 2.4%) of them were female. The majority of adults (70%; SE 4.3%) have never gone to school, or received an incomplete primary education. Households had a median monthly income of 39,866 airary (SE 5,282), which is equivalent to approximately 18 USD. The majority of individuals lived in houses made with poor-quality roofing materials (72%, SE 6.1%) or poor-quality flooring materials (74%, SE 6.6%), however these were not necessarily the same households. Only 58% (SE 7.1%) of individuals lived in homes made with both poor-quality roofing and poorquality flooring materials. Thirty-five percent (SE 4.1%) of individuals lived in households that reported owning a latrine.

Nutritional indicators and frequency of self-reported diarrhea for the population of the six villages are reported in *Table 2*. In the under 5 year-old population, 36% (SE 7.8%) were

underweight and 20% (SE 7.0%) were stunted. Approximately 16% (SE 3.8%) of the 5 to 17 year-old population had a low BMI-for-age z-score (< -1), and approximately 11% (SE 2.8%) of adults had a low BMI (<18.5). Overall, low BMI is reported for 13.5% (SE 2.4%) of the \geq 5 year-old population. Diarrhea, with or without blood was reported by almost 16% of the total population (SE 2.5%).

Characteristics of the human-livestock interface are reported in *Table 3*. The greatest number of households owned poultry (75%; SE 6.1%), while significantly fewer, but roughly equal amounts of households own zebu (16%; SE 5.7%) or pigs (16%; SE 5.4%). Only 4% (SE 3.4%) of households own both zebu and pigs, suggesting little overlap in ownership of these two species. Seventy-eight percent (SE 6.2%) of households own any livestock. In regards to animal protein consumption, just over 40% (SE 6.2%) of people eat animal protein less than once a month; however approximately 19% (SE 2.9%) eat animal protein more than once a week. Of the 221 individuals who live in households with poultry, roughly 48% (SE 9.0) keep their poultry inside the house at night. Within the month prior to the survey, 22% (SE 2.8%) of the \geq 5 year-old population had participated in the slaughter of livestock in the past 4 weeks, and 38% (2.4%) had participated in the cooking or preparing of meat. Almost 19% (SE 3.1%) of the \geq 5 year-old population reported handling animal feces at least once a month.

Bivariate Analysis

Bivariate analysis results for nutritional indicators are reported in *Table* 4. High-protein intake was protective against underweight in the < 5 population (OR 0.10; 95% CI 0.01,

0.81); however it was not associated with stunting (< 5 years), or low BMI in either the \geq 5 year-old or \geq 18 year-old populations. Low-protein intake was not significantly associated with any of the reported nutritional indicators in the bivariate analysis. Livestock ownership was evaluated as owning pigs and/or zebu, owning poultry, and owning any livestock. The only significant association was found for livestock ownership with low BMI in the \geq 18 year-old population, and it appeared to be protective (OR 0.33; 95% CI 0.11, 0.99). Few covariates were found to be significantly associated with the nutritional indicator outcomes; Poor-quality housing materials were negatively associated with underweight in children < 5 (OR 4.04; 95% CI 1.07, 15.27) and latrine ownership was negatively associated with low BMI in the \geq 18 year-old population (OR 5.63; 95% CI 1.33, 23.78).

Bivariate analyses for self-reported diarrhea are reported in *Table 5*. Self-reporting of diarrhea was significantly associated with handling animal feces at least once a month (OR 3.06; 95% CI 1.29, 7.30), but not with any other husbandry practices assessed. None of the additional covariates assessed were significant in the bivariate analysis for self-reported diarrhea.

Multiple Logistic Regression

Results of the regression analyses for all four nutritional indicators are presented in *Table* 6. All models are adjusted for household income and poor-housing materials, except for the model of the association of livestock and low BMI in the \geq 18 year-old population, which was adjusted for household income alone. Education was dropped from all models for low BMI in the \geq 18 population during the backwards elimination process. In general, high-protein intake was protective against poor nutritional status, but was only significant for underweight children < 5, who were 95% less likely to report consuming any animal protein more than once a week (OR 0.05; 95% CI 0.01, 0.26). Low-protein intake was generally associated with greater odds of poor nutritional status, but this was not significant for any of the nutritional indicator variables. Livestock ownership was negatively associated with low BMI in the \geq 18-year old population; adults living in households that owned livestock were 68% less likely to have a BMI < 18.5 (OR 0.32; 95% CI 0.10, 0.98).

Results of the regression analyses for the association of animal husbandry practices and the self-reporting of diarrhea are reported in *Table 7*. The final models for the associations with participation in livestock slaughter and the cooking/preparing meat for consumption were adjusted for age, sex, and household income; neither was significantly associated with self-reported diarrhea [(slaughter OR 0.79; 95% CI 0.30, 2.10), (cooking OR 1.14; 95% CI 0.33, 3.89)]. The final model for handling animal feces was adjusted for age, sex, household income, poor-housing materials, latrine ownership and livestock ownership; People over the age of 5 who handled feces at least once in the month prior to the survey were 2.61 (95% CI 1.03, 6.62) times more likely to report diarrhea than those who did not handle animal feces or handled animal feces less than once a month. For those living in households with poultry, the effect of keeping poultry in the home at night, adjusted for sex, age, household income, poor-housing materials and latrine ownership was also significant. Those living in houses with poultry and who kept their

poultry in the home at night had 2.46 (95% CI 1.09, 5.57) times greater odds of reporting diarrhea as those who kept their poultry outside.

V. DISCUSSION

This study provides a description of the rural population living in six villages located within 5km of the Ranomafana National Park in southeastern Madagascar. The study focuses on the human-animal interface, as defined by livestock ownership, husbandry practices, and dietary intake of animal protein. In rural communities where humans and their livestock live in close proximity and there is minimal sanitation or access to safe water, the ways in which people care for their livestock can be an important contributor to direct and indirect (i.e. through the environment) transmission of diarrheal zoonotic pathogens. Additionally, in countries with food insecurity, defined in part by indicators of poor nutritional status, livestock have been shown to play an important role in the economic and food security of low-income households (19). In order to fully investigate the relationships between livestock, zoonotic disease transmission and nutrition, studies must first describe the human-animal interface so that hypotheses on transmission pathways and livestock development interventions can be developed.

Based on the database provided by FAO (20), the most recent (2009) estimate for stunting in children < 5 in Madagascar is 49%, more than twice the prevalence found in this study (20%). The prevalence of underweight adults in 2005 was 19%, which is close to twice the prevalence found in this study (11%). The prevalence of underweight in children < 5 in this study (36%) was comparable to 2004 estimates (37%). The differences in values between the national population and the study population may result from true differences in food access or other factors such as improved access to health interventions through the influence of CVB as a large research station that provides both community health outreach and a source of employment; however, these factors could not be assessed by the study design. Alternatively, nutritional indicator estimates for the general population are between four and eight years old, so it is possible that there have been overall improvements in the nutritional status in Madagascar in the past 4-8 years. This seems unlikely, as the country has experienced significant political unrest since 2009, and many other indicators of economic growth have declined (2). Regardless of the reason, the WHO's cut-off values for public health significance still consider this population to have a "very high prevalence" of underweight children (very high prevalence >30%) and a "medium prevalence" of stunting (medium prevalence = 20-29%)(21).

Eating animal protein of any source more than once a week was protective against underweight in children < 5 in both the unadjusted and adjusted analyses. The association became stronger when the model was adjusted for household income and poor housing materials [unadjOR 0.10; 95% CI 0.01, 0.81) (aOR 0.05; 95% CI 0.01, 0.26)]. The odds of being underweight in a child who eats animal protein more than once a week is 95% less than for a child who eats animal protein less frequently. There are no universal guidelines that set the ideal level of animal protein intake, and standard protein recommendations do not distinguish between animal and plant sources (22); however, animal-based foods contain the highest amount of protein per unit energy and provide all the essential amino acids in appropriate proportions (23). Additionally, even small amounts of meat provide easily absorbable micronutrients, like iron, vitamin B12, and Vitamin A (22). Children that are able to eat meat or eggs more than once a week may be benefiting from the additional protein and micronutrients provided on a more regular basis.

Household ownership of livestock was protective against a low BMI in the adult population (aOR 0.32; 95% CI 0.10, 0.98). The adjusted OR and its 95% CI changed very little from the unadjusted OR (OR 0.33; 95% CI 0.11, 0.99). Interestingly, although owning livestock was not significant in any other analysis, the point estimates for its effect on stunting and underweight in children < 5 were well above the null, suggesting that living in a house with livestock may be detrimental to nutrition status in young children. Living in a household with livestock may increase the chance of zoonotic diarrheal illness, which has greater health effects in children than adults (24). For adults, owning zebu may translate directly into greater rice production from manure fertilization and draft power, which would improve food availability; however one would expect this to have a similar effect on children as well. Alternatively, owning livestock may be linked to more strenuous agricultural work, which builds muscle mass, and can increase weight-for-height ratios.

Two of the four animal husbandry practices evaluated were positively associated with self-reporting of diarrhea: keeping poultry in the house at night (aOR 2.46: 95% CI 1.09, 5.57), and handling animal feces at least once a month (aOR 2.61; 95% CI 1.03, 6.62). Even apparently healthy poultry can shed *Salmonella* and *Campylobacter* bacteria in their feces, which contaminates their feathers and skin, as well as the environment (25). Most of the housing structures in the six villages consisted of one or two rooms with little or no

furniture and most of the sitting and cooking was done on the floor of the house. If poultry are kept inside the house at night, they can contaminate the house floor with their feces creating a source of potentially pathogenic bacteria within the home environment.

Handling animal feces, which, for the purpose of this study includes feces from all livestock species, provides a direct route for zoonotic transmission of diarrheal pathogens. Although not determined by the survey directly, the majority of manure handled in agricultural communities is for the purpose of crop fertilization. Based on verbal communication with survey participants, livestock manure was used in either rice fields (mostly zebu manure), or in home gardens (mostly pig and poultry manure). In cattle manure, *Crypotosporidium parvum* can survive up to 8 weeks, *Salmonella* 12-28 weeks, *Campylobacter* 1-3 weeks, and *E. Coli* 0157:H7 over 100 days (26). Proper composting of animal manure has been shown to significantly reduce the amount of pathogens present (28); however, composting or other methods of manure management were not documented by this study.

VI. STRENGTHS AND WEAKNESSES

A major strength of this study is the unique collection of detailed data acquired by the survey; it captures many aspects of human health including anthropometric measures and self-reporting of symptoms, as well as livestock husbandry practices and animal protein intake. This combination of information provides a broad overview of the multiple effects that livestock have on communities in the RNP region. Although several summary papers suggest that livestock are important to both zoonotic disease and food security (22, 28, 29), studies often focus on either one or the other aspect of the human-livestock interface, when they are clearly linked. Poor, rural communities maintain livestock in order to feed themselves, whether through direct consumption of animal products, increased income from the sale of livestock, or improved agricultural production through the use of draft power and fertilization (22, 28). Husbandry practices employed by livestock keepers may facilitate zoonotic disease transmission though direct and indirect mechanisms. Investigating the dynamic processes within communities and households helps develop more complex hypotheses of how zoonotic transmission occurs and what prevention strategies may be most effective and culturally relevant.

In addition to the strength of the type of data collected, the study was performed using appropriate survey methods including enumeration of households for random selection, trained surveyors, and anthropometric measurements taken by a single, trained individual. Weights were applied to the survey data, so that the sample could accurately reflect the population of the six villages. There are several weaknesses of the study. Replacement of the single selected household that declined to participate may have introduced selection bias if the household represented a unique element of the population not represented by the replacement household. In addition, although very few values were imputed, imputation decreases true variability and may have biased both the descriptive and regression values.

The category created for frequency of protein intake may not have appropriately categorized those who ate meat more than once a week. There were 125 people who were not placed into the low protein intake category or the high protein intake category. Of these 125 people, 62 reported eating two types of animal protein 1-4 times a month, and 27 reported eating three types of animal protein 1-4 times a month. Some of these people may have actually fallen into the high protein intake category, and were therefore misclassified.

Complete dietary information was not obtained in this study; therefore there is a possibility that those who reported eating animal protein more than once a week may also be able to eat more food and of a greater variety in general. The addition of socioeconomic indicators to the model hopefully controlled for this confounding effect, however, access to quantity and variety of foods may not be directly linked to socioeconomic status. For example, the quantity of food available for consumption could vary by number of household members, or support from relatives in the community. Misclassification of self-reported diarrhea is likely high. Only 16% of all people reported having diarrhea within the past 6 months. Previous pilot data collected (unpublished) shows high prevalence of *E. coli* (48%), *Salmonella* (20%), *Shigella* (40%), and adenovirus (34%) using PCR methods in the population near RNP. In this case, self-reporting likely underestimates the prevalence, or reported diarrhea may only represent the more severe cases that are easier for participants to remember. This bias would presumably make finding associations with husbandry practices more difficult, leading to bias towards the null. Fecal samples were collected from household members during the fieldwork for this project and will be used in further analyses instead of self-reporting; however PCR results are unavailable at this time.

Significant associations between self-reported diarrhea and husbandry practices do not necessarily indicate causation, although we can imagine how transmission may occur. To further strengthen these associations, verification that livestock are shedding zoonotic pathogens and that these pathogens are found in household members during bouts of diarrhea is needed. Livestock fecal samples were also collected and are in the process of being analyzed. Future data analysis will assess pathogen links between humans and their livestock.

Finally, the small sample size and relative homogeneity of the population limited the study's ability to assess interactions between variables included in the model.
VII. FUTURE DIRECTIONS

This study was able to identify some key parameters for animal protein intake that suggest potential benefits for children's nutritional status through increased access to animal protein at least once a week. Future studies should identify access barriers to animal protein intake, such as cost of the products, or loss of livestock to disease. Additionally, a more complete dietary assessment should be performed to include information on plant-based sources of protein, micronutrient intake, and seasonal changes in nutrient intake to provide a more complete picture of food insecurity issues that need to be addressed. The links between access to livestock manure for crop fertilization and its relationship with improved crop production would also provide further information on the importance of livestock in human nutrition.

Identifying husbandry practices associated with self-reporting of diarrhea provides a starting point for future research in transmission pathways of zoonotic diarrheal pathogens. Of particular interest are the practices of keeping poultry in the house at night and using animal manure for fertilization of crops. Future studies should determine what zoonotic pathogens are present and when (season), as well describe manure management and use in greater depth. Development projects that involve building poultry houses outdoors and implementing manure-composting strategies could be assessed for their potential to decrease zoonotic diarrheal transmission.

VIII. REFERENCES

1. The World Bank. GDP Ranking. http://data.worldbank.org/data-catalog/GDP-ranking-table. Published 2013. Accessed November 10, 2013.

The World Bank. Madagascar: Poverty Assessment.
 http://go.worldbank.org/RKK12SPZU1. Published 2011. Accessed January 9, 2014.

 United States Agency for International Development. USAID Country Health Statistical Report: Madagascar. Washington, DC: Analysis, Information Management, & Communications Activity (AIM), 2009.

4. International Food Policy Research Institute, Concern, and Welthungerlife. *Global Hunger Index 2013*. Bonn/Washington, DC/Dublin, 2013.

5. Dr. Patricia C. Wright. Biography. http://home.patriciacwright.org/bio/_Accessed December 10, 2013.

6. Centers for Disease Control and Prevention and the United Nations World Food Program. *A Manual: Measuring and Interpreting Malnutrition and Mortality*. Atlanta/Rome, 2005. 7. Ritz BW and Gardner EM. Malnutrition and energy restriction differentially affect viral immunity. *Journal of Nutrition*. 2006;136(5):1141-44.

8. The World Health Organization. *Technical Report on protein and amino acid requirements*. WHO Technical Report Series, No 935. Geneva, 2007.

9. The World Health Organization. *Nutritional Landscape Information System country profile indicators: Interpretation Guide*. Geneva, 2010.

10. The World Health Organization. Mortality and burden of disease attributable to selected major risks, Part 2: Results. In: *Global Health Risks*. Geneva, 2009.

11. The World Health Organization. Child Growth Standards.http://www.who.int/childgrowth/standards/bmi_for_age/en/. Accessed February 5, 2014.

 Cole T, Flegal KM, Nicholis D, Jackson AA. Body mass index cutoffs to define thinness in children and adolescents: international survey. *British Medical Journal*. 2007;335:194.

13. O'Ryan M, Prado V, Pickering LK. A Millennium update on pediatric diarrheal
illness in the developing world. *Seminars in Pediatric Infectious Diseases*. 2005;16:125-136

14. Farthing, M. J., L. Mata, J.J. Urrutua, and R.A. Kronmal. Natural history of Giardia infection of infants and children in rural Guatemala and its impact on physical growth. *The American Journal of Clinical Nutrition*. 1986;43(3):395-405.

15. Checkley, W., L. D. Epstein, R. H. Gilman, L. Cabrera, and R. E. Black. Effects of Acute Diarrhea on Linear Growth in Peruvian Children. *American Journal of Epidemiology*. 2003;157(2):166-75.

16. Teka T, Faruqye AS, Fuch GJ. Risk factors for deaths in under-age-five children attending a diarrhoeae treatment center. *Acta Pediatr*. 1996;85:1070-75.

17. Majowicz S, Musto J, Scallan E, Angulo F, Kirk M, O'Brien SJ, et al. The Global Burden of nontyphoidal *Salmonella* gastroenteritis. *Food Safety*. 2010;50:882-89.

Centers for Disease Control and Prevention. National Health and Nutrition
 Examination Survey: Anthropometry Procedures Manual. Atlanta, 2007.

19. Food and Agriculture Organization. Chapter 1: Socio-economic consequences for poor livestock farmers of animal diseases and veterinary public health problems. In: *Improved animal health for poverty reduction and sustainable livelihoods*. Rome, 2002.

20. The Food and Agriculture Organization of the United Nations. FAOSTAT. http://faostat.fao.org. Updated 2012. Accessed March 20, 2014.

21. World Health Organization. WHO Global Database on Child Growth and Malnutrition. http://www.who.int/nutgroethdb/en/. Accessed February 27, 2014.

22. Food and Agriculture Organization of the United Nations. Livestock and food security. In: *World livestock 2011*. Rome: FAO Office of Knowledge Exchange, Research and Extension, 2011.

23. Schonfeldt HC, Hall NG. Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition*. 2012;108:s69-s76.

24. World Health Organization. Chapter 2: Diarrhoeal diseases: The basics. In: *Diarrhoea: Why children are still dying and what can be done*. Geneva, 2009.

25. Iowa State University. The Center for Food Security and Public Health: Zoonoses. http://www.cfsph.iastate.edu/DiseaseInfo/?transmission[]=006&lang=en. Accessed March 28, 2014.

26. Olsen ME. 2001. Human and animal pathogens in manure. Presented at the LivestockOptions for the Future National Conference, Winnipeg, Manitoba, Canada, June 25-27,2001.

27. Berry ED, Millner PD, Wells JE, Kalchayanand N, Guerini MN. Fate of naturally occurring Escherichia coli O157:H7 and other zoonotic pathogens during minimally managed bovine feedlot manure composting processes. *Journal of Food Production*.
2013 Aug;76(8):1308-21.

28. Thornton PK. Livestock production: recent trends, future prospects. *Phil Trans R SocB*. 2010;365:2853-2867.

29. Kelly AM, Ferguson JD, Galligan DT, Salman M, Osburn BI. One health, food security, and veterinary medicine. *J Am Vet Med Assoc*. 2013;242(6):739-743

36

Table 1. Demographic and household characteristics for the population of the six selected villages located within the Ranomafana National Park region, Madagascar; June 14th to August 9th, 2013

Characteristic (N=303)	n (weighted%)	weighted SE	
Demographics			
Sex (% female)			
Overall	173 (55.4%)	2.1%	
< 18 years old	92 (54.3%)	3.9%	
\geq 18 years old	81 (56.7%)	2.4%	
Age (years)			
< 5	50 (14.6%)	1.5%	
5 to 17	113 (38.0%)	2.5%	
≥ 18	140 (47.4%)	2.2%	
Education ¹			
Never/Some primary	95 (70.0%)	4.3%	
Completed primary	15 (11.2%)	2.7%	
Some secondary or higher ²	30 (18.8%)	3.4%	
Household Indicators			
Roof Materials			
Bamboo/Barrel/Thatch (poor-quality)	201 (72.4%)	6.1%	
Metal (high-quality)	102 (27.6%)	6.1%	
Floor Materials			
Mud, Unfinished Wood (poor-quality)	218 (73.5%)	6.6%	
Cement, Tile, Finished Wood (high-quality)	85 (26.5%)	6.6%	
Poor-quality Roof and Floor Materials	161 (58.2%)	7.1%	
Own Latrine	120 (35.1%)	4.1%	
Household Income in Airary			
Median	39,866³	5,282	
75 th Percentile	85,289 ⁴	12,341	

¹Reported for the \geq 18 year-old population who completed the individual survey (n=140)

² Includes high school and college

³ Approximately equivalent to 18 USD

⁴Approximately equivalent to 40 USD

Characteristic	n (weighted %)	weighted SE
Nutritional Indicators		
Low BMI ¹ (\geq 5 years)	30 (13.5%)	2.4%
Low BMI-for-age (5-17 years)	17 (15.5%)	3.8%
Low BMI (≥18 years)	13 (11.4%)	2.8%
Stunting ²	10 (20.1%)	6.1%
Underweight ²	17 (36.3%)	7.8%
Self-Reported Symptoms ³		
Diarrhea with or without blood	54 (15.6%)	2.45%

Table 2. Nutritional indicators and self-reporting of diarrhea for the population of the six selected villages located within the Ranomafana National Park region, Madagascar; June 14th to August 9th, 2013

¹Low BMI reported for the \geq 5 year-old population who completed the physical assessment and had either a BMI < 18.5 (\geq 18 years, n= 110) or a BMI-for-age z-score < -1 (5-17 years, n= 108)

 2 Stunting and underweight reported for z-score < -3 for the < 5 year-old population who completed the physical

assessment (n=50)

³Reported symptoms were current or had occurred within the past 6 months (n=303)

Characteristics (N=303)	n (weighted %)	weighted SE	
Livestock Ownership			
Zebu	46 (16.3%)	5.7%	
Poultry ¹	221 (74.8%)	6.1%	
Pigs	46 (16.5%)	5.4%	
Animal Protein Intake ²			
Eat Eggs			
Never/less than once a month	235 (80.1%)	5.2%	
1-4 times a month	46 (15.2%)	5.1%	
More than once a week	17 (4.7%)	1.2%	
Eat Pork			
Never/less than once a month	169 (62.3%)	5.1%	
1-4 times a month	78 (23.1%)	4.2%	
More than once a week	51 (14.5%)	2.6%	
Eat Poultry			
Never/less than once a month	226 (79.6%)	4.2%	
1-4 times a month	43 (12.3%)	3.5%	
More than once a week	29 (8.2%)	2.0%	
Eat Beef			
Never/less than once a month	141 (53.3%)	5.3%	
1-4 times a month	134 (40.7%)	5.0%	
More than once a week	23 (6.0%)	2.0%	
High Protein Intake			
Eat any animal protein more than once a week	69 (19.4%)	2.9%	
Low Protein Intake			
Eat animal protein less than once a month	104 (40.1%)	6.2%	
Animal Husbandry Practices			
Poultry kept inside house at night ³	99 (48.4%)	9.0%	
Participate in livestock slaughter ^{4,5}	56 (22.4%)	2.8%	
Females	10 (13.5%)	3.4%	
Participate in cooking/preparing meat ^{4,5}	101 (38.3%)	2.4%	
Females	77 (77.7%)	3.9%	
Handle animal feces ^{4,6}	52 (18.9%)	3.1%	
Females	29 (54.1%)	6.4%	

Table 3. Characteristics of the human-animal interface, as defined by livestock ownership, frequency of animal protein intake and animal husbandry practices for the population of the six selected villages located within the Ranomafana National Park region, Madagascar; June 14th to August 9th, 2013

¹Poultry include chickens, ducks, turkey and/or geese

² Reported for population ≥ 1 years of age (n=298)

_

³Reported for population owning poultry (n=221)

⁴ Reported for population \geq 5 years of age (n=253)

⁵At least once in the past 4 weeks ⁶At least once a month

Table 4. Associations of frequency of animal protein intake, livestock ownership, and covariates with underweight and stunting in children < 5 years, low BMI in the ≥ 5 population and low BMI in adults ≥ 18 years

	OR (95% CI)	Р	
Underweight (< 5 years) ^{l}			
High protein intake ^{2,3}	0.10 (0.01, 0.81)	*0.03	
Low protein intake ^{2,4}	2.96 (0.58, 15.07)	0.19	
Own zebu and/or pigs	2.85 (0.59, 13.84)	0.19	
Own poultry ⁵	3.72 (0.92, 14.96)	0.06	
Livestock ownership	2.43 (0.60, 9.80)	0.21	
Household income < 85,000	2.73 (0.58, 12.94)	0.65	
Own latrine	0.47 (0.12, 1.85)	0.28	
Poor-quality roof/floor ⁶	4.04 (1.07, 15.27)	*0.04	
Stunting $(< 5 \text{ years})^{1}$			
High protein intake ^{2,3}	0.25 (0.03, 2.07)	0.20	
Low protein intake ^{2,4}	3.68 (0.74, 18.26)	0.1	
Own zebu and/or pigs	1.64 (0.29, 9.27)	0.5	
Own poultry ⁵	3.42 (0.45, 25.93)	0.2	
Livestock ownership	2.30 (0.31, 17.27)	0.4	
Household income < 85,000	1.77 (0.31, 17.27)	0.1	
Own latrine	0.24 (0.04, 1.37)	0.1	
Poor-quality roof/floor ⁶	1.48 (0.32, 6.79)	0.6	
Low BMI (ages 5 and older) ⁷			
High protein intake ³	0.62 (0.20, 1.93)	0.40	
Low protein intake ⁴	0.96 (0.41, 2.26)	0.93	
Own zebu and/or pigs	1.17 (0.46, 2.97)	0.74	
Own poultry ⁵	1.09 (0.48, 2.49)	0.83	
Livestock ownership	0.90 (0.40, 2.08)	0.83	
Household income < 100,000	0.63 (0.23, 1.77)	0.33	
Own latrine	1.29 (0.57, 2.90)	0.54	
Poor-quality roof/floor ⁶	0.68 (0.30, 1.54)	0.3	
Low BMI (adults ≥ 18 years) ⁸			
High protein intake ³	0.66 (0.12, 3.56)	0.62	
Low protein intake ⁴	0.78 (0.19, 3.16)	0.72	
Own zebu and/or pigs	0.18 (0.02, 1.50)	0.1	
Own poultry ⁵	0.39 (0.13, 1.16)	0.0	

Livestock ownership	0.33 (0.11, 0.99)	*0.05
Household income < 100,000	0.56 (0.18, 1.72)	0.30
Own latrine	5.63 (1.33, 23.78)	*0.02
Poor-quality roof/floor ⁶	0.41 (0.14, 1.25)	0.12
Low education level ⁹	3.11 (0.33, 8.94)	0.96

* significant at p < 0.05

 1 n= 50

² Reported for children < 5 and ≥ 1 years of age (n= 45)

³ At least one type of animal protein (meat or eggs) reported more than once a week ⁴ Animal protein intake reported as never or less than once a month for all sources (meat and eggs)

⁵ Poultry include chickens, ducks, turkey and/or geese

⁶ Roof made of bamboo, barrel, or thatch and floor made of mud or combination of mud and unfinished wood

 7 n= 278

⁸ n= 110

⁹ Low education level includes no school ever, or incomplete primary

Table 5. Associations animal husbandry practices and covariates with self-reported diarrhea within the past 6 months.

	OR (95% CI)	Р
Self-Reported Diarrhea		
Poultry kept inside house at night ¹	1.58 (0.63, 3.97)	0.32
Slaughter any livestock ^{2,3}	0.92 (0.35, 2.42)	0.86
Cook/prepare meat ^{2,3}	1.64 (0.82, 3.29)	0.16
Handle animal feces ^{3,4}	3.06 (1.29, 7.30)	*0.01
Own zebu and/or pigs	0.78 (0.29, 2.08)	0.61
Own poultry ⁵	1.55 (0.57, 4.23)	0.38
Livestock ownership	1.73 (0.56, 5.30)	0.33
Female	1.42 (0.74, 2.71)	0.29
Under 18 years of age	0.86 (0.44, 1.68)	0.64
Household income < 85,000	0.40 (0.17, 0.96)	0.25
Own latrine	1.95 (0.91, 4.19)	0.08
Poor-quality roof/floor ⁶	0.77 (0.34, 1.73)	0.52
Low education level ⁷	0.36 (0.10, 1.33)	0.14

* significant at p < 0.05

¹ Reported for population owning poultry (n=221)

 2 At least once in the past 4 weeks

³ Reported for population \geq 5 years of age (n=249)

⁴At least once a month

⁵ Poultry include chickens, ducks, turkey and/or geese

⁶ Roof made of bamboo, barrel, or thatch and floor made of mud or combination of mud and unfinished wood

⁷ Low education level includes never or some primary school; Reported for adults \geq 18 years of age (n=140)

	OR unadjusted	OR _{adjusted} (95% CI)	P _{adjusted}
Underweight (< 5 years)			
High-protein Intake	0.10	0.05 (0.01, 0.26)	*<0.01
Low-protein Intake	2.96	1.29 (0.19, 8.64)	0.79
Livestock ownership	2.43	2.19 (0.48, 9.87)	0.30
Stunting (< 5 years)			
High-protein Intake	0.28	0.32 (0.05, 2.09)	0.23
Low-protein Intake	4.86	3.24 (0.63, 16.73)	0.16
Livestock ownership	2.30	2.47 (0.60, 10.13)	0.20
Low BMI (ages 5 and older)			
High-protein Intake	0.62	0.44 (0.11, 1.73)	0.24
Low-protein Intake	0.96	1.36 (0.50, 3.68)	0.54
Livestock ownership	0.91	1.00 (0.41, 2.43)	0.99
Low BMI (adults ≥ 18 years)			
High-protein Intake	0.66	0.53 (0.09, 3.31)	0.49
Low-protein Intake	0.78	0.99 (0.19, 5.13)	0.69
Livestock ownership	0.33	0.32 (0.10, 0.98)	*0.05

Table 6. Associations of protein frequency intake and livestock ownership with four nutritional indicators

¹Adjusted for household income and poor-quality housing materials

Table 7. Associations of ani	imal husbandry practices	and self-reported diarrhea

	OR unadjusted	OR _{adjusted} (95% CI)	P adjusted
Self-reported diarrhea			
Poultry kept inside house at night ¹	1.58	2.46 (1.09, 5.57)	*0.03
Slaughter any livestock ²	0.92	0.79 (0.30, 2.10)	0.63
Cook/prepare meat ²	1.64	1.14 (0.33, 3.89)	0.83
Handle animal feces ³	3.06	2.61 (1.03, 6.62)	*0.04

* significant at p < 0.05

¹Adjusted for age, sex, household income, and latrine ownership; Reported for population owning poultry (n=221)

²Adjusted for age, sex, household income and poor-housing materials; Reported for population \geq 5 years of age (n=249) ³ Adjusted for age, sex, household income, poor-housing materials, latrine ownership, and livestock

ownership; Reported for population ≥ 5 years of age (n=249)

X. Figures

Figure 1^{*}. The Ranomafana National Park boundary line and the 127 villages located within the 5km buffer zone



Ranomafana National Park, Madagascar

*Map created by Cassidy Rist, 2013. All rights reserved.

XI. APPENDIX A: Household Survey

Section 1: Household Composition							
01 Ankialosud 02 Ambodiaviavy 03 Ambatolahy 04 Bevohazo 05 Vohiparara 06 Sahavondronana							
A1a: Village number: A1b: Household number: A1c: Interviewer Initials							
A1d: Time Star	ted::	A1e: Time Finis	hed::				

A2: "Please list everyone who is a member of your household. This includes everyone currently living with you and th living with you, but contributing income to the household. Include their sex, age, and relationship to you."

Individual Number	Age (years)	Sex (M/F)	Relationship of this person to you	Currently living in your home?	Individual Survey Completed?

A3: What is the average total monthly income for the household? Include contributions from those not currently living with you:

_____ ariary

	Section 2: Livestock owned by household					
	"I will now ask about the number and type of livestock that all members of your household own."					
NO	QUESTION	RESPONSE				
B1	Does your household own Zebu?	00 No (Skip to B2) 01 Yes				
B1a	How many zebu does your household own?					
B1b	Where are your zebu kept at night?	 01 Inside the house 02 In the yard, not in a pen 03 In the yard, in a pen 88 Other:				
B1c	Where do your zebu go during the day?	Circle all that apply 01 They stay in the yard or in a pen 02 In or near the rice paddies 03 Forest 04 Farming land (actively growing crops) 05 Recently cleared land (not in use yet) 06 Side of the road 88 Other 99 Don't Know/Refused				
B1d	Where are your zebu slaughtered?	Circle all that apply: 01 In the house 02 In the yard close to the house (within sight of the house) 03 In the yard but far from the house (unable to see the house) 04 Take to slaughterhouse 05 Slaughter elsewhere in the village 88 Other: 99 Don't Know/Refused				
B2	Does your household own Pigs?	00 No (Skip to B3) 01 Yes				
B2a	How many pigs does your household own?					
B2b	Where are your pigs kept at night?	01 Inside the house 02 In the yard, not in a pen 03 In the yard, in a pen 88 Other: 99 Don't Know/Refused				
B2c	Where do your pigs go during the day?	Circle all that apply: 01 They stay in the yard or in a pen 02 In or near the rice paddies 03 Forest 04 Farming land (actively growing crops) 05 Recently cleared land (not in use yet) 06 Side of the road 88 Other 99 Don't Know/Refused				
B2d	Where are your pigs slaughtered?	Circle all that apply: 01 In the house 02 In the yard close to the house (within sight of the house) 03 In the yard but far from the house (unable to see the house) 04 Take to slaughterhouse 05 Slaughter elsewhere in the village 88 Other: 99 Don't Know/Refused				
B3	Does your household own Poultry? <i>"Poultry include all chicken, ducks, turkeys and geese"</i>	00 No (Skip to C1) 01 Yes				

B3a	How many poultry does your	
	household own?	
B3b	Where are your poultry kept at	01 Inside the house
	night?	02 In the yard, not in a pen
	-	03 In the yard, in a pen
		88 Other:
		99 Don't Know/Refused
B3c	Where do your poultry go during the	Circle all that apply
	day?	01 They stay in the yard or in a pen
		02 In or near the rice paddies
		03 Forest
		04 Farming land (actively growing crops)
		05 Recently cleared land (not in use yet)
		06 Side of the road
		88 Other
		99 Don't Know/Refused
B3d	Where are your poultry slaughtered?	Circle all that apply:
		01 In the house
		02 In the yard close to the house (within sight of the house)
		03 In the yard but far from the house (unable to see the house)
		04 Take to slaughterhouse
		05 Slaughter elsewhere in the village
		88 Other:
		99 Don't Know/Refused

	Section 3: Material Goods Owned by	Household			
	"I will now ask about goods owned by your household."				
NO.	QUESTION	RESPONSE			
C1	Does anyone in your household own a radio?	00 No 01 Yes 99 Don't Know/Refused			
C2	Does anyone in your household own a bicycle?	00 No 01 Yes 99 Don't Know/Refused			
C3	Does anyone in your household own a car?	00 No 01 Yes 99 Don't Know/Refused			
C4	Does your household own bed nets?	00 No (Skip to B5) 01 Yes 99 Don't Know/Refused (Skip to B5)			
C4a	How many bed nets does your household own?				
C5	Does your household have a functional latrine? "Do not count latrines that are not in working order"	00 No (Skip to C6) 01 Yes 99 Don't Know/Refused			
C5a	How important are the latrines for your family? Interviewer: Please read answers out loud to participant	 01 Not important at all 02 A little important 03 Moderately important 04 Very important 05 Extremely important 99 Don't Know/Refused 			

C5b	How often do members of your household use the latrine? Interviewer: Please read answers out loud to participant	 01 Never 02 Rarely (not every day) 03 Sometimes (almost everyday) 04 Often (everyday) 99 Don't Know/Refused
C6	Does anyone in your household own a cell phone?	00 No 01 Yes 99 Don't Know/Refused

POST-INTERVIEW: DO NOT ASK OUT LOUD:

D1: Observe the house- Circle all that apply 01 Mud and clay walls 02 House on stilt poles 03 Metal sheet roof 04 Brick walls

05 Bamboo roof 06 Barrel roof

XII. APPENDIX B: Individual Survey

Section 1: Individual Information					
01 Ankialosud	02 Ambodiaviavy	03 Ambatolahy	04 Bevohazo	05 Vohiparara	06 Sahavondronana
A1a: Village nu	A1a: Village number: A1b: Household number: A1c: Individual Number:				
A1d: Interview	er Initials: A	1e: Time Started: _	: A:	1f: Time Finished:	;;

NO	QUESTION	RESPONSE
A2	How old are you?	years
A3	What is your gender?	00 Male 01 Female
A4	What is your current status as a student?	 I am not currently in school I am in school part-time I am in school full-time No Answer/Refused
A5	What is the highest level of education you have <u>completed</u> ?	

		ction 2: Occupation/Income
trad		t your occupation. Your occupation is what you do for money or majority of your time doing even if these activities do not produce ker."
NO.	QUESTION	RESPONSE
B1	What is your primary occupation? "Your primary occupation is the job you <u>spend the most time</u> working. You should list student or homemaker here if you spend more time in one of these	 01 Livestock Trade (sell livestock/products for money) 02 Livestock Care (tend livestock, but do not sell them) 03 Farmer/Trade (crops, not livestock) 04 Business/Trade (not livestock or crops) 05 Field Assistant 06 Health Care 07 Student
	activities compared to your job."	08Homemaker09Civil Servant (work for the government)10Tourism (Centre Val Bio, Hotel, Guide)11Craft Work (weaving, embroidery)12Gold mining88Other:99No Answer/Refused
B2	Do you have a second occupation? "Your second occupation is the job you spend the second most time working. You can also answer student or homemaker here, if you did not list them in the previous question."	00I have no secondary profession01Livestock Trade (sell livestock/products for money)02Animal Care (care for animals, but do not sell them)03Farmer/Trade (rice or vegetables)04Business/Trade (not livestock, rice or produce)05Field Assistant06Health Care07Student08Homemaker09Civil Servant (work for the government)10Tourism (Centre Val Bio, Hotel, Guide)11Craft Work (weaving, embroidery)12Gold mining88Other:99No Answer/Refused
B3	How much is <u>your monthly</u> income from all sources combined? "This does not include income from any other household member, goods or services you receive in trade"	, ariary
B4	Are you ever paid in goods or trade instead of money for some or all of your work?	00 No 01 Yes 99 Don't Know/Refused

Section 3: Animal-Human Interaction

"I will now ask you some questions about your interaction with livestock. When asking about 'poultry,' I am asking about all chickens, geese, ducks or turkeys."

Use the code below to answer the next questions C1-C3:

- 00 Never
- 01 Less than once a month
- 02 1-4 times this month, includes once a week
- 03 More than once a week, but not everyday
- 04 Everyday
- 99 Don't Know/ Refused

"How often have <u>you</u> participated in the following activities in the <u>past 4 weeks</u>?"

	a: Slaughter	b: Milking	c: Help with birth	d: Provide food	e: Provide water	f: Take to forage	g: Cooking	h: Collect eggs
C1: Zebu								
C2: Poultry								
C3: Pigs								

	Definitions for activities above			
ACTIVITY	DEFINITION			
a) Slaughter	Actively restrain an animal for slaughter, or participate in cleaning/preparing the carcass for consumption or sale			
b) Milking	Manually collecting milk for personal/household use or for sale			
c) Help with birth	Assist in removing a newborn from the birth canal or have contact with the placenta or other birth fluids during the birth process			
d) Provide food	Collect feed for the animal and bring it to the animal			
e) Provide water	Collect water for the animal and bring it to the animal			
f) Take to forage	Take the animal to a water source or to a food source (to graze)			
g) Cooking	Prepare food made of the animal's flesh, blood, bone, or internal organs			

NO.	QUESTION	RESPONSE
C4	In the <u>past year</u> have you helped to	00 No
	slaughter any zebu, pigs or poultry?	01 Yes
		99 Don't Know/Refused
C5	In the <u>past year</u> have you ever helped	00 No
	to milk a zebu?	01 Yes
		99 Don't Know/Refused
C6	In the <u>past year</u> , have you ever helped	00 No
	with the birth of a zebu or pig?	01 Yes
		99 Don't Know/Refused
C7	On average, how often do you eat	00 Never (Skip to C8)
	Poultry?	01 Less than once a month
		02 1-4 times a month, includes once a week
	"Poultry includes chickens, ducks,	03 More than once a week, but not everyday
	geese and turkey"	04 Everyday
		99 Don't Know/Refused
C7a	-Please name all the places where the	Circle all that apply:
	poultry you eat comes from.	01 Animals from my household
		02 Animals from the village
		03 Animals from another village
		04 Local market (village unknown)
		88 Other
		99 Don't Know/Refused
C8	On average, how often do you eat Pig?	00 Never (Skip to C9)
		01 Less than once a month
		02 1-4 times a month, includes once a week
		03 More than once a week, but not everyday
		04 Everyday
		99 Don't Know/Refused
C8a	Please name all the places where the	Circle all that apply:
	pig you eat comes from:	01 Animals from my household
		02 Animals from the village
		03 Animals from another village
		04 Local market (village unknown)
		88 Other
<u> </u>		99 Don't Know/Refused
C9	On average, how often do you eat zebu?	00 Never (Skip to C10)
		01 Less than once a month
		02 1-4 times a month, includes once a week
		03 More than once a week, but not everyday
		04 Everyday 00 Den't Know / Refused
C9a	Please name all the places where the	99 Don't Know/Refused Circle all that apply:
C9a		01 Animals from my household
	zebu you eat comes from:	02 Animals from the village
		03 Animals from another village
		04 Local market (village unknown)
		88 Other
		99 Don't Know/Refused
C10	On average, how often do you eat eggs?	00 Never (Skip to C11)
		01 Less than once a month
		02 1-4 times a month, includes once a week
		03 More than once a week, but not everyday
		04 Everyday
		99 Don't Know/Refused
C10a	Please name all the places where the	Circle all that apply:
	eggs you eat come from:	01 Animals from my household
		02 Animals from the village

-		0.0	
		03	Animals from another village
		04	Local market (village unknown)
		88	Other
		99	Don't Know/Refused
C11	On average, how often do you drink	00	Never (SKIP to C12)
	milk?	01	Less than once a month
		02	1-4 times a month, includes once a week
		03	More than once a week, but not everyday
		04	Everyday
		99	Don't Know/Refused
C11a	Please name all the places where the	Cire	cle all that apply:
	milk you drink comes from:	01	Animals from my household
		02	Animals from the village
		03	Animals from another village
		04	Local market (village unknown)
		88	Other
		99	Don't know/Refused
C11b	Is the milk you drink pasteurized or	00	No
	boiled before you drink it?	01	Yes
		99	Don't Know/Refused
C12	How often do you touch zebu, pig or	00	Never
	poultry feces?	01	Less than once a month
		02	1-4 times a month, includes once a week
		03	More than once a week, but not everyday
		04	Everyday
		99	Don't Know/Refused

Section 4: Current and Past Symptoms

"Now I will ask you about symptoms you <u>are currently experiencing</u> and for how long you have had them." Only answer 'Yes' for symptoms that you have today."

- **01** 1-2 days
- **02** 3-6 days
- **03** 1 week-2 weeks (7-14 days)
- **04** More than 2 weeks-3 weeks (15-21 days)
- **05** More than 3 weeks
- 99 Don't know/Can't remember

Symptoms	Are you <u>currently</u> experiencing any of the following symptoms?	How long have you had this symptom?
D1: Diarrhea, no blood	00 No (SKIP to D2) 01 Yes	a) 01 02 03 04 99
D2: Diarrhea with blood	00 No (SKIP to D3) 01 Yes	a) 01 02 03 04 99
D3: Vomiting/Nausea	00 No (SKIP to D4) 01 Yes	a) 01 02 03 04 99
D4: Fever	00 No (SKIP to D5) 01 Yes	a) 01 02 03 04 99
D5: Headache	00 No (SKIP to D6) 01 Yes	a) 01 02 03 04 99
D6: Abdominal pain	00 No (SKIP to D7) 01 Yes	a) 01 02 03 04 99
D7: Cough	00 No (SKIP to D8) 01 Yes	a) 01 02 03 04 99

"Now I will ask you about symptoms you have had in the past 6 months. These do not include symptoms you have today. However, if you currently have a symptom, and you also had the symptom in the past 6 months, but it resolved, you can say 'Yes' to the symptom for both current and past."

Symptoms	Have you experienced any of these symptoms in the <u>past 6</u> months?	Но	w lon	g dic	l you	have	e this	symptom?
D8: Diarrhea, no blood	00 No (SKIP to D2) 01 Yes	a)	01	02	03	04	05	99
D9: Diarrhea with blood	00 No (SKIP to D3) 01 Yes	a)	01	02	03	04	05	99
D10: Vomiting/Nausea	00 No (SKIP to D4) 01 Yes	a)	01	02	03	04	05	99
D11: Fever	00 No (SKIP to D5) 01 Yes	a)	01	02	03	04	05	99
D12: Headache	00 No (SKIP to D6) 01 Yes	a)	01	02	03	04	05	99
D13: Abdominal pain	00 No (SKIP to D7) 01 Yes	a)	01	02	03	04	05	99
D14: Cough	00 No (SKIP to D8) 01 Yes	a)	01	02	03	04	05	99

D15	Name all the places where	Circl	e all that annly:			
D15 Name all the places where you sought care or		Circle all that apply: 00 I have not sought care for my symptoms				
	treatment for the symptoms		Doctor/health clinic (name):			
	you answered "Yes" to in the		Traditional Healer			
	questions above.	-	Other:			
	questions above.		Don't Know/Refused			
D16	If you used medication to		e all that apply:			
210	treat any of the symptoms		I have not taken any medications for these symptoms			
	you answered, "Yes" to		Doctor/health clinic			
	above, please name all of the		Local shop/pharmacy			
	places where you obtained		From a friend/neighbor			
	the medication(s).	88 (Other:			
		99 1	Don't Know/Refused			
D17	In your opinion, how much		No money or trade goods (Skip to D9)			
	has it cost to treat <u>your</u>		A small amount (money or trade)			
	symptoms in the past six		A moderate amount (money or trade)			
	months?		A large amount (money or trade)			
	Interviewer: read options	99 I	Don't Know/Refused			
	to this question aloud.					
D18	What is the approximate		Ariary:			
	amount treatment for your		Goods/trade:			
	symptoms has cost you?	99 I	Don't Know/Refused			
D19	How much time have you	00 I	No time missed			
	had to miss from school or	01	1 – 3 days			
	work as a result of your		4 – 6 days			
	symptoms in the past 6		7 days or more			
	months?		Don't Know/Refused			
D20	How much have the		Not at all			
	symptoms you have		A small amount			
	experienced in the past 6		Moderately			
	months impaired your		A great amount			
	ability to fulfill your daily		I cannot fulfill my daily activities without assistance			
	activities?	99 1	Don't Know/Refused			
	Interviewer: read options					
D 04	to this question aloud.	0.0	AT			
D21	How often have you slept		Never Less than once a week			
	under a bed net in the <u>past 4</u>	-				
	weeks?		More than once a week, but not every night Every night			
			Don't know/Refused			
D22	Do you feel that your health	01	•			
022	is:		Fair			
	Interviewer: read options		Good			
	aloud		Excellent			
For Wo	men Only:	01 1				
		00 1				
D23	D23 Have you given birth in the		No (Skip to END)			
	past 2 years?		Yes			
B <i>GT</i>		99	Don't Know/Refused (Skip to END)			
D23a	Please think back to when		No (Skip to END)			
	you were pregnant. Did you		Yes			
	ever sleep under a bed net?		Don't Know/Refused (Skip to END)			
D23b	How often did you sleep		Occasionally			
	under the bed net?		Most of the time			
	Interviewer: read options		All of the time			
	to this question aloud.	99 1	Don't Know/Refused			

SECTION 5: Individual Health Assessment					
Rapid Detection					
Tests:	E1: Malaria RDT I	Date/time of test:			
	00 Neg				
	01 Pos P. fa	al			
	n-P. fal				
	03 Pos both				
	99 Not done	ie			
	Fecal Sample Date	e/time of test:			
	E2: Adenovirus	E3: Rotavirus			
	00 Neg	00 Neg			
	01 Pos	01 Pos			
	99 Not Done	99 Not Done			

E5	Weight:	kg		
E6	Height:	cm		
E7	Temperature:0F			00 Normal 01 >100.4 02 Not taken
E8	Resp Rate:/ min			00 Normal 01 Abnormal 02 Not taken
E9	Heart Rate:/ min			00 Normal 01 Abnormal 02 Not Taken
E10	Blood Pressure: mmHg			00 Normal 01 Abnormal 02 Not Taken
	Systems	Normal	Abnormal	Comments
F1	General	00	01	
F2	Appearance	00	01	
F3	Integument	00	01	
F4	HEENT	00	01	
F5	Dental	00	01	
F6	CV	00	01	
F7 F8	Resp GI	00 00	01 01	
го F9	GU	00	01	
F9 F10	Neuro	00	01	
F11	Msk	00	01	