RESEARCH PROTOCOL OUTLINE

Instructions: Protocols should be formatted according to the following outline and include all of the elements indicated.

Title of Project: EFECAB: Improving pig management to prevent epilepsy in Burkina Faso

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Abstract

This proposal extends the work of our pilot project, "Epidemiology and Burden of Neurocysticercosis in Bukina Faso" (R21 NS055353) which was successfully conducted due to the development of strong research collaboration and the dedication of our group to improving the living conditions of the Burkinabé people. Our ultimate goal is to develop a sustainable, cost-beneficial intervention strategy to reduce the incidence and prevalence of neurocysticercosis (NCC) and associated neurological conditions across the lifespan. Inclusion of epilepsy and NCC in the list of notifiable diseases for Burkina Faso will be pilot tested as a surveillance mechanism. Based on our pilot study findings, we estimate that an educational intervention targeting pig farmers could reduce the incidence of infection in humans and pigs by one-half. We also estimate that this could result, in the long term, in reducing the incidence rate of recurrent seizures in Burkina Faso by 25% in villages where pigs are raised. We will use the PRECEDE PROCEED approach to develop educational materials to maximize the monetary benefits of farmers through healthy pig raising. A pre-post randomized community trial will be conducted in 60 villages located in three provinces, using a 18-month baseline and 18-month post-intervention follow-up of 80 participants per village. The effectiveness of the educational materials in reducing the incidence rate of human and porcine cysticercosis, and of recurrent seizures and severe headaches, will be estimated. If effective, this sustainable intervention could be exported to other pig-raising countries in Sub-Saharan Africa to reduce the frequency of cysticercosis and epilepsy.

A. Specific Aims

The ultimate goal of the proposed project is to develop a sustainable, cost-beneficial and cost-effective intervention strategy to reduce the incidence and prevalence of neurocysticercosis (NCC) and associated neurological disease across the lifespan. We have identified three aims focusing on ongoing research capacity building and five research aims.

The capacity building aims include: 1) strengthening prior collaborations initiated during the R21; 2) establishing the Cysticercosis Working Group for Western and Central Africa (CWGWCA) headquarters in Burkina Faso; and 3) developing and testing the inclusion of seizures, epilepsy and NCC in the list of notifiable diseases routinely collected by the Health Information System managed by the Ministry of Health in Burkina Faso.

The research aims are to: 1) Use the PRECEDE PROCEED approach to develop a training package to educate farmers on cysticercosis and raise awareness of villagers about sanitation; 2) Estimate the effectiveness of the educational intervention developed in Research Aim 1 in reducing the cumulative incidence and prevalence of human cysticercosis through a randomized community-based controlled trial; 3) Estimate the effectiveness of the intervention in reducing the incidence rate of porcine cysticercosis and in improving fertility in pigs (number of piglets per sow and number of litters per sow); 4) Estimate the 3-year cumulative incidence of seroconversion to the antigens of cysticercosis in humans and in pigs, and of neurological disorders (epilepsy, acute seizures and severe progressive headaches).

B. Background and Significance

NCC results from CNS invasion by the larval stage of *T. solium*. The type of CNS disease produced depends on both the location and evolutionary stage of the cyst (Prabhakar and Singh, 2002). Clinical manifestations of parenchymal cysts, the most common location, include seizures, severe, progressively worsening headache, and focal neurological deficit, the first two being the most common. Seizures occur at some stage in 66-90% of NCC cases. Thus, identification of persons with seizures is a logical starting point for identification of persons with NCC.

There have been numerous cross-sectional studies conducted on the prevalence of cysticercosis in humans and pigs and of NCC in humans, but, to our knowledge, there have been only two short reports published on cohort studies of seroconversion with antibodies to *T. solium*. Both studies were conducted in Latin America. No cohort study of human cysticercosis and NCC, as measured by the presence of antigens, has ever been conducted. In addition, there has never been a cohort study in Africa of cysticercosis infection in humans. There are only two controlled studies on the effectiveness of educational interventions to prevent cysticercosis. The lifecycle of this organism is well documented and its association with significant neurological conditions, such as epilepsy and severe headache, has been established. It is now time to test sustainable interventions to control this preventable cause of epilepsy and other neurological disorders that significantly affect the lives of people of all ages in areas endemic for *T. solium*, which includes most of Africa.

C. Preliminary Studies/Progress Report

The severe underdevelopment in Burkina Faso makes conducting research in this country challenging. Nonetheless, a strong research collaboration and belief in the need for improving the living conditions of the Burkinabé people made our pilot project a success. A critical element of our success was the collaboration of the local population in actively participating in

the study. In the past *three* years, we have made excellent progress in building research capacity and in demonstrating the feasibility of research in neurological disorders in Burkina Faso. We describe below how each of the specific aims of our earlier R21 grant were met.

Our preliminary results indicate a high prevalence of lifetime and "active" epilepsy. In addition, 37.2% of those with recurrent seizures who received a CT-scan had lesions suggestive of NCC. In villages where pigs are raised, this proportion was between 50% and 55%. We also found a high prevalence (*11.7*%) of blood antigens of the larval stage of *T. solium* in Batondo. The seroprevalence to the larval stages of *T. solium* was also high in pigs (36% and 45%) in the two villages where pigs were raised. Based on these findings, we will target the area surrounding Batondo where cysticercosis is most endemic.

D. <u>Research Design and Methods</u>

In the interest of space, we will only describe the research methods in this section.

D.1 Development and testing of the educational program (*Research aims 1-3***)**

PRECEDE stands for Predisposing, Reinforcing, and Enabling Constructs in Educational Diagnosis and Evaluation. We will conduct Focus Group Discussions (FGD) and in-depth interviews with pig farmers and villagers from 3 pilot villages located in the 3 provinces targeted for the RCT. The questionnaires used by Dr. Ngowi in Tanzania will be adapted to the socio-cultural characteristics of Burkina Faso and to add questions on sanitation. Based on the PRECEDE model, we will organize the data from the FGD and the questionnaires to identify predisposing, reinforcing, and enabling behavioural factors to be targeted for the intervention. Final targets for the intervention will be identified during this phase considering resources and support available, organizational constraints, and barriers that could influence the intervention in the areas. PROCEED is an abbreviation for Policy, Regulatory, and Organizational Constructs in Educational and Environmental Development. We will target 2 major audiences: a) the public health extension officials and b) the smallholder pig farmers. We will divide the interventions into 2 main types: a) the provision of education through the group approach by organizing related seminars and training sessions; and b) the dissemination of illustrated and audio-visual materials such as leaflets, posters, comic books and the production of a video-tape. The impact of our intervention strategy will be assessed by comparing the pre-post randomization Knowledge, Attitudes and Practices (KAP) between the intervention and control groups. The effectiveness of the intervention will be measured as described later.

D2. Randomized community trial (*Research Aims 2-3*)

We will conduct a randomized community-based controlled trial (RCT) to estimate the effectiveness of an educational package in reducing the incidence rate of human and porcine cysticercosis in 60 villages in 30 of the 31 departments located in the Provinces of Boulkiemdé (15 departments) and Sanguié (10 departments) in the Region of Centre-Ouest, and Nayala (6 departments) in the Region of Boucle du Mouhon. According to the 2006 census, there were 498,008, 297,230 and 162,869 people in these provinces, respectively. The RCT will be conducted over 3 years, including a 1-year baseline follow-up period. The RCT will be followed by 2 years of post-intervention follow-up. Pigs are common in those Regions. Boulkiemdé and Sanguié are inhabited by the Mossi and Gourounsi ethnic groups, who were involved in our pilot project. Nayala, inhabited by Samo people, is 1 of the 3 provinces out of 5 in that region where pigs are bred. Traditionally, the method of pig-keeping is different according to the

region: in the Centre Ouest pigs are not kept in pens whereas in Nayala, pigs are put in pens that are also used by household members as toilets.

In each department, we will select two non-neighboring villages and invite the chief to consult with the community council to ask for their willingness to participate. The inclusion criteria for villages are: 1) the presence of some pig farming, which is probably the case in all villages of the 3 provinces, and 2) at least 80 concessions (a collection of households which shares a common area) in the village. In each randomly selected village, we will first conduct a census of all concessions as was done in the pilot study and randomly sample 80 concessions. A similar random sampling method as described for the selection of the concessions will be used to randomly select one member of the concession. Both the heads of the households and of the concession will be asked for their consent to participate. Based on our pilot experience, we expect nearly 100% participation at the concession level. All 80 selected individuals (1 from each concession) will be invited to answer a screening questionnaire on seizures and headaches. Of those 80, 60 selected at random will be invited to also participate in a serological examination for the detection of the larval stages of *T. solium*, which will involve blood sampling. Participants who do not have neurological disorders at baseline will be invited to participate in a follow-up study for a period of 3 years. Participants free of neurological disorders at baseline will be invited to also participate in a serological follow-up. Consent for children less than 16 years will be sought from the mothers. Children aged 10 - 16 years will be asked for their assent to participate in the study. Based on the pilot study, we expect a participation proportion of 86% at the individual level for the serological component of the study. If the person selected at random in the concession refuses to participate, another number from the same concession will be selected at random until we get one person willing to participate. We expect 100% initial agreement for participation in the follow-up of neurological disorders only. Individuals younger than 5 years of age will be excluded from the sampling frame.

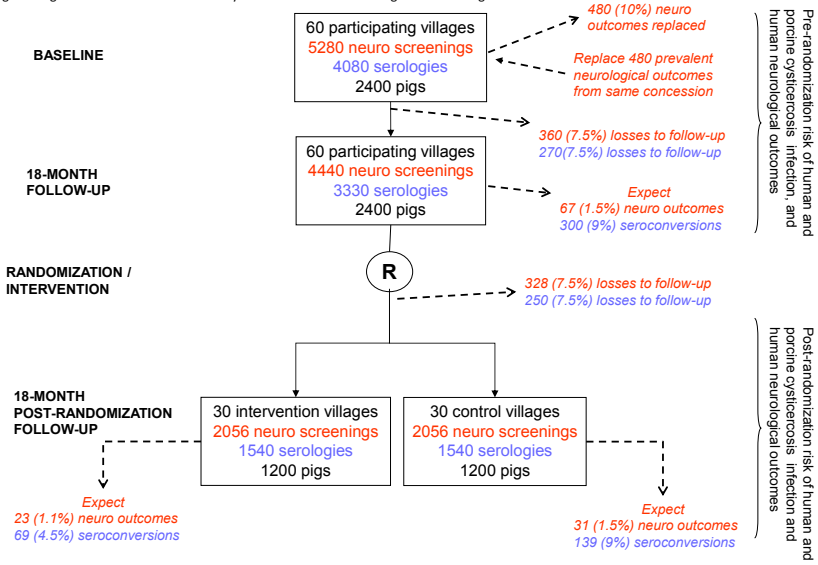
The diagnostic of epilepsy will be determined in a similar way to what was done during the pilot study. The field staff will first interview the participants with the screening questionnaire. The 18-month follow-up questionnaire will exclude the socio-demographic section of the questionnaire and ask only about work absenteeism and the development of new symptoms. At baseline and at 18-months follow-up, the physician will collect a 10 mL blood sample from 60 of the eligible participants on the day of, or the day following, the interview. The blood samples will be placed on ice in coolers until the evening when they will be centrifuged and refrigerated.

When the screening is positive for epilepsy or headaches, the study physician will examine the participant to confirm or reject the probable occurrence of epileptic seizures or severe headache which may be secondary to NCC. All participants with CNS seizures or severe, progressively worsening headache as confirmed by the physician will be offered a cranial CT-scan and treatment for the duration of the study if seizures are recurrent. The CT-scan will be done at the CHUYO. The images obtained during the pilot will be used to train the radiologists at the CHUSS in NCC diagnosis.

Figure 4 illustrates the design of the RCT. Numbers in red correspond to the sample size for the follow-up of neurological disorders and in blue for the serological follow-up. These numbers represent sample sizes only and not mutually exclusive groups; i.e., those in the serological follow-up are part of the larger, neurological follow-up.

The process for randomizing villages into the intervention group (30 villages) or control group (30 villages) will take place prior to the 18-month post-baseline follow-up. We will use a block randomization strategy with departments as the blocks. Village leaders and pig farmers from the participating concessions will be invited to attend a seminar offered by the local trainers. Illustrated educational materials will be distributed at the end of the seminar.

Fig. 4 Design of the randomized community-based trial for the neurological and serological outcomes



Following the intervention, participants will be followed-up after 18 months. The same process as that described for the baseline survey and 18-month follow-up will be followed for the last visit. Prevalence at each visit and cumulative incidences between each visits will be calculated.

E. Statistical Methods

The primary analysis will assess the effectiveness of the intervention and the effect of monitoring alone in reducing the incidence rate of human cysticercosis. We will first calculate estimates of the effectiveness of the intervention and the monitoring alone as incidence rate differences (IRDs) between the intervention and control groups. The variances for the 95% confidence intervals (95% CI) of the crude effectiveness will be calculated for the control and the intervention groups, assuming a normal distribution. We will use a Bayesian hierarchical log binomial regression model for the number of incident cases of cysticercosis in humans. The model will include independent variables for the intervention, monitoring alone (pre or post period), village random effect, and any other confounding variable that was not well balanced after randomization. We expect to observe considerable between-village variability in cumulative incidences and prevalences, and will therefore plan to use a hierarchical model to account for these differences. These types of Bayesian hierarchical generalized linear models have been used extensively by the PI. All Bayesian analyses will be conducted using WinBugs 1.4©.

F. Gender/Minority/Pediatric Inclusion for Research

All inhabitants of the participating villages meeting the inclusion criteria based on age will have the opportunity to be included in this study. Thus, women and minorities meeting the eligibility criteria will have the possibility of being included.

Pregnant women will be excluded from the CT-scan of the brain, due to the risk to the fetus.

F.1. Distribution of Subjects by Sex/Gender and Racial/Ethnic Groups

Targeted/Planned Enrollment Table

Study Title: EFECAB: Improving pig management to prevent epilepsy in Burkina Faso - a randomized controlled trial

Total Planned Enrollment: Maximum of 18288

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino			
Not Hispanic or Latino			
Ethnic Category: Total of All Subjects *			
Racial Categories			
American Indian/Alaska Native			
Asian			
Native Hawaiian or Other Pacific Islander			
Black or African American	*9744*	*8544*	*18,288*
White			
Racial Categories: Total of All Subjects *	*9744*	*8544*	*18,288*

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

F.2. Selection Criteria

A total of 60 villages from the provinces of Sanguié, Boulkiemde and Nayala in Burkina Faso will be invited to participate in the randomized controlled community trial. In each village, a total of 80 subjects will be invited to take part in the follow-up study. Subjects to be included in the study will be selected at random, and there are no selection criteria based on sex/gender or racial/ethnic groups. The only criterion is that the included villages have some pig production.

This may lead to the exclusion of mainly Muslim areas where pigs and cysticercosis are less likely to be an issue.

F.3. Rationale for Exclusion of Any Sex/Gender or Racial/Ethnic Group

There is no such exclusion. The study is conducted in Burkina Faso where all the population living in rural areas is black.

This proposal aims to establish the frequency of cysticercosis and NCC-associated neurological outcomes (single seizures, recurrent seizures and severe headaches) across the lifespan. Also, there is very little known about the incidence of infection or its neurological complications in children. Preliminary results in piglets suggest that age, as an indicator of immunological maturity, could have a major impact on the establishment of cysts in the body, including in the CNS. Pregnant women will also be included since their immunological response may be altered. However, pregnant women and children less than 5 years of age or those requiring sedation will not receive a CT-scan of the brain.

F.4 Inclusion of children

Since the entire village will be included in the study, children living in the village are eligible to be included. For the blood sampling and interview components of the study, children aged 5 years and older will be included. The age of 5 years old was selected for inclusion in the study following discussions with the head of the ethical board for the Centre MURAZ (IORG0003562), Dr. Ouédraogo. This age was considered acceptable to ask for a blood sample and also for a CT-scan, as long as it does not require sedation.

All data collection, except for the CT-scan, will take place at the house of the child, which will minimize the stress levels. In our pilot study, the random selection of study participants led to the inclusion of 20% who were children less than 16 years old. This means that a sufficient number of children will be included to allow for a better understanding of the prevalence and incidence rates of *T. solium* in this population and the ages at which primary exposures can occur.

Children older than 10 years of age will be asked for their assent to participate in the study. Individuals aged 16 years or older will be asked for their consent to participate in the study.

Our research team is comprised of researchers and clinicians with considerable experience working with children in research and in clinical environments. Subjects' recruitment, including children, and their follow-up will be conducted and supervised by research staff with training and experience working with children. In particular, Hélène Carabin, DVM, PhD is an infectious disease epidemiologist with experience working with pre-school children and staff in daycare centers to evaluate the effectiveness of an intervention to control infections. Dr. Cowan was the PI on a large epidemiological study of epilepsy in children in Oklahoma. Dr. Millogo, a neurologist, has numerous pediatric patients. Drs Ganaba and Hounton, as active research members of the IMMPACT project, have experience working with mothers in Burkina Faso.

G. PROTECTION OF HUMAN SUBJECTS

Human subject involvement and characteristics are summarized here. The purpose of the portion of the research project that involves human subjects is to conduct a randomized

controlled community-based trial to test the effectiveness of an education program focusing on improved pig management to reduce the incidence rate of human cysticercosis and associated neurological symptoms of neurocysticercosis (NCC) (single seizures, recurrent seizures and severe headaches) in 60 villages from 30 departments in three provinces of Burkina Faso. The initial 18 months of the study will be used to establish the baseline (18 months) cumulative incidence of cysticercosis infection and of the target neurological outcomes (single seizures, recurrent seizures or epilepsy and severe headaches). After the 18 month follow-up measurements, the 60 villages will be randomized to receive the education program (30 villages) or to serve as a control (30 villages). All participants will be followed for an additional 18 months. The control group will be offered the intervention at the end of the follow-up.

G.1. Provide number, age range, and health status of the participant population. Identify criteria for inclusion or exclusion.

a. Anticipated number of subjects

We are planning to follow two overlapping cohorts of people during the randomized community trial. One cohort will be followed for the development of the target neurological outcomes. We are planning to screen a total of 5280 people (4800+480) at baseline for assessing eligibility for the follow-up. The other cohort, which is a sub-set of the first, will be followed for the presence of antigens to the larval stages of *Taenia solium* in their blood. We are planning to enroll 3600 people at baseline for the serological component of the study (included in the 5280). Also, people with neurological disorders will be tested serologically, since the definition of NCC requires a serological test. This leads to 4080 serological tests to be conducted at baseline.

Only people who are free of the target neurological outcomes (expected n=4800) will be followed-up for a period of three years, until they develop one of these outcomes, they drop out from the study, or the study ends. Assuming that 10% of people will have one of the target neurological outcome at baseline, a 5% annual proportion of losses to follow-up, an incidence rate of neurological outcomes of 1.5 per 100 persons every year, and a reduction in the incidence rate of 25% in the intervention group, we are expecting to measure the target neurological outcomes in 4440 and 4112 people at 18 and 36 months of follow-up, respectively.

Subjects who have one of the target neurological outcomes -- confirmed by a physician -- at baseline or who develop one of them during the three year follow-up period, will be invited for a CT-scan of the brain. We anticipate referring a total of 656 subjects for a CT-scan of the brain during the study.

For the serological component of the study, people will be followed-up for a period of three years, until they seroconvert, they drop out, or the study ends. Assuming a 5% annual proportion of losses to follow-up, we expect to follow-up 3330 and 3080 people at 18 and 36 months, respectively. Please refer to the Figure in the methods section for a flow chart of the expected number of participating subjects at each stage of follow-up.

A random sample will also be drawn of 40 concessions in which participants reside in order to collect data on and blood samples from their pigs. A total of 2400 pig managers will be interviewed. The person in charge of cooking for the household for each of the 5280 subjects screened for their eligibility will be questioned on household cooking habits. Finally, the head of

the household of each of the 5280 subjects screened will be asked about possessions and other factors used to measure the socio-economic level of the household. Some of those subjects may participate in the follow-up component of the study as well.

During the preparation phase, 48 people from three villages not included in the randomized community trial (16 in each village from each of the study provinces), will be invited to participate to a focus group discussion on pig management, taeniasis, cysticercosis and epilepsy. Results are used to develop the intervention materials.

Please note that the enrollment form submitted with this proposal reflects the maximum number of people from whom we are expecting to collect any type of project-related data, including those individuals we are planning to follow as part of the randomized community trial. The numbers in the enrollment form were estimated as follows: 5280 (4800 originally selection plus 480 replacements of prevalent cases with the study neurological outcomes) participants screened at baseline with a sub-sample followed-up through time (assuming 50% men and 50% women), 2400 pig manager interviews (assuming 75% women) from the households of the selected participants, 5280 interviews of the person from the selected households who are in charge of cooking (assuming 100% women), from 4800 to 5078 interviews of the head of the concession (assuming 100% men). This will be in addition to the 250 people (129 women and 121 men) included in the focus group discussions. This results in a maximum number of persons from whom study data are collected (interviews and/or participants to the trial) of between 18,005 and 18,288 with an estimated 8161 to 8439 men and 9849 women. It is very possible that some of the interviewees will be included in more than one group resulting in a smaller number of total number of individuals participating in either interviews for collection of household data, focus groups or the trial itself.

b. Age range of subjects

The minimum age for the follow-up of the neurological outcomes and for the serological study will be 5 years of age. This is considered acceptable in Burkina Faso according to local investigators. There is no limitation on the maximum age. All participants who have one of the study neurological outcomes at baseline or who develop them during the follow-up will be offered a CT-scan of the brain. The minimum age for the CT-scan of the brain depends on the need for sedation. No CT-scan of the brain will be performed if sedation is required. More details on ages for providing informed consent and assent are given in the section, “Inclusion of Children”.

c. Health status of subjects

At baseline, we will select participants at random from the 60 participating villages. Hence, all village members are eligible to be included in the baseline study.

As outlined in section (i) above, we expect that about 10% of the subjects will have one of the target neurological outcomes at baseline and will therefore be excluded from the follow-up. All other subjects are expected to be free from these outcomes. We expect that *12%* of the subjects will be seropositive to the larval from of *Taenia solium*. Seropositive individuals at baseline will be included in the follow-up.

d. Eligibility (inclusion and exclusion) criteria

The proposed project is a randomized community-based controlled trial. The first level of eligibility is the community. At the community level, provincial and departmental capitals (chef-lieu), villages located on national roads, villages not appearing on the official National Census map, villages in the vicinity of Koudougou or Ouagadougou, and neighboring villages (<5 km) will be excluded from the study. This is to prevent "contamination" of the intervention between villages. Village-level inclusion criteria are the presence of pig farming and of at least 1000 inhabitants.

At the individual level, the only exclusion criterion is being younger than 5 years of age.

As described in (a) above, people who have a target neurological outcome at baseline or who develop them during the study period will be excluded from the next follow-up assessment. For the serological component of the study, all participants will be followed-up for both the serological and neurological outcomes.

Pregnant women and subjects with one of the target neurological outcomes who would require sedation in order to obtain a CT-scan of the brain will be excluded from the radiological examination.

G.2 Identify sources of research material in the form of specimens, records or data.

Several interview questionnaires, which have been validated in the pilot study, will be further refined and used in this study. These include: 1) a screening questionnaire for seizures, recurrent seizures and severe headaches which includes demographic and socio-economical questions (screening questionnaire); 2) a household-level questionnaire to be administered to the head of the household (household questionnaire); 3) a "cooking" habits questionnaire to be administered to the mother of the household of the participant (cooking questionnaire); 4) a pig management questionnaire to be asked of the owner of the sampled pig (pig questionnaire); 5) a questionnaire to measure the costs associated with the treatment of epilepsy, to which questions on severe headaches will be added (cost questionnaire); a form which documents the findings of the history and physical examination by the study physician of those who screen positive for seizures or severe headache, to which questions on severe headaches will be added (medical questionnaire). A questionnaire for the focus group discussion (focus group questionnaire) will be used in three pilot villages to develop the educational material. All the material will be translated (and back-translated) to the local languages of the study regions (primarily Gourounsi). Not written version of those languages exists so this exercise will be done orally.

The data from the CT-scans of the brain will be evaluated at the CHUYO by Prof. Cissé. The CT-scans will be saved on CD-roms and stored at the research office in Bobo Dioulasso. A sub-sample of the de-identified CT-scans will be sent to the IENT as a quality assurance system.

A maximum of four 10 ml blood samples each may be collected on any given subject participating in the serological component of the study: at baseline, at 18 months follow-up (and time of randomization), at 18 months post-randomization. All material necessary for the blood sampling and for the serological tests will be provided by the project. A centrifuge and generator will be purchased for the centrifugation of the sera in the field. A freezer and refrigerator and all storage materials will be purchased by the project for the IRSS for storing the sera.

All sera will be tested for circulating antigens of the metacestode of *T. solium* using the enzyme-linked immunosorbent assay (ELISA) (Brandt JRA, Geerts S, De Deken R, Kumar V, Ceulemans F, Brijs L, Falla N. A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. *Int J Parasitol* 1992; 22:471-7). The test was found to have a sensitivity of 90% (95%BCI: 80-99%) and a specificity of 98% (95%BCI: 90-99%) to detect current infection in a study conducted in Ecuador (Praet N, Verweii JJ, Meape KE. Bayesian modelling to estimate the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis. Trop Med Int Health 2013, 18: 608-614). These analyses will take place at the IRSS in Bobo Dioulasso, Burkina Faso.

Sera from all individuals confirmed to having severe chronic headaches or epilepsy or single seizures and a sub-sample of individuals without such samples matched for age group, sex and village residence will be tested with rES33 for the detection of taeniasis antibodies and rT24H for the detection of cysticercosis antibodies (Handali et al. Clin Vacc Immunol 2010; 17: 631-7). These analyses will take place at the Center for Disease Control and Prevention in Atlanta, USA. Once these analyses will have been completed, the remainder of the sera will be sent to Oklahoma for storage.

Educational materials in the form of leaflets, posters, comic books and a movie will be produced for the intervention. The movie will be played using a generator during the educational sessions that are part of the intervention.

A Sports Utility Vehicle will be purchased for the project to allow the research team to move between villages and to transport study materials.

Each participant will be assigned a research identification number. Data retained on participants will only be identified by their research ID. Information linking participants to their research ID will be stored in secured files in the research office in Bobo Dioulasso and at the University of Oklahoma Health Sciences Center and will only be accessible to authorized research staff who have completed the Human Subjects Training required by the Institutional Review Board.

All of the biological specimens and the interview assessments listed above will be collected specifically for the purposes of the proposed research project. The participants will be asked for their consent to bank the sera for a maximum of 5 years for potential future analyses on parasitic infections or inflammatory markers, <u>but not for HIV</u>.

<u>G.3. Plans for recruitment and consent procedures to be followed</u>

The first element of outreach will be a “launching” ceremony to which local policy makers and politicians will be invited. This will provide us with the opportunity to explain the goals of the study and its potential benefits to the region and the country. The second step will be to meet with the village leaders of each of the 60 eligible villages selected at random. The third step is the random selection of 80 concessions in each village and the consent of the concession “chief” to participate. The concessions will have been numbered before the random selection process. The fourth step is the selection of one person at random in the concession for participation in the 4-year study. The head of the household and mother of the household of

the participating subject will also be recruited for answering questions on the economical and cooking practices of the household.

Three provinces in Burkina Faso will be selected based on location in an endemic area, proximity to a reference hospital and the known presence of pigs. One team consisting of one medical doctor, six interviewers and one veterinarian will visit 60 villages located in three provinces on four occasions: baseline, 18-months follow-up pre-randomization and 28-month follow-up post-randomization. The visiting of all 60 villages is expected to last less than 12 months on each occasion. The project will first be launched publicly during a ceremony to be held in one of the three provinces. Then, Drs Millogo and Ganaba will supervise the team that will be introduced to the village head by a person of trust from the area. The research team and research project will then be introduced to villagers by the village heads. The village head and/or the village administrative committee will first be asked for their willingness to participate as a community in this initiative.

We will modify the consent forms used during our pilot study and add a new one for the head of the household and for the focus group. The consent forms will include:
1) Mothers: for an interview collecting data on cooking practices and household building characteristics.
2) Head of household: for a short interview providing a census of household members and on possessions of the household.
3) Villagers: for participating in the neurological outcome component of the study over a period of 4 years, with yearly screening interview for seizures, epilepsy and severe headaches and a clinical exam for those who screen positive and for the presence of subcutaneous nodules. This interview and clinical exam are expected to take around 20-30 minutes in total to complete.
4) Villagers: for participating in the serological component of the study which involves taking a blood sample to test for *T. solium* infection on four occasions, one year apart. The blood sample is expected to take no longer than 5 minutes.
5) Pig owners: for taking a blood sample and for completing a short questionnaire on pig management practices. The blood sampling of each pig should be completed in 10 minutes.
6) Subjects screened positive for seizures and severe headache: for further testing with a brain CT scan in Ouagadougou, all fees reimbursed. The participant may need to take a day off work for this examination.
7) A sub-sample of subjects screened positive and negative for seizures, epilepsy or severe headaches: for answering questions regarding costs associated with treatment of these conditions.
8) A sub-sample in 3 villages one in each of the 3 participating provinces will be asked to participate in a focus group. Each focus group may take up to one hour.

Informed consent will be obtained for all participants. All consent forms will include a first section where the objectives of the study are clearly stated. This will be followed by the description of what participation in the study will involve for the individual. Because we expect that a large proportion of the population will be illiterate, we expect the explanation to be read to the potential participants. Subjects that know how to sign their name or mark will be ask to do so on a form that clearly explains, in simple words of the local language, the objectives of the study. Children older than 10 years of age will be asked for their assent to participate in the

study. Individuals aged 18 years or older will be asked for their consent to participate in the study. The consent will clearly state that Individuals may terminate participation at any time.

a. Location where consent is most likely to take place

During our pilot project, all consents successfully took place in the household of the participant. We will use this same approach for this study.

b. Provisions for recruiting non-English speaking participants

All participants will be non-English speakers. We will recruit research staff who are familiar with the local languages and native speakers.

c. Measures to decrease coercion of participants

All research staff will be Burkinabè people. We will also make an effort to recruit people from the research area to be part of the research staff. The study protocol will be submitted to the Centre MURAZ ethical committee which is recognized by the Office of Human Research Protections (OHRP) (Centre MURAZ (IORG0003562)). This committee has replaced the previous IRB which was linked to the CHUSS through the Federal Wide Assurances (FWA) during the pilot (FWA00009934). We will renew our FWA with the new International IRB for Burkina. Their review will be essential to make sure that our approach is not coercive. The study will be clearly explained to each participant orally in their own language (if they cannot read, which we expect in the majority of people). There will be a clear statement that participation is optional and there is no penalty for non-participation.

All participants will be given a bag of sugar (600 CFA or $1.45) each time that they answer questions and/or provide a blood sample as a compensation for their time. In a very deprived environment such as that of Burkina Faso, sugar is a most welcomed compensation. The village leaders will also be given a 10,000 CFA (~$24) compensation to purchase something for the community each time the village is visited. These compensations have been recommended by our local collaborators and are not considered to be of a magnitude that is coercive.

G.4. Risks and assessment of likelihood and seriousness.

The potential risks associated with this protocol are minimal. There are minor risks associated with drawing blood such as possible bruising, infection, and fainting. Subjects participating to the serological component of the study will be asked for their consent to inform the local health care provider of their serological status if they test positive for *T. solium*. This is because the results of the serology will only be available a few weeks after the samples have been drawn. The local health care provider will be given albendazole to treat persons who are antigen positive but do not have neurological symptoms. The project will provide these treatments. Provision of this free treatment is likely to be a benefit to the participants.

Brain CT scans will only be offered to individuals with unprovoked single or recurrent seizures or severe headaches. This is often part of the normal workup for identifying the causes of these disorders. Given that most people in Burkina Faso cannot afford this test, the brain CT scan will most likely be a benefit to them. Pregnant women and children needing sedation will not be offered the CT-scan.

We will use a consent form, modified to include severe headaches, like that used during our pilot study. The following statement regarding the risks associated with the CT-scan reads:

"*I am being asked to have a special test on my brain because I have seizures. I am being asked to go to Ouagadougou for a brain-scan study (computerized tomography or CT scan). The brain scan is the best way to determine whether my seizures are caused by the worm, T. solium, in my brain. The brain scan might also help to determine other possible causes of my epilepsy. The CT scan requires that I lie still for a short time while pictures are taken of my brain. I understand that I will feel no pain with the brain scan, but that I may be bothered by the noise and the closed-in-space. Someone will be watching me all the time and I will be able to speak to them, so they will stop the scan at any time if I ask them to. I also understand that the CT scan uses an amount of radiation that is somewhat above the amount I receive normally from the environment. During this special test, I will be injected into a vein with a dye called iodine. This will help the doctors to see any abnormal areas of my brain. I understand that the iodine causes some people to have an unusual taste in their mouth and a feeling of warmth throughout their body, but only for a very short time. I also understand that some people rarely have a mild headache, nausea, vomiting, chills, fainting or allergic-type reactions. If I am a woman and I am pregnant, I should not have iodine injected in one of my veins for the CT scan examination. I will have a free urine test to rule out pregnancy if there is a possibility that I may be pregnant. I am agreeing to disclose medical information related to my health.*"

Only minimal risks are associated with the interviews and include possible distress to persons recalling their seizure histories.

To avoid economical losses to their owners, we will not attempt to take blood samples from pregnant sows or piglets younger than 2 months to avoid harmful stress to the animal and potential spontaneous abortion of the sows (stress associated).

All individuals who are identified as suffering from single seizures, epilepsy or severe headaches will be treated accordingly to the recommendations of Dr Millogo for the duration of the research project (money has been allocated in the budget to do this). Those identified by CT-scan to have NCC will also be treated for their condition as appropriate. At the end of the research project, these subjects will be encouraged to obtain treatment from the local health care provider. This approach was successful during the pilot study, especially in Batondo, a village located in one of the three provinces targeted for the proposed study.

G.5 Procedures for protecting against or minimizing potential risks

Subjects who consent to participate in the study will be identified by name and with an alphanumeric code. Only one coding sheet linking the names to the codes will be created and it will be password protected. Every effort will be made to keep the subject's personal data confidential**.** As the study progresses, data will be entered in Burkina Faso into a password-protected database. All consent forms and the coding sheet will be maintained in a locked file cabinet in the study's secure research office. In keeping with local practice, the office will also be monitored by security staff working for the project. Any data sent to the COPH will be anonymous and with alphanumeric codes for the subjects, pigs, households and village names. Published reports will not use names or any other information that could be used to identify individual study participants.

During our pilot study, none of the 77 subjects who received a CT-scan of the brain experienced any side effects from the procedure, either with or without the use of contrast.

Among those, there were 8 children aged between 7 and 9 years old. No sedation was required for these children.

At the CHUYO, for the entire 2-year interval between April 2006 and April 2008, out of 885 patients who received a CT-scan with contrast, there were 30 cases of vomiting (3.4%) and five cases of vertigo (0.6%). The staff of the Department of Radiology at the CHUYO interviews patients a few days prior to conducting the CT-scan to obtain information regarding risk factors for side effects. Those at high risk will not receive the contrast media. Those with a history of iodine allergy are excluded; those with drug allergies or seasonal allergies are given anti-histamines several days prior to the examination. The radiology department is located in the hospital with access to an emergency room and also has a provision of hydrocortisone, oxygen and adrenaline to treat potentially severe side effects.

Subjects who may require sedation for the CT-scan of the brain will be excluded from this part of the study. Pregnant women will be excluded from the CT-scan of the brain to avoid any risk to the fetus.

G.6. Potential benefits and importance to the participants and others

The benefits of the information gained from the study outweigh the minimal risk involved.

All participants will be given a bag of sugar (600 CFA or $1.45) each time that they answer questions and/or provide a blood sample as a compensation for their time. In a very deprived environment such as that of Burkina Faso, sugar is a most welcomed compensation. The village leaders will also be given a 10,000 CFA (~$24) compensation to purchase something for the community each time the village is visited.

Subjects screening positive for seizures or severe headaches based on the individual questionnaire will receive a free physical examination to confirm the diagnosis and to look for the presence of subcutaneous nodules. Subjects with confirmation of their seizures or severe headaches will be invited for a brain CT scan in order to better understand the basis of their symptoms. These individuals will be offered appropriate, free treatment for the remainder of the project. They will be monitored through the usual care provided at local clinics. The initial course of treatment will be recommended by the medical intern under the supervision of Dr. Millogo. All participants live in Burkina Faso, Africa; no subjects will be studied at Oklahoma Health Sciences Center sites.

Subjects will be asked for their consent to inform the local health care provider of their infection status based on the AgELISA. Those found seropositive will be offered a course of albendazole treatment.

Owners of pigs found infected with *T. solium* will be informed of the risk of consuming this meat raw or undercooked. They will be advised to either destroy the meat or to cook it very well. This approach has been found acceptable in regions of southeastern Africa.

If the educational intervention is effective, we expect to reduce the incidence rate of cysticercosis infections by 50% in humans and pigs. We also expect to reduce, in the long term, the incidence rate of seizures, recurrent seizures and severe headaches by at least 25%. Given the important stigmatization associated with epilepsy and the monetary value of pork these results would have a tremendous impact on the health and well-being of these communities.

Potential benefits to others include the development of a cost-beneficial intervention to prevent a large proportion of epilepsy and severe headaches in developing countries where pigs are raised. It will also potentially increase the income of pig producing farming communities.

The results from this study could have a tremendous impact on reducing the prevalence of epilepsy in African countries where cysticercosis is endemic. Other benefits include the possible increase in income for pig farmers through improved management, which could lead to improvement growth and fertility of pigs, and ultimately to an improvement in nutrition and increasing school attendance. This is because women are typically in charge of pig raising in that part of the world and the benefits from selling pigs are managed by them. If the education program also improves sanitation, the project may also reduce the rate of diarrhea and parasitic infections. To assess this, we have included a question on diarrhea in our questionnaire.

Discuss why risks are reasonable in relation to benefits.

The basis of this assessment are discussed in detail above.

H. Data and Safety Monitoring Plan

This study is not a clinical trial. None of the DSMP mechanisms apply to this study. Brain CT scans will only be offered to individuals with unprovoked single or recurrent seizures or severe headaches. This is often part of the usual diagnostic workup for identifying the causes of these disorders.