

Brucellosis Education Project and Epidemiological Survey 2009 Project Journal

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May 29th – Meeting with Dr. Khurlbaatar, Ministry of Health

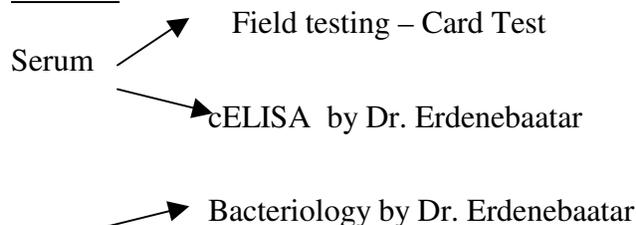
This morning we met with Dr. Khurlbaatar at the Ministry of Health. We have been working with him on the brucellosis grant and today brought him the Memorandum of Understanding to sign. The National Health Sciences University in Mongolia has already signed the MOU with Montana State, and Dr. Khurlbaatar agreed to sign the MOU along with another board member at the Ministry. There seemed to be some misunderstanding in regards to the grant proposal. Dr. Khurlbaatar thought we had obtained funding for the project, while our understanding was that he was applying for a source of funding. He agreed to look for funding from the World Bank or US-AID.

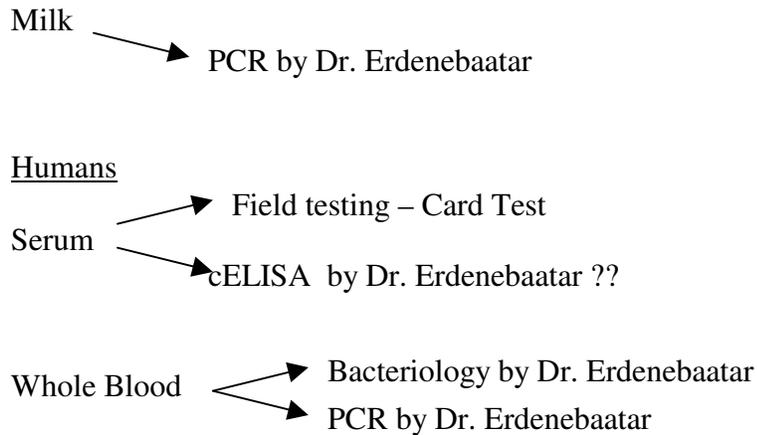
In our discussion with Dr. Khurlbaatar about our brucellosis work plan for this field season, everything did not go as favorably. We received IRB approval from MSU for human brucellosis screening. The National Health Sciences University in Mongolia signed the IRB as well, but we were not able to obtain a signature from Dr. Khurlbaatar. The Ministry of Health has their own IRB committee that we should have submitted our research proposal to. We came to an agreement that our project could go forward if we tested only serum from blood drawn by local doctors in Renchinlhumbe rather than drawing blood samples ourselves.

Meeting with Dr. Erdenebaatar, Veterinary School

After meeting with Dr. Khurlbaatar from the Ministry of Health to discuss the human-side of our brucellosis project, I met with Dr. Erdenebaatar, Head of Public Health at the Veterinary School to discuss the animal-side of our project. This meeting went very well. I had previously sent Dr. Erdenebaatar our work plan, so that he would be familiar with our plans. He supports our work and was very enthusiastic about the potential for collaboration between BioRegions, the Veterinary School, and the Ministry of Agriculture. In our meeting we discussed the possibility of transporting some samples to Ulaanbaatar for further testing. This is very exciting, as this will not only allow Dr. Erdenebaatar to carry out his own research, but will allow us to gain important information from tests that we are unable to perform in the field. The plan going forward is to have Mishig contact Dr. Erdenebaatar to discuss transport of samples to UB. If we can keep samples cold until they can be transported to the aimag testing center in Murun, this laboratory can then arrange transport to UB. The following are the samples that Dr. Erdenebaatar would like and their intended uses:

Animals





Serum samples for Dr. Erdenebaatar will be used for competitive ELISA (cELISA), which also only tests for exposure to Brucella, but will be useful in checking card test results and most importantly, is not influenced by animal vaccination. Thus, if we are finding a large percentage of animals to test positive by the Card Test, we can use cELISA to investigate whether these are false positives resulting from animal vaccination.

Milk samples from cows and whole blood from humans will be used for detection of the actual presence of Brucella in the animal / human, as well as biotyping of Brucella species. This will be done by traditional bacteriology and PCR. The results from these tests will be useful for our research as they could indicate whether detected exposure to Brucella is often associated with actual infection. Biotyping results will provide information on the species of Brucella infecting cows and humans. BioRegions, as well as Dr. Erdenebaatar, is interested in determining whether cows are infected with *B. melitensis* and what species of Brucella typically infect people in the Darhad. Dr. Erdenebaatar is also interested in looking for *B. abortus* biovar 7 in cattle and human populations. The WHO / FAO had declared that this subspecies of *B. abortus* no longer existed, but in previous research Dr. Erdenebaatar found biovar 7 in Mongolian cattle herds. Finally, another professor at the veterinary school, the head of the microbiology department, expressed interest in BioRegions collecting ticks from the Darhad Valley for her research on tick borne diseases. This researcher has even tested ticks for the presence of Brucella since they can be a possible source of disease transmission.

June 3rd – Meeting with Mishig, Renchinlumbe Veterinarian

This morning the health team met with Mishig to discuss our brucellosis work plan, as well as the larger health team plan and screenings. Mishig seemed supportive of animal and human testing and happy that BioRegions is beginning to work together with the Darhad community on the control of brucellosis. Mishig stressed that education was one of the most important things. Mishig also provided some information on the local situation regarding brucellosis. One of the main challenges in the Darhad is that the government has supplied only enough vaccine to vaccinate a third of the female calves, kids, and lambs born each year. The

lack of vaccine is limiting the ability to control brucellosis in the Darhad. Mishig also provided interesting insight into the symptoms of brucellosis observed in animals in the Darhad. According to Mishig, symptoms observed in sheep are quite severe. Their joints become very stiff to the point that they cannot move. Brucellosis, thus, results in significant mortality in sheep. This is interesting since worldwide brucellosis typically does not lead to severe symptoms in animals. According to Mishig, symptoms are less severe in cattle. Swelling of the joints is observed in cattle, but it is typically not severe enough to restrict joint movement and contribute to mortality. Mishig agrees that it is during calving season that the majority of people contract brucellosis. He believes that people primarily contract brucellosis from cows. Herders typically only assist with calving, not kidding or lambing. Cows are more valuable, are fewer in number, and typically have more trouble calving. These factors combine to create a situation in which brucellosis can be transmitted to herders. It will be interesting to investigate Mishig's belief that cows are the primary source of brucellosis infection in people. This goes against the conventional belief of small ruminants being the typical source of human infection. If cows are infecting humans in the Darhad and are responsible for the severe chronic symptoms observed in humans in this region, this might indicate that cows here are infected with *B. melitensis*.

June 4th – Meeting with Dr. Purevsuren, Director of Renchinlumbe Hospital

We visited the Renchinlumbe hospital and talked with Dr. Purevsuren and some of his staff today. During the meeting, we made arrangements for field testing including use of the hospital's centrifuge. The plan is to bring two centrifuges with us, one that we will power with a generator and one that is turned by hand. Two doctors and a lab technician from the hospital will accompany the health team and have agreed to draw blood from people for our brucellosis screening. Dr. Purevsuren was very happy that we had come to help screen for brucellosis. BioRegions has been discussing this with Dr. Purevsuren for several years after he identified brucellosis as one of the primary health concerns in the area. According to Dr. Purevsuren, many people in the Darhad have brucellosis. Animals with brucellosis used to be separated from the rest of the herd, but this is no longer practiced. As a result, Dr. Purevsuren believes brucellosis incidence in people is increasing. The hospital in Renchinlumbe no longer tests people for brucellosis. During the period of Soviet influence, brucellosis screening was performed in the Renchinlumbe hospital, but now anyone with symptoms (and the necessary financial resources) must travel to Murun for testing. Dr. Purevsuren believes Bag 4, and also to a lesser extent Bag 3, has served as the origin of brucellosis for the rest of the Darhad Valley. There are high incidences of brucellosis in these two bags.

June 8th –

The final schedule for our health screenings and education sessions has been set (although it will likely change several more times before we finish testing) and is as follows:

- June 8 – Travel to Bag 1
- June 9-11 – Health Education Workshops, Brucellosis workshop on June 9th
- June 12 – Travel to Bag 2, Hogrog
- June 13 – Screenings in Bag 2 with group from Murun
- June 14 – Travel to Tengis

June 15 – Travel to Bag 2, Southern location
June 16 – Screenings in Bag 2, Southern location
June 17 – Travel to Bag 3
June 18 – Screenings in Bag 3
June 19 – Return to Renchinlumbe

June 9th – Bag 1, Doctor and Herder Education, Sample Collection Begins

The health team began their three day First Aid and medical training workshop today. Fifteen bag doctors were in attendance, traveling to the workshop from all over the Darhad Valley. We began the workshop with brucellosis education. This two hour education component covered the following topics: disease symptoms in animals and humans, mechanisms of disease transmission from animal to animal, mechanisms of disease transmission from animal to human, and suggestions on ways to limit each type of disease transmission. The bag doctors were very attentive, copiously taking notes during the discussing and taking down points from our posters. The workshop turned into an interesting discussion with the doctors, allowing for valuable exchange of knowledge between our groups. The doctors were very interested in learning about brucellosis in the U.S. and the steps we have taken to control brucellosis as a possible model system for brucellosis control in Mongolia. We discussed the advantages and disadvantages of various control strategies including slaughter, separation of sick animals, and vaccination. The doctors were also understandably very interested in diagnosis and treatment strategies. We discussed treatment regimes used in the U.S. and the success rates of these strategies. The doctors asked about the specific dosage for the antibiotics prescribed, carefully writing down the suggested treatments in their notes. Doxycycline and gentomycin are available in the Darhad. The bag doctors were not familiar with rifampicin, a substitute for doxycycline that is used for treatment in children, but co-trimoxazole, a suitable substitute is available. During our discussion, I was somewhat concerned about sharing detailed knowledge on treatment regimes with the doctors in fear that they would start treatment before definitive diagnosis was made. Also, gentomycin and streptomycin can cause severe complications in patients with kidney and liver problems, and thus organ function should be carefully monitored during treatment. These concerns were shared with the doctors, and it seemed clear that they would not offer treatment unless patients traveled to Murun for brucellosis diagnostic testing. The doctors realized the value of disease prevention and public education, however, at the same time I can sense that these strategies offer no hope for those currently suffering from brucellosis. In order to share the information we discussed with the families they serve, the doctors from each bag asked for 200 copies each of education materials. We promised to prepare these copies for them in UB and distribute them to the doctors in July.

In the afternoon I traveled with the soil and grazing team to the Bayanhangai Valley where the 23 family user group lives. This group is interested in trying new strategies and “thinking outside of the box” and has served as a sort of case study for some of BioRegions’ activities in the Darhad. Today the user group has gathered to celebrate the dedication of their new artisan building, and we took advantage of this gathering of people to offer a brucellosis education workshop following the ceremony. Again the educational workshop went well and the group was very attentive. The workshop delved into a discussion of herding and animal husbandry practices that could be applied to limit disease transmission. In response to our suggestions to create a separate area for caving and to separate goats and sheep from cattle

during the calving season, the herders seemed knowledgeable that these were good ideas; however, they expressed the opinion that these practices were not feasible. During the period of Soviet influence, the herders explained that they had a separate enclosure and pasture for calving, but now they have only enough money to build a single fence. The herders said that many good practices existed during Soviet times. They used to bury aborted fetuses and placentas, but this is no longer practiced. They would move the aborted fetuses with wire to avoid touching the tissues. Now they have a different method of disposing of placentas. Some herders will pick up the placenta with one finger (thus demonstrating some knowledge that contact with this tissue should be avoided), boil it with some salt and consume the placental “meat” as a meal. In response to our discussion of animal slaughter and disposal of the uterus and udder, the herders replied that they fed these organs to their dogs. This raises the question of whether dogs could be a source of disease transmission in the area. There is some evidence in support of this form of disease transmission in other parts of the world. We left the workshop with mixed feelings. While the herders were very interested in what we had to say and asked many questions, they expressed the sentiment that it was impossible to prevent the spread of disease with so many diverse routes of transmission that exist.

To finish off the day, we collected our first blood samples from one family’s sheep and goat herd near our health team camp. This first attempt at sample collection was slightly disorganized and we were busy until midnight centrifuging samples.

June 10th – Sample Collection Continues with Livestock in Bag 1, Human Sampling Begins

Today started at 5:30 am with Mishig revving the engine of the big monster truck outside my tent door. We were in a rush to collect samples before the animals were released from their enclosures. We managed to reach four families this morning with 17 sheep, 8 goats, and 7 cows sampled. After an hour of centrifuging over salty milk tea and bread, we returned to camp. The collection process went much smoother this morning overall. I am having some trouble finding the tail vein on cows, however, and the needles we brought are too short to get a good stick in the jugular.

During lunch break, the bag doctors taking part in the First Aid workshop enthusiastically gave blood for brucellosis testing, and with this we have our first 19 samples of human blood. The lab technician from the Renchinlumbe hospital is taking care of human blood draws, and we are left only to process the blood samples and ask patients our survey questions. We were also able to pinpoint the current place of residence of most doctors sampled, and we hope to use GIS software in later analysis of test results.

Tonight we collected our final animal samples from this location in Bag 1, visiting three more families to obtain blood from sheep and goats. It was another late night processing blood especially after waking up at 5:30 this morning. When the blood was being spun down, we worked on preparing our controls for the first run of the Card Test that we have planned for tomorrow afternoon. We attempted to do everything possible to create a clean workplace and keep everything as sterile as possible, though kneeling on the dirty floor of Mishig’s truck and using a seat for a workbench could only approximate a lab. We worked by the light of my headlamp.

June 11th – Last Day in Bag 1 First Aid Camp, First Card Test Run

This afternoon we ran our first set of brucellosis Card Tests on the animal and human samples collected over the past two days. After the long trip from the States and the many temperature changes endured, we were quite nervous as we ran our three controls. The controls worked perfectly and we proceeded with our samples. Of the samples run, we found one test positive goat and one test positive human. The bag doctors were waiting for their tests results, and I was apprehensive about sharing the test results with them given the ambiguous conclusions that can be drawn from the data. The hospital technician called the bag doctor who had tested positive into our lab, and we sat down with Susan and Dr. Katie to discuss the possible meanings of a positive test result. The discussion with the patient went very well, and since he suffers greatly from joint pain, he seemed to embrace our suggestion to travel to Murun for further testing.

June 13th – Testing in Bag 2, Hogrog Location, with Team from Murun

Today was the health team's first official day of screening. We have met up with a medical group from Murun and the congregation of doctors has attracted more than one hundred herders to the screening. The doctors from Murun were taking blood samples for HIV and STD testing and were happy to share the samples with us for brucellosis screening. The cooperation allowed us to obtain approximately one hundred human serum samples. Unfortunately, there was too much confusion to ask people to locate their current place of residence on our maps, hindering prospective GIS analysis. We were also only able to ask a small subset of the people sampled our survey questions.

In the evening we sampled one family's goats, sheep, and cows as well as cows from a second family. Animal sampling is going well, and I am beginning to realize that the challenge in obtaining blood from the tail vein in cows occurs only in animals around two years of age and younger. The older animals are much easier to get a good stick on and some tolerate the blood draws surprisingly well.

June 14th – Card Test Run #2

A long day of travel to the Tengis is planned today, and we woke up at 5 am to run our tests before departing. Again our controls worked perfectly, and with the exception of some numb fingers from the frigid early morning air and the need to keep the antigen warm in our pocket between tests, everything ran smoothly. The small number of animal samples run all tested negative. There were several test positive humans in the hundred samples run, including two strong positive results. Interestingly, these two strong positives occurred in very different subsets of the population – a 71 year old male and a 26 year old female. The results were shared with a doctor from Murun as well as the bag 2 doctor. They will communicate the results to the people tested. It is interesting to observe everyone's eagerness at obtaining the test results. During the screening, those giving blood would repetitively inquire about when the brucellosis test results would be back, with seeming disregard for the other blood tests being run.

June 16th – Testing in Bag 2 South and Another Brucellosis Education Workshop

This morning we collected blood from our host family's cows, as well as helped Mishig with the annual Pasturella vaccinations. After the hospital technician failed to arrive with her centrifuge at 11 am for health screenings, we experimented with the tube-on-a-string centrifugation technique. Following much swinging of tubes around our heads and some sore arm muscles, we have come to the conclusion that this technique produces very poor separation of serum.

The centrifuge and the lab technician eventually arrived via motorcycle and spinning of the cattle samples, as well as sampling of humans commenced. In the mean time, we organized a meeting of the herders arriving for health screenings. The meeting began with a brucellosis education workshop. The discussion of methods to limit animal to animal disease transmission digressed into a rather heated conversation about vaccination. The herding methods suggested to limit disease transmission (including the separation of animals about to give birth from nonpregnant animals, the separation of different families' herds during calving season, and the separation of cattle from small ruminants) were not perceived to be feasible solutions by the herders. Like the members of the 23 family user group in Bag 1, they explained that these herding methods were practiced during the time of Soviet influence, but now there is insufficient pasture space to separate animals and the construction of separate enclosures is too expensive. I have a sense that the herders' argument is partially genuine. However, the community and grazing team has learned through interviews that during Soviet times, animals were herded more intensively. Herders spent the entire day with the herd, and they would graze their animals for ten to fifteen days in one area before moving the animals to neighboring pastureland to allow the forage to rest. After a series of these moves, the herders would then migrate to their next camp. It is intriguing how such well-founded practices and teachings evaporated after the fall of the communes. There seems to be a lack of incentive among many herders to work so hard now, intensively tending to their animals. We believe it is up to the herders to realize this. BioRegions' role is to step in and offer training and share knowledge when the community asks for it. Herding methods that can be adopted for disease control purposes are closely related to those that lead to healthy pastureland. As the soil and grazing team of BioRegions works with families on herding and ecosystem health, brucellosis disease control strategies could easily be interjected into the training.

In regards to the discussion of vaccination as a disease control strategy, I think many herders seize upon this idea because it is an "easy fix." The reaction of many herders is disturbing in another way – many respond with what seems like complete astonishment that a brucellosis vaccine actually exists. When I survey herders and ask if they use such a vaccine on their herd, the majority of herders reply that they do not vaccinate. Some of the older individuals surveyed explain that they vaccinated prior to 1990 during the time of Soviet influence, but no longer administer the brucellosis vaccine. These responses come during the ninth year of a ten year National Brucellosis Control Program that the Mongolian government instituted in 2000. Vaccination of all herds is mandatory, and the brucellosis vaccine is to be supplied free of charge by the Mongolian government. The difference between what appears to be occurring to the outside observer and what actually occurs is rather disturbing. When I met with the head of the National Brucellosis Control Program, Dr. Erdenebaatar, in 2007, he described that vaccination in Khovsgol aimag began in 2001 with booster vaccination of all female animals occurring in 2004. Female young stock were to be vaccinated every year at four to six months of age. On the local level, from the veterinarians in RENCHINHUMBE, I learned that vaccination did not first occur until 2004 with no booster vaccination planned. In 2007 one of the veterinarians in

Renchinlumbe explained that brucellosis vaccination was mandatory and coverage was near 100%. Now after talking with the same individual and delving deeper into the local situation, I have learned that brucellosis vaccination coverage is actually only about 30%. For all vaccines there is a general shortage. Last year, for example, there was only sufficient vaccine to administer to 11,000 of the Darhad Valley's 29,000 head of cattle. A small part of the problem is at the local level. Herders seem to underestimate the number of livestock they own, resulting in an insufficient number of vaccine doses requested to the national government. The primary problem is the national government itself, however. It is supplying only 30% of the necessary doses, and for other vaccinations – anthrax and pasteurized – some of the vaccines supplied were not properly produced. These vaccines caused disease in the livestock and had to be destroyed. In regards to the brucellosis vaccine, Mishig has tried to purchase additional doses beyond what has been supplied by the government. Mishig has had trouble even purchasing the vaccine, but that which he has obtained he offers to families at a cost. While vaccine supply is an important factor in brucellosis control, it is something BioRegions cannot significantly impact, as this is a bureaucratic issue.

To wrap up the day we sampled our host family's goats and sheep, with plans of running the Card Test tomorrow before leaving for the next screening location in Bag 3.

June 17th – Card Test Run #3

This morning before packing up for Bag 3, we ran the card test on the human samples collected during screening in the southern location of Bag 2 as well as the cow, goat, and sheep samples collected from our host family in Bag 2. We were unable to reach other families for animal screening due to transportation logistics and the absence of neighboring gers. The tests ran well again with the trend of a relatively high number of positive human samples (10-15%) and no test positive animals continuing. We rushed through our tests as Maggie tried to kick us out of our "lab," aka the big Boojum truck, so that they could pack for our next move . . . on to Bag 3.

June 18th – Bag 3 . . . Rain, snow, sleet, and no testing

Six AM testing of animals did not go off as planned today. There was heavy rain overnight, causing the medical tent to collapse only two minutes after I went in to find needles and blood tubes. Soon the rain had changed to driving snow, and the screening was off as well as animal testing. After the initial consensus that we would be stuck in Bag 3 for several days waiting up the river to go down, we made it back to Renchinlumbe permanently calling off the health screenings in Bag 3.

In the evening we arranged for transportation to Bag 4, and Kestrel and I drove off with Otqu and his family with evening and early morning animal screenings planned. We successfully took samples from one family's cows, goats, and sheep in the evening. Sampling of cattle is getting easier and tonight I learned that yaks too have a tail vein. After arranging to pick up a milk sample from the family the following morning, we drove up to Gendengonchig's cabin to centrifuge samples before retiring to bed.

June 19th – Continued sampling in Bag 4 and Card Test Run #4

Early morning testing went well – three family’s cattle were screened as well as goats and sheep from two of these three families. We also collected our first milk samples, stopping on the way back to town to chop ice from the river. Hopefully a big flour bag full of ice plus what would fit in our Styrofoam cooler will last until we reach Murun.

The remainder of the day was spent processing samples, running the Card Test, and meeting with Dr. Purevsuren for a final wrap-up session with the medical team. The Card Test of Bag 4 samples produced one weakly positive result – our first positive cow and only our second test positive animal. I was particularly interested in screening in Bag 4 since Dr. Purevsuren previously mentioned that he believed this bag to be the location where brucellosis originated in the Valley and where many of the cases occur. It is unfortunate that we could not sample humans in Bag 4.

June 20th – Bag 1, 23 Families Wrap-Up

I parted ways with the health team today and left for the Bayan Valley and the 23 family user group. A wrap-up session with the grazing and community team was scheduled, and I hoped to conduct one final brucellosis screening. We had a nice meeting with Nyamrenchin, the leader of the user group, and other members of the community. The work of each member of BioRegions was highlighted, and I shared the progress we had made with our brucellosis project and asked about the potential for animal and human screening the following morning. This was greeted with enthusiasm, and we planned on 5:30 am screening of Nyamrenchin’s animals and herds of the neighboring families.

June 21st – Last Day in the Darhad, Bag 1 Screening and Card Test Run #5

The morning started off well. I was able to get blood samples on eight of nine cows attempted. This finished off our supply of animal needles, and I planned on using the smaller 21 gauge x 3/4” butterflies left over from human screening for the sheep and goats. This started off working quite well, but after the fourth goat, I could no longer get the samples I needed. I seemed to be in the vein and would get an initial flash of blood in the butterfly, but it would quit after that. After getting very frustrated with myself and worried that I was threatening to stretch Nyamrenchin’s patience and respect, I decided to call it a day and work with the samples I was able to collect. I think part of the problem with the needles was that the blood more easily formed a clot in the narrow bore of the needle / tube. Also, the short length of the needle made redirecting difficult. The samples were centrifuged, and we set off to find the bag doctor to collect human blood. Ten people eagerly volunteered for screening and we were able to collect whole blood, as well as blood to be spun down for the Card Test. The whole blood samples, as well as milk samples we collected this morning from cows, will be sent to UB for bacteriological screening and PCR.

There were some interesting cases among the animals and humans we screened. Three of the goats tested had aborted this year, and I was anxious to see the results of the Card Test. Of the humans tested, we had our first two blood samples from children. Also the bag vet was sampled. This individual was an older woman who tested positive for brucellosis in 1979 and received ten months of antibiotic treatment at this time. I am unsure if antibodies from this previous infection 30 years ago would persist causing a positive test result, regardless of any more current exposure to Brucella.

The results of the Card Test revealed a continuing trend of seronegativity in animals, with all animals having a negative test result including the three goats with reported abortions. Although only a very small percentage of the human population of the Bayan Valley was tested, of the ten people screened, 30% were seropositive. One of these individuals was the bag vet who had brucellosis in 1979. The children screened were negative, although one child was the son of a test positive woman. The high seroprevalence in this small subset of the population of Bayan suggests that we should work closely with them in the future on brucellosis. The close relationship we have developed with the user group should facilitate this future work.

June 22nd – Murun, Final Meeting with Mishig

We arrived in Murun today, successfully keeping our samples chilled on the river ice. Upon arriving we touched base with Dr. Erdenebaatar in UB, and made plans for the samples to travel by van with Tom James to UB. They should arrive the morning of June 24th.

In the evening we met with Mishig for the last time. I had a chance to ask him for his opinion regarding the high number of abortions occurring in goats in the Darhad. Families I surveyed often had ten goats in their herd aborting, with one family having 30 of 60 goats aborting this year. Interestingly, no sheep reportedly aborted and abortions in cattle were also rare. Mishig had two possible explanations for the presence of a high number of abortions in goats. First, he believed it may indeed be due to brucellosis. Alternatively, it could be due to the poor condition of goats during the winter. Reportedly, goats produce more urine than manure, at least in comparison to sheep. During the winter, the manure layer that builds up in the enclosure that sheep and goats are kept in provides a critical layer of insulation keeping the animals warm during the winter. As herders have increased the proportion of goats in their herd (due to the value of cashmere), the amount of manure build-up in the winter enclosures has decreased, and the animals have trouble staying warm and maintaining energy stores during the winter. It is the goats that are affected rather than the sheep, since sheep have thicker fleece and more body fat.

June 24th – Last Day in Mongolia, Meetings with Sean Armstrong and Dr. Erdenebaatar

This morning Cliff, the health team, and I met with Sean Armstrong to discuss the brucella project and how to go forward from this year's work. First we discussed what is necessary to obtain ethics approval from the Ministry of Health. This will be necessary to receive if we decide to become more involved with human brucellosis research. Ethics approval is a complicated three step process. The first step is to gain approval from Burma at the Ministry of Health. This step of the process involves an oral review of the research proposal. The proposal must be translated carefully because the entire project can be thrown out based on a single translation error. For the oral review, either a member of the project or a representative like Dr. Khurlbaatar must be present. If the project receives approval, it is passed on to the Scientific Review Committee. This second part of the approval process involves another oral review. The third step of approval is passage by the board of the Ministry. A project representative must be present for each step of the ethics approval process. During ethics review, the Ministry will inquire about funding and expects a detailed research budget to be included. A group from Harvard University successfully went through the ethics approval process to pursue research on Vitamin D in Mongolia. It took this group three years to obtain approval, and they are a valuable contact. This group should be able to explain necessary forms

that need to be completed and examples of challenges they faced. The primary investigator on this project is Dr. Ganmaa who has an office at the Health Sciences University I believe.

We also briefly discussed funding sources for a project. The possible sources of funding to pursue would vary depending on the exact direction we would like to take with the brucellosis project. NIH funding is difficult to receive if the project does not have broad scope. In regards to the WHO, the Mongolian sector of this organization seems to be focused on HIV and TB work and likely would not be interested in funding brucellosis work. US-AID has never funded a health project in Mongolia. Organizations that have funded projects in Mongolia include the Asia Development Bank and the Millennium Challenge Account.

After these details were discussed, the conversation changed focus to a more general discussion on the situation of brucellosis control in Mongolia and the different approaches that can be taken to pursue brucellosis control / research. Below is a list of different components that could be included in a potential project:

- Observatory / participatory approach to health work. (This is described in a PhD dissertation by a Fulbright Scholar, Jaspal).
- Behavioral Science Model
- Social Development
- Education
- Historical Perspective - Soviet system of brucellosis control
- National Vaccine Supply and Production
- Holistic Management Approach
- Yellowstone / US brucellosis parallel
- Scientific sampling – including epidemiological studies and molecular techniques, biotyping, etc.
- Animal and Human Components

The conversation with Sean illustrated how complex the issue of brucellosis control is in Mongolia and how involved our project could become. It is exciting, as well as overwhelming, to think of all the different components and the people of different backgrounds and expertise we could potentially bring into the project. We also started thinking of what my personal role in the project would be, which ultimately will likely influence how involved the brucellosis project becomes and what direction BioRegions will take with brucellosis research.

In the afternoon we met with Dr. Erdenebaatar and brought him our samples. He was very pleased with the samples and planned on beginning the analysis of them tomorrow. cELISA as well as the Rose Bengal Test with Mongolian antigen will be performed on the serum samples. Dr. Erdenebaatar was most interested in the samples from the three goats with known abortions and the cow that was Card Test positive. Nested PCR will be performed on the milk and human blood samples. Multiplex PCR will then be used on any samples that are positive to test for the presence of each species of brucella – *B. abortus*, *B. melitensis*, and *B. suis*. Bacteriological testing will also be performed on milk and human blood samples, with the samples first added to liquid culture medium in an attempt to culture any Brucella present.

Dr. Erdenebaatar was intrigued by the Card Test positive human sample from the older woman who was diagnosed with brucellosis in 1979. He believed it was possible that antibodies from this previous infection have persisted, but it would be somewhat unusual and that the current positive test result may also indicate a more recent infection. Dr. Erdenebaatar seemed to think that the relatively high seroprevalence observed in humans in the Darhad (11%) could be explained by the quite low seroprevalence observed in animals (2%). A small quantity of infected animals can cause a high degree of human infection. Also, he agreed that antibody response is often more pronounced and easier to detect in humans. Thus human incidence data is a more sensitive means of discerning animal incidence. In regards to the herds in which the test positive animals were found, it is quite possible that the other animals in the herd are infected despite their negative test results. The general protocol would be to retest all animals in these herds repetitively over the next several months since antibody production can change throughout the time course of infection. It is sometimes the case that animals do not produce antibodies once the brucella is localized to the reproductive tract. The antibody response varies between individual animals.

Dr. Erdenebaatar promised to be in touch with us regarding results of the cELISA, Rose Bengal, PCR, and bacteriological testing and we briefly discussed publication possibilities as well as future collaboration.