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Tuberculosis (TB) is a serious disease caused when bacteria attack the respiratory system. There are three types of TB - human, avian, and bovine. Human TB is rarely transmitted to non-humans, avian TB is typically restricted to birds (pigs and occasionally other animals have been found to be susceptible), and bovine TB - or cattle TB - is the most infectious, capable of infecting most mammals. Bovine TB is caused by the bacterium *Mycobacterium bovis* (*M. bovis*) which is part of the *Mycobacterium tuberculosis* complex.

**Distribution**

Although bovine TB was once relatively common in cattle in the U.S., it has historically been a joint project between USDA APHIS Wildlife Services and Indiana DNR Division of Fish and Wildlife

Special points of interest:
- Bovine TB
- Wabash River Sturgeon
- 2009-2010 AI surveillance season changes
- Rabies prophylaxis
- 2008-2009 AI surveillance summary

**Inside this issue:**

- Wabash River Sturgeon research
- Changes to AI Surveillance
- No TB in wild deer in Indiana
- Pre-exposure rabies prophylaxis available
- Indiana 2008-2009 AI surveillance summary
- Midwest Wildlife Disease Update

**Disease in Focus**

**Bovine Tuberculosis**

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**Distribution**

Although bovine TB was once relatively common in cattle in the U.S., it has historically been conducted surveillance for bovine tuberculosis in Michigan. (Photo: USDA)

a very rare disease in wild deer. Prior to 1994, only eight wild white-tailed and mule deer had been reported with bovine TB in North America.

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**Tuberculosis Case Identified on Indiana Cervid Farm**

INDIANAPOLIS (15 May 2009)—The Indiana State Board of Animal Health (BOAH) is investigating a case of bovine tuberculosis (commonly called “TB,” or more formally known as *Mycobacterium bovis*) in a farm-raised cervid herd in Southeastern Indiana. “Cervid” is a category of animals that includes elk and various species of deer.

A BOAH veterinarian found the TB infection in a red deer being processed for meat. A U.S. Department of Agriculture (USDA) laboratory test confirmed the disease.

The animals in the cervid herd, which include elk, red deer, fallow deer and Sika deer, are part of an on-going targeted surveillance program. The farm sits in close geographic proximity to a beef cattle herd that was traced to a TB-positive cow in December 2008.

“Since December, BOAH has been working to determine if this disease is present in that region,” explains Indiana State Veterinarian Bret D. Marsh. “In addition to two whole-herd tests on the initial beef farm, we have tested animals at all adjacent properties. All have yielded negative results.”

With this new finding, BOAH is collaborating with state, federal and industry partners to determine a course of action. More information will be released as it becomes available.

Indiana has held a bovine tuberculosis-free status since 1984 with the USDA. Under federal guidelines, a TB-positive cervid does not affect the status for cattle producers. Before the December 2008 case, the last time a Hoosier herd tested positive for the disease was in the 1970s.

More information about the disease and the investigation, as it develops, will be available on the BOAH website at: www.boah.in.gov.
Intersex Male Sturgeon From The Wabash River

Intersex is referred to as a gonadal abnormality characterized by the presence of, sometimes microscopic, oocytes in predominately testicular tissue. In the United States, this condition has been observed in wild populations of several fish species, the most notable being smallmouth bass (Micropterus dolomieu) from the Potomac Watershed, West Virginia. Smallmouth bass males with “testicular oocytes” were first discovered in 2003 by accident when examined under the microscope and in a recent study over 80% of male smallmouth bass sampled from this watershed were intersex (Blazer et al., 2007). Intersex male fish are not restricted to the United States, and in fact the first reports came from feral fish sampled downstream of sewage treatment plants in the United Kingdom.

The cause(s) of intersex in male fish is not completely understood and several hypotheses have been suggested. For example, exposure of fish to potent synthetic estrogens such as ethinyl estradiol from birth-control pills and to other estrogen-like chemicals, such as bisphenol-A, a by-product from plastics, both released from sewage treatment plants have been suggested as the causative agents. However, intersex fish have also been observed downstream from paper mill effluents and near agricultural fields. For instance, intersex in smallmouth bass from the Potomac was highest in males inhabiting heavily farmed areas.

In order to assess the prevalence of intersex in shovelnose sturgeon (Scaphirhynchus platorynchus) from Indiana, 61 fish were captured from the Wabash River in May 2008 at river mile 300 near Lafayette, Indiana (N40° 26.185’ W86° 53.802’), using a Smith-Root electroshocking boat. This site is located in relatively close proximity to a couple of sewage treatment plants. All fish were transported live to the Indiana Animal Disease Diagnostic Laboratory at Purdue University where they were euthanized with a lethal dose of FINQUEL MS-222. Gonad samples were collected post-mortem and fixed in 10% buffered formalin for histological analysis. Formalin-preserved gonadal tissues were dehydrated in alcohol and embedded in paraffin. The embedded tissues were then sectioned at 5 µm and subsequently stained with hematoxylin and eosin.

The population of shovelnose sturgeon sampled for this study was dominated by males. A total of 61 fish were captured, of which only 9 were females. Testicular oocytes were observed in nearly 7.5% of the males (Fig. 1). Although numerically greater, male gonad wet weight and gonadosomatic index (GSI, 3.7 ± 2.5%) were not significantly different from the gonad weight and GSI (2.8 ± 1.1%) of intersex males. Histological examination of intersex males revealed the presence of immature developing oocytes embedded in testicular tissue (Figs. 1B and 1C). Testes from these males contained seminiferous tubules with abundant amounts of sperm.

Of the 52 males captured, 7.5% were determined to be intersex males, whereas Colombo et al. (2007) found that only 4.7% of the male shovelnose sturgeon captured in the Middle Mississippi River were intersex. Interestingly, intersex male shovelnose sturgeon were smaller than females and slightly, but not significantly, smaller than males. This slightly smaller size may be indicative of younger fish and some species of fish will change sex naturally as they grow (Wu et al. 2008). Unfortunately, ages of the shovelnose sturgeon sampled in our study were not known.

What are the implications of these findings to fish populations? The answer is: we don’t really know. A study conducted dosing a lake in Ontario, Canada, with the potent estrogen ethinyl estradiol found that very low doses of this pharmaceutical were enough to seriously impact entire fish populations (Kidd et al. 2007). Others have reported altered breeding behavior (Coe et al. 2008), decreased sperm counts (Edwards and Guilette 2007) and altered growth (Shved et al. 2008) in intersex male fish. These changes in

Continued on pg. 5
Tuberculosis (continued from pg 1)

only other time TB had been found in a wild deer in Michigan was in 1975, when a hunter killed a 9-year old bovine TB infected female white-tailed deer in Alcona County, Michigan.

Starting in 1995 hunter harvested, road killed, and other dead deer were examined for bovine TB infection. White-tailed deer in Michigan have since been tested year round for bovine TB. Testing revealed that most of the TB positive animals were located in a core area in the northeastern part of Michigan’s Lower Peninsula. The core area is located around the four corners where the counties of Montmorency, Alpena, Oscoda and Alcona meet. Antrim, Crawford, Emmet, Iosco, Mecosta, Osceola, Otsego, Presque Isle, and Roscommon Counties have also had animals test positive for bovine TB.

In Michigan bovine TB has been found in white-tailed deer, elk, black bear, bobcat, coyote, opossum, raccoon, and red fox.

Transmission and Development

Bovine TB is spread primarily through the exchange of respiratory secretions between infected and uninfected animals. This transmission usually happens when animals are in close contact with each other. Thus, animal density plays a major factor in the transmission of M. Bovis. Bacteria released into the air through coughing and sneezing can spread the disease to uninfected animals. Research suggests that bovine TB can also be contracted from ingesting contaminated feed. Survival of M. Bovis in the environment is primarily affected by exposure to sunlight. Reports on the length of survival of M. bovis vary from 18-332 days at temperatures ranging from 54-75 F. In a number of studies under laboratory conditions, M. Bovis has been isolated for up to 8 weeks from various feeds kept at 75 F and 14 weeks from various feeds kept at 32 F. However, under field conditions, it is difficult to isolate M. bovis from pastures grazed by animals known to be infected with bovine TB. Non-cervid animals most likely contract TB from feeding on infected tissues from deer carcasses.

Bovine TB infected deer not showing lesions in the chest cavity can be diagnosed by performing a visual inspection of the lymph nodes in the deer’s head. Affected lymph nodes, when cut, will contain one or more necrotic nodules. These nodules may vary in size and be filled with yellow-green or tan pus.

Tuberculosis is a chronic, progressive disease that can cause gradual debilitation, emaciation, depression, and intolerance to exercise. Coughing, nasal discharge, and difficulty breathing can result in cases where the lungs become severely affected. In some instances, superficial lymph nodes in the neck will develop large abscesses that may rupture and drain through the skin.

Continued on pg. 4
Tuberculosis (Continued from pg. 3)

Diagnosis

After performing a visual inspection of the lymph nodes, any suspicious looking lymph nodes are removed for further testing. M. Bovis is unique among the bacteria because they have a lot of waxy material in their cell walls. Because of the waxy material (known as mycolic acid), the usual stains for looking at bacteria with a microscope do not work. The mycolic acids give the Mycobacterium the ability to hold onto special bacterial stains, allowing them to be seen with a microscope. The special stain is called an acid-fast stain. The stain causes the Mycobacterium to look like very small red rods that are called acid-fast bacilli.

The rest of the sample is transferred to culture (growth) media which will allow any acid-fast bacilli which are present to multiply. Over the next two months, culture media is examined regularly looking for growth typical of M. bovis. This normally appears in 10-14 days. The growth is tested using genetic probes to determine whether the culture contains M. tuberculosis complex, of which M. bovis is a member. Additional biochemical testing, which requires three to five weeks, will confirm the final identification.

Treatment and Control

Since there are no effective vaccines for disease prevention and no effective medications for treatment of bovine TB in wild deer, a combination of wildlife disease surveys and deer management strategies are being used to eliminate the disease in wild deer. The wildlife surveys monitor the spread and occurrence of the disease, while hunters are asked to examine their deer from all areas of the state.

Humans can be skin-tested to determine if they have been exposed to TB. These tests can be done at either the local health department or a private physician's office. A positive skin test, however, does not identify the source of the infection. Remember, most people get the infection from other people.

Significance

In the U.S. today, the threat of humans contracting bovine TB from animals is extremely remote. Health officials have confidence in the state's meat and milk supply.

At risk are Michigan's deer herd and other wildlife species with their many social, ecological, and economic values as well as Michigan's livestock industry. By continuing to eliminate TB-infected animals from wild and domestic animal populations, paying close attention to the meat inspection and pasteurization processes, using proper food handling, and good management practices, the chance of bovine TB transmission from animals to humans is virtually eliminated.

Source: Michigan DNR Wildlife Disease Manual found at http://www.michigan.gov/dnr/0,1607,7-153-10370_12150_12220---,00.html (last accessed on June 1, 2008).
**Intersex Male Sturgeon (Continued from pg. 2)**

Physiology can have devastating impacts on fish populations that are endangered or that are commercially targeted for one gender (e.g., female sturgeon for caviar).

More studies are needed that further examine the prevalence of intersex in male shovelnose sturgeon as well as from other fish species inhabiting the Wabash River. For this purpose, we have begun a preliminary study evaluating gonads of largemouth bass. It is also of great importance to conduct studies that seek to identify the cause(s) of this problem as well as evaluate its potential impact on reproductive and general health of affected fish.

**Acknowledgements**

We would like to thank everyone that participated in the collection and processing of the fish used in this study, especially the staff of the Indiana Department of Natural Resources and Animal Disease and Diagnosis Laboratory (Dr. Tsang Long Lin) of Purdue University for donating the tissues used in this study. We also are thankful to all of the graduate and undergraduate students that helped in the collection of data and tissues.

**References**


**Wildlife Disease Surveillance**

**No Bovine Tuberculosis in Indiana Wild Deer**

**Monitoring by DNR and BOAH**

Due to the spread of tuberculosis in deer in northeastern Michigan, the Indiana DNR and Board of Animal Health are monitoring Indiana’s deer herd for signs of the disease for the past several years. To date, there have been no wild deer that have tested positive for TB in Indiana. You can help with this effort to protect Indiana’s wild animal populations by helping to verify that Hoosier deer continue to be disease-free.

**Inspect your deer!**

1. While field dressing your deer, look for white or red-blister-like sores (lesions) on internal organs or inside of the carcass (see article on page 1 of this issue). In the unlikely event you see lesions, exercise caution in handling the animal.

2. Do not proceed with further processing until the carcass is examined by a State Board of Animal Health veterinarian. Refrigerate (or ice down) the carcass if possible.

3. Keep the animal, including the head, intact until examined. To contact a veterinarian, call 1-877-747-3038 (toll free). This number is answered Monday-Friday, 8 a.m. to 4 p.m. Messages left on weekends or holidays will be returned as soon as possible. A veterinarian will advise you, free-of-charge, about the appropriate use of the animal and may collect tissue samples for further testing. By reporting any suspicious lesion, you are helping to protect the health status of Indiana’s white-tailed deer resource.

If you submit your deer for further testing, the DNR will replace your permanent/temporary deer tag to allow revalidation of your existing license.

**Be safe - wash your hands!**

After field dressing or handling any carcass or other raw meat, wash your hands with soap and water. Hand washing removes disease-causing bacteria, including tuberculosis. This practice should always be followed, even if the animal appears healthy.

Source: Indiana DNR
For the couple of years or so, the pre-exposure prophylaxis for rabies has been difficult to come by, expensive, and/or both. This was due primarily to a limited amount of production by the drug companies. However, in the last month, production has resumed and the pre-exposure prophylaxis is once again available at a reasonable price.

Pre-exposure rabies prophylaxis is administered for several reasons. First, although pre-exposure vaccination does not eliminate the need for additional therapy after a rabies exposure, it simplifies management by eliminating the need for RIG and decreasing the number of doses of vaccine needed -- a point of particular importance for persons at high risk for being exposed to rabies in areas where immunizing products might not be available or where lesser quality biologics might be used placing the exposed person at increased risk for adverse events. Second, pre-exposure prophylaxis might protect persons whose post-exposure therapy is delayed. Finally, it might provide protection to persons at risk for unapparent exposures to rabies.

Primary Vaccination
Three 1.0-mL injections of HDCV or PCEC vaccine should be administered intramuscularly (deltoid area) -- one injection per day on days 0, 7, and 21 or 28. Vaccine preparations for intradermal administration are no longer available in the United States.

Booster Doses of Vaccine
Persons who work with rabies virus in research laboratories or vaccine production facilities are at the highest risk for unapparent exposures. Such persons should have a serum sample tested for rabies antibody every six months. Intramuscular booster doses of vaccine should be administered to maintain a serum titer corresponding to at least complete neutralization at a 1:5 serum dilution by the RFFIT.

The frequent-risk category includes other laboratory workers (e.g., those performing rabies diagnostic testing), spelunkers, veterinarians and staff, and animal-control and wildlife officers in areas where animal rabies is enzootic. The frequent-risk category also includes persons who frequently handle bats, regardless of location in the United States. Persons in the frequent risk group should have a serum sample tested for rabies antibody every 2 years; if the titer is less than complete neutralization at a 1:5 serum dilution by the RFFIT, the person also should receive a single booster dose of vaccine.

Rabies Pre-exposure Prophylaxis Available

Veterinarians, veterinary students, and terrestrial animal-control and wildlife officers working in areas where rabies is uncommon to rare (infrequent exposure group) and at-risk international travelers do not require routine pre-exposure booster doses of vaccine after completion of primary pre-exposure vaccination.

What will I be given for rabies pre-exposure prophylaxis?
For persons who have never been vaccinated against rabies previously, post-exposure anti-rabies vaccination should always include administration of both passive antibody and vaccine. Persons who have been previously vaccinated or are receiving pre-exposure vaccination for rabies should receive only vaccine. The combination of human rabies immune globulin (HRIG) and vaccine is recommended for both bite and non-bite exposures, regardless of the interval between exposure and initiation of treatment.

Where Can I Get the Pre-exposure Treatment?
The treatment can be received from several locations including personal physicians, local occupational health clinics, outpatient clinics specializing in travel medicine, and other health care providers. Some physicians and clinics may not be familiar with the treatment, especially in smaller communities. Also, if price is a concern, be sure to call to different clinics, because prices can vary greatly. In Lafayette, Indiana prices ranged from $250-$350 per dose (as of May 2009).

Indiana Avian Influenza 2008 Surveillance Report

Since 1998, U.S. Department of Agriculture (USDA) scientists, in cooperation with the U.S. Department of the Interior (DOI), have monitored wild migratory birds for avian influenza (AI) viruses. By early 2006, the agencies tested more than 12,000 birds in the Alaska flyway, and between 2000 and early 2006, they tested more than 3,000 birds in the Atlantic flyway.

In 2006, the U.S. Geological Survey (USGS), U.S. Fish and Wildlife Services (USFWS), DOI, USDA, and state wildlife agencies began working to conduct surveillance for the early detection of highly pathogenic H5N1 avian influenza, which included all of the major migratory flyways. This program serves to provide an early warning to the agriculture, public health, and wildlife communities should migratory birds be found to carry this particular virus. Details about the surveillance in Indiana were in previous issues of the Indiana Wildlife Disease News.

The avian influenza surveillance in wild birds continued in Indiana in 2008 and 2009. USDA Wildlife Services and the Indiana Department of Natural Resources (DNR) implemented the surveillance plan for the H5N1 strain of high path avian influenza in wild birds in July.

During the 2008-2009 surveillance season, which ran from April 1, 2008 until March 31, 2009, USDA APHIS Wildlife Services and the Indiana Department of Natural Resources Division of Fish and Wildlife exceeded their goal of 800 samples from live and dead birds throughout Indiana.

Sample Numbers
A total of 811 samples were collected from 797 birds during the 2008-2009 sampling season (Table 1). Last June, USDA APHIS Wildlife Services collected samples from 187 resident Canada geese during several of the Indiana DNR Division of Fish and Wildlife goose banding projects. After samples were collected from the geese, they were released on-site.

In July and August, approximately 27 free-ranging wood ducks were sampled by the Indiana DNR. Ten samples from were collected at Goosepond Fish and Wildlife Area from live trapped shorebirds as part of a project between Wildlife Services and a class from the Purdue School of Veterinary Medicine.

During the waterfowl hunting season (September through the end of January), samples were taken from 559 birds with cooperation from hunters.

The remaining 14 samples were collected as part of the mortality surveillance. These included one American coot, five Canada geese, seven mallards, and one pied-billed grebe that were reported to the Indiana Wildlife Conflicts Information Hotline.

Test Results
All results have been negative for highly pathogenic H5N1 avian influenza. Ninety-eight of the 811 samples were either positive or suspected of being positive for the avian influenza matrix. Of those, 4 were positive for H7 and 19 were positive for H5. Each of those samples were forwarded to the National Veterinary Services Laboratory in Ames, Iowa for further testing. Of those 19 H5 positive samples, nine were confirmed to be a low pathogenic strain of H5. Of the four positive H7 samples, two were confirmed as low path H7.

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Table 1. Target and actual live and dead bird numbers from April 1, 2007 through February 25, 2008.
Midwest Wildlife Disease Update

May 2009

Indiana Cervid Rule Changes - The new cervid CWD program is underway and changes are beginning to take effect. Currently, herds are being distinguished between CWD-susceptible and CWD-non susceptible. The susceptible species are white-tailed deer, elk, moose, sika, red deer and hybrids of the listed species. Regulations for non susceptible cervids are minimal, although herd owners may voluntarily join the CWD Certification Program (or CWD Monitoring). The Certification Program allows cervid herds to gain status in a CWD program so they can meet interstate requirements. The Monitoring Program provides minimal regulations for pet and hobby herds not interested in moving interstate.

PLEASE REMEMBER to check the state of destination import requirements for cervids when writing CVIs (Certificate of Veterinary Inspection). Requirements vary from state to state. Official identification must be in the cervid’s ear and recorded on the CVI before the animal leaves the state. (Source: BOAH Issues Quarterly Newsletter, April 2009.)

Indiana Aquaculture and VHS - THE USDA Interim Rule on viral hemorrhagic septicemia (VHS) has been delayed indefinitely. As a result, BOAH’s VHS requirements, when importing fish into Indiana, remain unchanged. See the BOAH web site for details concerning the current requirements. BOAH, THE Indiana Department of Natural Resources and Purdue-ADDL are cooperating with USDA to accomplish surveillance testing for VHS in Indiana’s fish populations. Sampling is currently underway, but no results have been returned. Thus far, the disease has not been found in any inland Indiana waters. (Source: BOAH Issues Quarterly Newsletter, April 2009.)

Rabies Reports Up In Kentucky - Health officials say they’ve found a rabid bat in Lexington, Kentucky making it the 9th animal in the city to test positive for the disease this year [2009]. Eight other animals in the city have tested positive for the viral disease this year, including 6 skunks, a fox and a horse. The number of cases more than doubles the 4 confirmed cases of rabies in Lexington in all of 2008.

The Lexington-Fayette County Health Department announced the findings Thursday [21 May 2009] and said it has posted signs in the neighborhood reporting the incident and stressing the importance of getting pets vaccinated. (Source ProMED, May 21, 2009)

Sick Raccoon – Not Always Distemper - On March 13, 2009 a female raccoon was reported stumbling and standing around in a yard during daylight hours by a homeowner near West Lafayette, Indiana. USDA Wildlife Services personnel responded, euthanized the animal and submitted the carcass to the Purdue Animal Disease Diagnostic Lab. This could well have been another case of the viral disease, canine distemper. However, pathologist, Dr. Pam Mouser at the ADDL, “reported that the neurologic signs in this raccoon are the result of the severe necrotizing encephalitis discovered in the brain. The described protozoal organisms observed associated with the inflammation and necrosis are most consistent with Sarcocystis sp.. No viruses were isolated, and therefore, concurrent canine distemper infection was unlikely.” The raccoon was tested for rabies and canine distemper and was negative. In simple terms, the raccoon had an inflammation of the brain caused by a significant presence of the parasite Sarcocystis sp.. (Source: Dean Zimmerman, Purdue ADDL Report 4/7/09, edited)

Drugs, Fish, Contaminated Waterways - Fish from five U.S. rivers were found to be tainted with traces of medications and common chemicals, according to a new study from the U.S. EPA and Baylor University. The common antihistamine diphenhydramine (Benadryl), an anticonvulsant and two types of antidepressants were among the seven types of pharmaceuticals found in the tissue and livers of fish from waterways in or near Chicago, Dallas, Philadelphia, Phoenix and Orlando, Florida. Each river is considered “effluent-dominated” because they receive large amounts of wastewater discharge from nearby sewage treatment plants.

While federal standards exist for treated wastewater, they do not address pharmaceuticals or most personal care products, and little is known about the effects they have on the environment and wildlife. This study is part of a federal strategy to address the issue. Previous research has concluded that behavior vital for fish survival, such as mating and fighting, can be affected if too much antidepressant residue collects in their systems.

Other medications and products found were the cholesterol drug, Lopid; Cardizem, a medication that helps control high blood pressure; Tegretol, a drug used for epilepsy and bipolar disorder; the antidepressant Zoloft; and galaxolide and tonalide both of which are odor-enhancing ingredients in soap and other hygiene products. The latter chemicals were found in highest concentrations in fish tissue, while the other products were more concentrated in the liver which processes foreign substances that enter the body. (Source: ProMED, 03-31-09, edited)

Photo: courtesy Lil’ Grizz traps
Bovine TB Rate Up Slightly In Michigan Deer - Michigan wildlife officials say deer in a four-county area of northeastern Michigan were infected with bovine tuberculosis at a slightly higher rate in 2008 than during the previous year. The Michigan DNR reports that 1.8 percent of deer tested in the area last year were infected with TB, as compared to 1.4 percent in 2007. DNR wildlife veterinarian Steve Schmitt says the infection rate has decreased over the long term and appears to have stabilized.

Skunk Tests Positive For Distemper - A striped skunk from Tippecanoe County was submitted for necropsy on February 26, 2009. It was reported to have been unafraid of humans, immobile, and had tremors. Purdue Animal Disease Diagnostic Lab pathologist, Dr. Grant Burcham, reported the skunk was strongly positive for canine distemper virus within sections of lung, and was the primary cause of death. Additionally, numerous protozoa (Sarcocystis sp.) were observed within the lung, liver, brain, spleen, and nasal tissue. Systemic protozoal infections can occur as secondary infections in immune-compromised animals. Canine distemper causes immune-suppression.

(Source: Dean Zimmerman, Purdue ADDL report 3/18/09, edited)