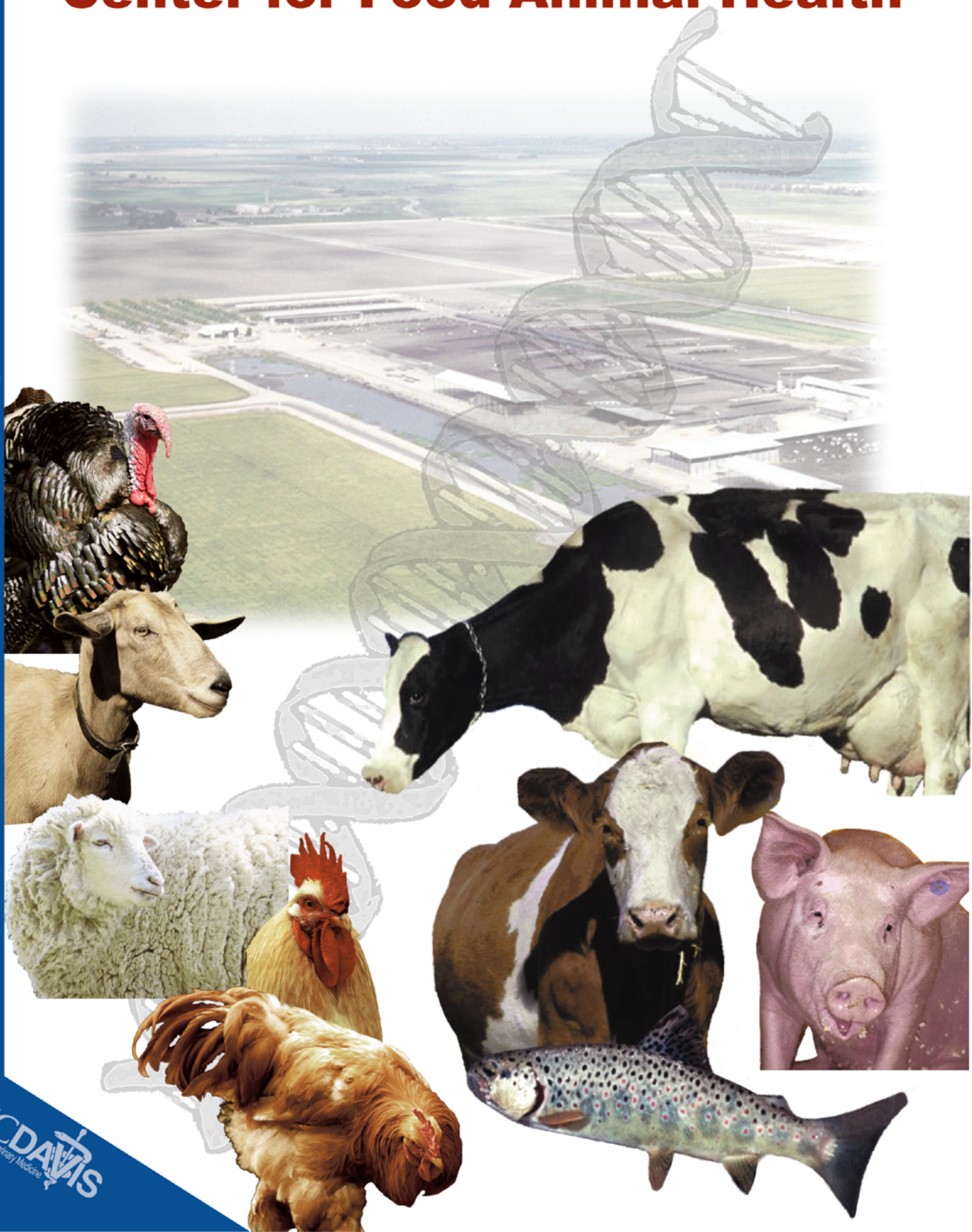
Center for Food Animal Health

School of Veterinary Medicine



UC DAVIS School of Veterinary Medicine

Center for Food Animal Health

Research Highlights

2001-2002 Edition

Office of Research and Graduate Education Programs
School of Veterinary Medicine
University of California Davis

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Introduction by Dean Bennie I. Osburn

The Center for Food Animal Health (CFAH) is an organized research unit in the School of Veterinary Medicine at the University of California Davis. Established in 1972 and formerly known as the Livestock Disease Research Laboratory, this program serves as the veterinary medical component of the Agricultural Experiment Station (AES). Although the CFAH program is managed on the Davis campus, the Division of Agriculture and Natural Resources (DANR) serves as a conduit for the reporting process required by the United States Department of Agriculture (USDA).

The mission of the CFAH is to create, apply and disseminate new knowledge that will enhance the current and future health and well-being of food producing animals, promote the safety of foods of animal origin, and provide a healthy environment for food animals and humans.

Faculty in the School of Veterinary Medicine who design and conduct CFAH research projects include basic scientists, clinicians, extension veterinarians and diagnosticians from the California Animal Health and Food Safety Laboratory. The vital link between School researchers and county farm advisors, practicing veterinarians, animal producers and consumers is provided by Veterinary Medicine Extension faculty. Extension specialists provide teaching, research and service programs on disease prevention, production quality control, biotechnology, food safety and animal well being. Many of the CFAH projects are collaborative efforts and combine multiple disciplines and shared resources.

Research in the CFAH is organized along commodity lines: dairy and beef cattle, small ruminants (sheep, goats, swine, etc.), poultry and aquaculture. Other projects affect multiple commodity groups such as epidemiology research or studies which impact trade regulations, public health and food safety. CFAH funds are designed as Aseed@ monies to enable researchers to obtain preliminary data. CFAH funds also supplement research support received from extramural sources such as federal and state agencies, private corporations and organizations, marketing boards, commodity groups and gifts. CFAH projects are selected for funding through an intramural competitive grant review. Proposals must be scientifically sound as well as meet priority needs of the livestock industries in the State of California, including:

- Sustainable Production Systems (Includes Animal Well-Being)
- Food Safety
- Environmental Health (Includes Water and Waste Management)
- New and Emerging Diseases (Including Infectious Diseases)
- Exotic Pests and Diseases / Bio Security
- Genomics.

Principal Investigators provide an annual progress report for each CFAH project. This booklet highlights the most recent progress reports and research activities.

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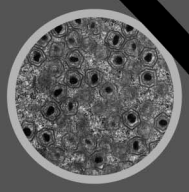
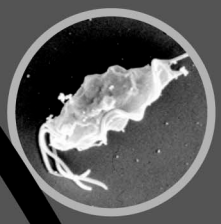
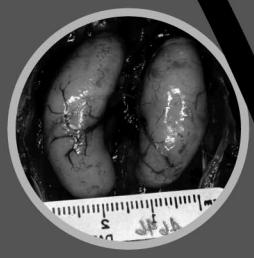
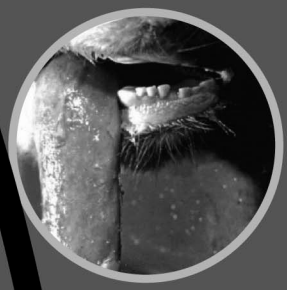
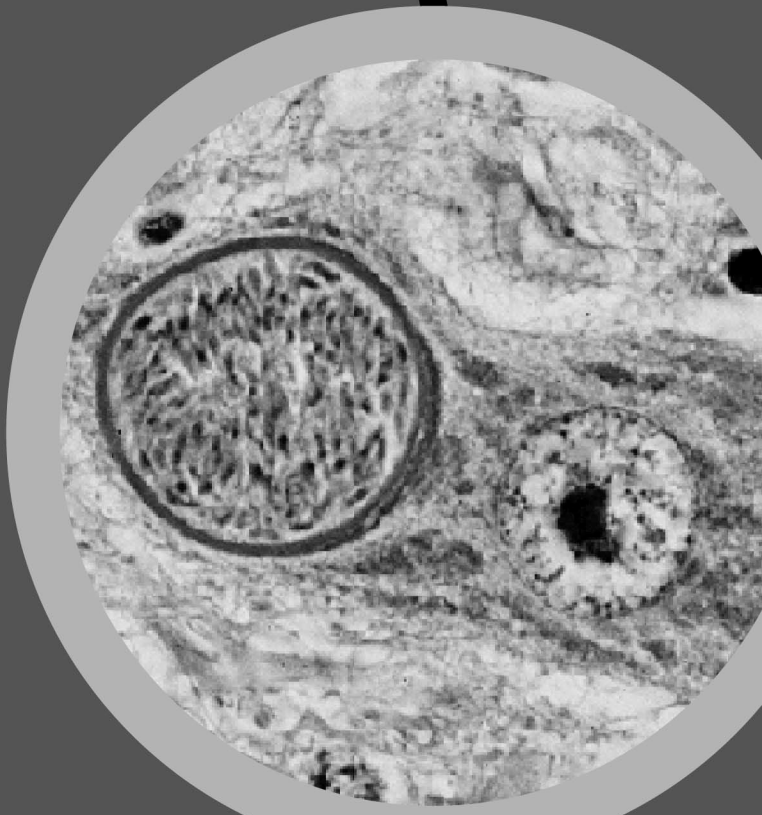
Dairy Research











Dairy Research Highlights

DAIRY RESEARCH HIGHLIGHTS

Research addressing the concerns of the dairy production industry in the state of California account for the majority of projects supported by the CFAH. Research is aimed at maintaining healthy animals and thereby reducing production costs. Highlights of the dairy research programs in the CFAH include:

- **Bovine Neosporosis** is the major diagnosed cause of abortion in cattle throughout the world. CFAH researchers are studying the pathogenesis of this disease and improving methods for diagnosis and control.

- **Bovine Viral Diarrhea Virus (BVDV)** studies by CFAH researchers use molecular techniques combined with epidemiological methods to study the diagnosis and control of this disease. Efforts to further understand the transmission of BVDV in herds have focused on improving detection methods, and improving cost effectiveness for BVDV diagnosis.

- **Johne's Disease** (pronounced "*Yo-nees*") is a contagious bacterial disease of the intestinal tract afflicting cattle. Mycobacterium avium paratuberculosis, the bacteria that cause Johne's disease in cattle, resemble those organisms that cause tuberculosis in animals and humans and there is evidence linking this as well with Crohn's disease. CFAH researchers are developing new strategies to combat a recent statewide increase in this disease.

- **Papillomatous Digital Dermatitis (PDD) or Hairy Footwarts** is a significant health problem in dairy cattle, causing pain, affecting an animal's ability to stand or walk, decreasing appetite and water intake and resulting in decreased milk production and major weight loss. CFAH researchers have found that spirochetes play a primary role in the genesis of PDD.

- **Salmonella** is the most common zoonotic disease associated with human consumption of beef and dairy products. The use of antibiotic treatment reduces animal mortality but does not eliminate chronic infections or Salmonella shedding. CFAH researchers are studying all aspects of salmonella including vaccine development.

- The **Dairy Food Safety Laboratory** (DFSL) provides consistent, rapid response to dairy herd health and on-farm food safety problems. Applied research projects include mastitis diagnosis and prevention, chemical and microbial contamination of milk and meat, vaccine development, validation of chemical and microbial residue test kits including E. coli 0157:H7 and evaluation of natural antibiotics. The impact of the DFSL has been significant. The J-5 E. coli vaccine alone saves the California dairy industry between $11-$24 million annually.

- **Leptospira** infection of dairy cattle is a major source of economic loss for which commercial whole cell vaccines do not provide protection. CFAH research is aimed at cloning, identification and expression of genes that protect against Leptospira infection in dairy cattle.

- **Vocalization Studies** are being conducted by CFAH researchers on non-bST dairies to determine if this technique impacts or increases milk production.

Pathogenesis of Repeat Congenital Neospora Infection in Dairy Cattle. (Mark L. Anderson, Ann Melli, Aurelie Andrianarivo, Joan D. Rowe and Patricia A. Conrad)

The newly recognized parasite named Neospora caninum has been identified as the greatest cause of abortion in cattle herds throughout the world. An interesting aspect of this disease is that many adult cattle acquire the infection from their dam as a fetus during gestation. The disease can be vertically transmitted through successive generations whenever the congenital infection, rather than causing abortion, results in the birth of an infected fetus. We have shown that these fetuses are chronically infected and will pass the infection on to their offspring. Our current investigations have been focused on where this persistent infection localizes in chronically infected cattle. To achieve this, we identified and purchased chronically Neospora infected cows. In their subsequent pregnancies, 7 of 8 pregnant cows transplacentally infected their fetus. The standard methods to demonstrate the parasite in various tissues, including culture, histopathology, immunohistochemistry and specific molecular procedures, were all inconclusive. We did not identify the tissue or tissues where the parasite can persist in the adult cow though we did observe microscopic changes in the nervous system suggestive of infection. Our lack of success using these procedures may be due to insufficient sensitivity due to very low numbers of parasite tissue cysts. A new test method, focused Neospora PCR, which will enable us to perform the specific molecular procedures on specific sites in tissues, is expected to improve the sensitivity of the existing procedures because we can focus our search to those tissues that have changes indicating that they are the possible site of infection. Initially, the procedure was not successful so a second method was adapted utilizing a different primer. The second primer was successful in detecting Neospora DNA in known infected tissues but the level of sensitivity for the test was relatively low. Due to the low sensitivity of the procedure, the samples from the chronically infected cows have not been tested. We are repeating the procedure on positive and negative control samples to improve the sensitivity. A sensitive test procedure that will identify rare Neospora parasites in the tissues of the infected cow will add to our knowledge of the parasite life cycle by demonstrating where the parasites reside during latency in the adult host. Knowledge of which tissues are chronically infected is fundamental in the rational development of treatment or control programs for vertical transmission of this disease.

Neospora Oocyst Production with a Bovine Isolate and Horizontal Transmission to the Cow (B.C. Barr, P.A. Conrad, M.L. Anderson and J.D. Rowe)

In 1998 McAllister et al reported they had proven that the dog was the definitive host for Neospora caninum, a protozoal parasite that is a major cause for abortion in dairy cattle in California and throughout the world. This finding is of major significance to the dairy industry as locating the definitive host for this parasite is the first major step forward in developing some means for prevention and control of this bovine disease by eliminating contact between cattle and dog feces infected with Neospora parasites. The purpose of this study was to independently corroborate the findings of McAllister et al and to show that the dog was also the definitive host for Neospora caninum isolated from dairy cattle in California, as McAllister et al had studied Neospora caninum isolated originally from dogs. We followed the methods of McAllister et al, infecting large numbers of mice with Neospora caninum to produce Neospora caninum tissue cysts in the mouse brain. These tissue cysts are considered to be the optimal Neospora biological stage necessary for infection of the definitive host. We examined the brains from these infected mice to make certain that sufficient numbers of Neospora tissue cysts were present in these infected brains, and at the time, they were fed to dogs that were not previously exposed to Neospora caninum. We then collected the feces from these inoculated dogs, examining them for the production of fecal oocysts, the biological stage of Neospora that would be produced by the Neospora definitive host. We also processed pooled feces from these dogs to inoculate into unexposed mice and calves to

see if they became infected with this processed dog feces. If Neospora oocysts were found in dog feces, or if we could detect Neospora caninum infections in the mice and calves inoculated with processed feces, then we would have corroborated that the dog is the definitive host. ***IMPACT***: The results of our study were inconclusive and we could not either confirm or refute the dog as the definitive host. We did not find any conclusive evidence of oocysts in the infected dog feces nor of infection in mice or calves inoculated with processed feces from these dogs. However, we also determined that there were very few Neospora tissue cysts present in the mouse brains fed to the dogs, and this finding makes it possible that the dogs were not given an adequate Neospora caninum inoculum to allow for production of sufficient Neospora oocysts in the dog feces. Our results do indicate that the mouse is a poor experimental model to rely on for consistent production of the critical Neospora tissue cyst stage and that use of this mouse model may be a major problem for any researchers trying to produce fecal Neospora oocysts in dogs.

Neospora Wildlife Serodiagnostic Test, Fecal PCR Test Development & Latent Bovine Neospora Infections (B. Barr)

The results of this project suggest that experimental infection of naive cows always protects against subsequent fetal infection, even with re-exposure during subsequent pregnancies. The results in naturally infected cows are unclear, however, it appears that most naturally infected cows were not affected by Neospora challenge. The marked difference in results between naive and naturally infected cows suggests that the immune response different in these two groups and that naturally infected cows may be immunosuppressed. Also the results suggest a live vaccine may be protective for naive cows.

Pathogenesis of Bovine Neosporosis (P.A. Conrad)

The intracellular parasite Neospora caninum is a major cause of infectious bovine abortion. Knowledge of the immune responses associated with N. caninum infection is a prerequisite in designing effective control programs, based on vaccination or chemotherapy. The present study in experimentally infected dams did not show a correlation between immune responses in the dams and the ability to transmit infection to the fetus. Our parallel studies of bovine fetal immune responses following experimental infection of the dams were not as well conclusive. More detailed immunological studies encompassing the entire period of pregnancy are currently being undertaken in pregnant naturally infected cattle to assess the immune mechanisms associated with reactivation of a latent N. caninum infection and vertical transmission.

Improved Diagnosis and Control of Bovine Neosporosis (P.A. Conrad)

Neosporosis, caused by the protozoan parasite Neospora caninum, is a major cause of infectious abortion in cattle. There is no prevention or treatment available for bovine neosporosis. To stop the cycle of vertical transmission and reduce the economic impact of Neospora abortion, we must investigate methods for increasing protective immune responses in naturally infected cattle. We report here the first vaccine efficacy trial of a POLYGENTM-adjuvanted killed Neospora caninum tachyzoite preparation in naturally infected cattle. This preparation was previously found to induce significant production of interferon-gamma (IFN-g), a cytokine known to be critical for resistance of mice to N. caninum infection. The study is still ongoing.

Reproductive Performance in Domestic Ruminants (M. Thurmond and S. Hietala)

A method has been developed that provides an estimate of the probability of infection, given an animal's serologic test result value, without the need for estimates of sensitivity and specificity. The method uses probability functions for Neospora caninum ELISA values and for BVDV SN titers, one for known infected animals and one for known uninfected animals, the

prevalence of infection and Bayes' formula to derive the probability of infection. The method can be used for any assay, serologic or otherwise, in which the test result is measured as a continuous variable. ***IMPACT***: This approach permits assessment of the probability of infection, rather than simply seropositivity, which has direct application to risk and hazard assessment where estimates of the probability of infection are required. The method permits an assessment of the risk or probability of infection directly from serologic test results that are measured on a continuum.

Diagnosis and Control of Bovine Viral Diarrhea Using Molecular Techniques (M. Thurmond)

The objective of the study was to use nucleic acid sequence analysis of partial BVD virus genome to differentiate BVDV vaccine strains from BVDV wild type virus. BVDV RNA was collected from 100 BVDV field isolates, in addition to BVDV reference strains and vaccine strains. The field isolates were obtained from an equal representation of persistently infected animals, clinical, and non-clinical acute infections. The viruses were genotyped as BVDV type I or BVDV type II (an approximate 2:1 ratio), as determined by nucleic acid sequencing of the genetically stabile 5' UTR region of the BVDV genome. Analysis indicates that direct automated cycle sequencing is possible and reproducible in stabile regions of the genome, but is not able to distinguish wild type from vaccine strains. Sequencing in the variable regions of the genome does not have sufficient sensitivity or reproducibility to detect all wild type isolates. For the purposes of diagnostic differentiation of vaccine and field strains, it appears that it will be critical to combine sequence information from both stabile and variable fragments of the BVDV genome. Development of a multiplex format to aid in this approach is being explored. There have been no publications or presentations to date. The BVDV isolates and RNA collected for the project have been stored and are available for ongoing evaluations. ***IMPACT***: The ongoing development of this approach is expected to improve our ability to distinguish between vaccine exposure and wild type exposure to the virus, and lead to improved diagnosis and epidemiologic understanding of BVDV.

Epidemiology of Bovine Viral Diarrhea Virus Infection in Dairy Cows - Screening (M. Thurmond and S. Hietala)

This project developed pooling methods for least-cost strategies to screen for rare diseases like bovine viral diarrhea virus persistent infection, and estimated sensitivity and specificity of the technique. This approach allows improved diagnosis and large-scale surveillance of rare diseases, previously too cost prohibitive to routinely manage. On several herds studied, we identified high rates of BVDV postnatal transmission in vaccinated herds, typically with high congenital infection rates of 10 percent. These results are critical to future control of diseases and economic impacts associated with BVDV in dairy herds. We estimated BVDV colostral antibody decay and effects of BVDV vaccination on transmission, and are applying these data to modeling control of BVDV transmission on dairies. Analytic methods for BVDV and abortion diagnostics being developed include Bayes probabilistic assessment of infection, and Bayes case-control and accelerated failure time procedures. These new analytic methods will permit improved epidemiologic research and diagnostic evaluation of herds suspected of being adversely affected by BVDV, and similar infectious agents. A BVDV 2-year renewal study is ongoing on several herds to assess affects of BVDV on fertility and abortion. Preliminary data for one herd indicate significant stillbirths associated with BVDV type II. ***IMPACT***: The pooled sample testing method is now being used in the veterinary diagnostic community to cost-effectively diagnose and aid in control of BVDV PI. Further applications, using the same approach are now being developed for surveillance of nationally important diseases such as Johne's disease. The new data involving BVDV congenital

infection, transmission, and colostral decay will provide the scientific basis for strategies for herd-specific control and national management of this economically important agent in cattle.

Epidemiology of Bovine Viral Diarrhea Virus Infection in Dairy Cows - Transmission (M. Thurmond, S. Hietala)

The objective of this study was to estimate decay of BVDV SN colostral antibodies for dairy calves raised under typical dry-lot management conditions, and to incorporate the estimates in a model describing BVDV transmission. No difference was observed in the ages at which calves lost colostral BVDV antibodies and became seronegative (titer<1:4) to BVDV type I or II between calves vaccinated with BVDV type I at 15 and 45 days of age and calves not vaccinated. Results indicate that the early BVDV vaccination practiced by many producers may not significantly affect decay of BVDV colostral antibodies, and that as much as 50% of calves may become susceptible to clinical disease from BVDV infection as early as 113 days (BVDV type I) or 75 days (BVDV type II) of age. The information on age at which calves loose colostral antibodies has been incorporated into a transmission model that identifies optimal vaccination strategies for BVDV. Two manuscripts are in preparation. In addition, characterization of when and where vaccinated dairy heifers become exposed to BVDV on 2 dry-lot dairies has been completed and submitted for publication. Very high exposure risk periods were identified for both BVDV type I and type II, which may depend on corral density, PI prevalence, and vaccination program efficacy. ***IMPACT***: These findings provide information necessary in characterizing the waning calf herd immunity resulting from decay of colostral BVDV antibodies and give estimates of ages when calves might respond favorably to BVDV vaccination. The model will permit development of vaccination strategies tailored to the specific needs and practices of a herd.

Evaluation of the Variability in Test Results in the Use of a Commercial Johne's Disease ELISA Test Kit (John Adaska and Sharon Hietala)

The goal of this project was to determine the variability in test results inherent in a commercially available Johne's disease ELISA kit (Herd-Chek, Mycobacterium paratuberculosis Antibody test Kit by IDEXX). We selected 201 serum samples from a bank of samples that had all been previously tested using the same test. Each sample was tested five times in each of two labs for a total of ten replicates. We found that the variation for the test results was much higher than desired when all ten replicates were used and we also found that within each of the labs the variation in the test results was typically greater than desired. The two labs differed both in the mean sample results they found and in the degree of variability they found in the sample results. The results of this project indicated to us that the results provided by this test are extremely variable. In response, the California Animal Health and Food Safety Laboratory no longer reports results as simple positive/negative. We now provide test results as actual numerical results and include a paragraph indicating that we recommend low results (S/P ratio of 0.2 or less) be considered negative, a mid-level result (0.2 to 0.35) be considered suspect, with repeat testing in the future recommended, and a high test result (0.35 or greater) be considered positive. This allows us to minimize the likelihood of misclassifying the infection state of animals and provides submitting veterinarians and producers to make decisions based on better information.

Seroprevalence of Johne's Disease in California Dairy Cattle (John M. Adaska, Sharon Hietala and Randy Anderson (CDFA))

The goal was to determine the approximate seroprevalence of Johne's disease in adult dairy cattle in California. Johne's disease is a chronic debilitating enteric disease of ruminants caused by the bacterium Mycobacterium avium subspecies paratuberculosis. The disease causes decreased milk production in dairy cows often before other clinical signs, such as diarrhea, become evident

and therefore can negatively impact production at both the individual animal and herd levels. In this study we used 2000 serum samples from a collection created during the course of another study involving adult dairy cows. The sampled cows were from a total of 65 dairies which were representative of herds throughout the state. We found that overall there was a 4.56% seroprevalence for Johne's disease as determined using the Herd-Chek, Mycobacterium paratuberculosis Antibody test Kit by IDEXX and the manufacturers suggested positive cut-off point. Within the state, the northern region had a seroprevalence of 6.92%, the central region had a prevalence of 3.72%, and the southern region had 5.18%. When an individual herd was classified as being positive if it had 1 or more seropositive animals 67.7% of the herds tested were positive. If more rigorous standards were used and a herd was considered positive only if two or more animals were seropositive then 35.4% of the herds tested were positive.

An Economic Evaluation of Johne's Disease Control on 3 California Dairies (T.E. Carpenter)

The objective of this research was to model the risk of introduction of Mycobacterium avium subsp. paratuberculosis infection into a dairy herd by purchase of replacement cattle, and quantify the effects of infection prevalence in the source herd(s) and use of single or multiple tests (ELISA and fecal culture) as risk mitigation strategies. A hypothetical dairy herd, free from M. a. paratuberculosis, which replaced 30 (1 lot) of its cows per year, was considered. Probability distributions were estimated for the sensitivities and specificities of ELISA and fecal culture, the proportion of infected herds and within-herd prevalence for a randomly-selected replacement source herds (high prevalence) and a herd in level-3 of the VJDHSP (low prevalence). Sensitivity analysis was performed to assess the effect of test dependence and altering the herd prevalence and ELISA sensitivity assumptions. A total of 120,000 iterations was performed for the 12 alternatives.

Results are presented here for the initial model assumptions that the ELISA sensitivity was 45%. Simulation results predicted that 1% to 10% of the M. a. paratuberculosis-infected lots would fail to be detected by the ELISA. A similar percentage of infected lots, would be undetected by a subsequent fecal culture (FC) test, assuming all ELISA negative cattle were FC-tested. The negative predictive value (NPV) (the probability that given the total lot-test result is negative, all the animals in the lot are truly not infected) ranged from 77% for ELISA results to 83% for fecal culture, if the lot was comprised of animals from high prevalence (randomly-selected) herds. On the other hands, the NPV's were both 99%, for lots of cattle purchased from low-prevalence (level-3) herds. It was predicted that approximately 2% and 44% of these lots would have at least 1 animal not detected by the ELISA, and 1% and 32% would not be detected on a subsequent fecal culture, if animals were from low- and high-prevalence herds, respectively.

The benefit of testing introduced cattle with ELISA alone or in combination with fecal culture was found to be minimal if cows were purchased from known, low-prevalence (level-3) herds. The value of testing by ELISA alone or in combination with fecal culture increased greatly in high-prevalence herds. If a lot of cattle from level-3 herds had negative ELISA and fecal culture test results there was <1% chance that this lot had an infected animal. However, this probability increased to 17% (1 in 6 lots) if the cows were from randomly-selected herds. Although our findings are specific for 30-cow lots, they emphasize the importance of limiting risk of introducing Johne's disease into dairy herds by purchase of replacements from herds that have a test-negative status, i.e., low to zero M. a. paratuberculosis-infection prevalence. Testing of random source cattle, bought as herd replacements, can partially mitigate the associated risk, but not as effectively as by using test-negative herds as the source, with or without testing.

Certification of California Cattle Herds as Free of Johne's Disease and Assessment of Disease Introduction Risk Associated with Animal Trade (I.A. Gardner)

In this project, a simple Bayesian model for certification of herds for Johne's disease based on ELISA test results and prior herd information has been programmed. In the model, which uses Gibbs sampling, herds are assumed to be infected or non-infected with fixed probability. Infected herds are assumed to have prevalences that vary according to a single beta distribution and all infected animals are assumed to be in stage-2 disease. The probabilities of test outcome results when the herd is either infected or not infected are obtained by Monte Carlo integration. The posterior probability of infection is plotted as a function of the prior probability of infection. These plots show which prior probabilities will designate the herd as Ainfection free@ for the given test outcomes. For model inputs, we used expert opinion of 2 nationally-recognized experts. We have used our initial model to evaluate whole-herd ELISA test results for several dairy herds with promising results. We are currently developing the model to include multiple disease stages, use of fecal culture in addition to ELISA, and improved probability calculations that incorporate different sampling schemes.

Studies on the Epidemiology Treatment & Etiology of Papillomatous Digital Dermatitis in Dairy Cattle (D. Hird, R. Walker, D. Read, S. Barry and J. Maas)

This is a long-term study which was undertaken following an outbreak of PDD in dairies in Southern California in 1991. Because of the compelling histopathologic evidence of invasiveness by spirochetes, we focused our efforts on isolating these organisms. Our studies indicated that the isolated spirochetes were antigenically related to the invasive spirochetes, not merely commensals or "innocent bystanders" colonizing nonviable parakeratotic debris. Our inability to experimentally transmit PDD unless the feet were predisposed by prolonged hydropic maceration and closure demonstrates that PDD is a multi factorial disease with an infectious component. This also helps to explain why high recurrence rates occurred in some of our treatment and control trials performed on dairies with muddy corrals. The interactions between host, infectious and environmental factors in PDD demands a concerted multi-disciplinary approach in order to establish a rationale for prevention and control. The UC Davis Vet School has facilitated such an approach by establishing a Footwart Taskforce with continues to focus on etiology, pathogenesis, epidemiology, treatment and control. ***IMPACT***: There is evidence that bacteria play an important role in the pathogenesis of Papillomatous digital dermatitis. In addition, we have learned that PDD is pathologically identical to digital dermatitis DD, which was first described in Italy in 1974 by Dr. Mortellaro.

Molecular Epidemiology of Pathogenic Microorganisms in California Dairies and Humans (John Adaska)

The goal of this project has been to compare the molecular characteristics of pathogenic microorganisms isolated from humans and dairy cattle in California. We have completed studies of Salmonella eneterica serovar Typhimurium (S. Typhimurium) collected during 1996-1999. These studies have used four different typing techniques targeting DNA (plasmid typing, ribotyping, IS200 typing and pulsed-field gel electrophoresis) and two techniques that target phenotypic characteristics (phage-typing and antibiotic sensitivity testing). We have found that two clusters of isolates predominated during the time period under study and these were represented by DT104 and PT193 strains. The isolates found in humans and dairy cattle had similar genetic and phenotypic patterns with the exception that 100% of human isolates of DT104 had a 90 kB plasmid while the same plasmid was found in only 30% of the bovine isolates. This 90 kB plasmid is the so-called "virulence plasmid" of S. Typhimurium and has been found to play an important role in murine infections with this agent. This difference between the human and bovine isolates of DT104, which is otherwise a highly homogenous organism, may indicate a degree of host

preference by the bacterial strains. ***IMPACT***: Such host preference could have important implications for farm-to-fork food safety efforts in that they may indicate that focusing on generic Salmonella would have less impact on the incidence of human food-borne salmonellosis than would a strategy that specifically targeted those strains most likely to cause human disease.

The Impact on Antimicrobial Use in Livestock (J.K. House)

This project has illustrated numerous important epidemiological features of salmonella on dairy farms. Important observations include: The prevalence of salmonella varies seasonally with a peak prevalence during the summer. Fecal Salmonella shedding varies during the production cycle with post partum cows shedding salmonella more frequently than dry cows or cows in mid lactation. The environment acts as a dynamic reservoir of Salmonella. Under appropriate conditions salmonella can proliferate in the environment attaining numbers of 10,000,000 salmonella per gram of bedding. Waste water perpetuates cycling of Salmonella. Irrigation of forage crops with waste water contaminates crops. If the ensilage process does not attain optimal conditions salmonella can survive in the feed and be cycled back to cattle. Salmonella contamination of milk occurs frequently. The risk of salmonella contamination of milk appears to be linked to salmonella contamination of the environment.

Relating in-vitro Attributes to Genotype Class for Field Isolates of Salmonella enterica Serotype Typhimurium (W.M. Sischo)

A cell culture laboratory was established and personnel trained to conduct the assays. Basic in-vitro assays were established to measure invasion. The assays were based on Hep-2 cells. In previous and on-going research we have collected a diverse collection of Salmonella enterica serotype Typhimurium from human and bovine sources. A portion of these isolates has been evaluated in the invasion assay and demonstrates differences in the isolates to invade in this cell system. ***IMPACT***: The funding from this project have led us to several new opportunities. We have developed a collaboration with Dr. Stanley Falkow at Stanford University a leading researcher in Salmonella virulence. We have standardized our assay to conform to his laboratory's assay and now can compare results between the two laboratories. We are currently developing an adherence assay. Both of these assays will be applied to a large set of isolates and used to differentiate phenotypic differences in the set.

Ecologic Assessment of Salmonella Enteritidis var Typhimurium in a Dairy Milk Shed (W.M. Sischo, E.R. Atwill, J.S. Jeffrey, J.M. Adaska, J.H. Kirk)

Specific Aims: 1) Determine the genetic and phenotypic diversity of S. Typhimurium isolates from human and bovine sources in a region with a concentrated population of dairy cattle. 2) Determine the spatial and temporal dynamics of S. Typhimurium isolates from human and bovine sources in a region with a concentrated population of dairy cattle. 3) Determine the risk factors for the persistence of S. Typhimurium on large dry lot and free stall dairies. Results, the system for routinely collecting ST isolates is in place. The three county health departments are providing us with isolates and epidemiologic data from reported clinical cases. The animal diagnostic laboratory is providing us with isolates from bovine (adult and calf) clinical cases. We are working closely with the milk receivers in the region and have assayed more than 400 bulk tanks for the presence of ST (85 percent of the dairies in the region). We are beginning collecting environmental samples, primarily water source. All the microbiologic assays have been optimized and detection limits determined. The objective of these assays is to develop tools to facilitate epidemiological studies where large numbers of microbiologic samples need to be processed in a short period of time. The milk assay uses conventional salmonella enrichment and plating media but requires 5 separate collections of the same sample in order to detect the pathogen at low levels

(1CFU/ml or 0.1CFU/gm) using low volumes of sample. The current milk volume used in the assay is 1-ml with 5-times sampling from serial milk collections. The assay has been evaluated on fresh and 48-hour spiked samples. Recovery is similar for the two sample types except that the 48-hour sample is less predictable over time, i.e. recovery does not follow a smooth logistic, declining function as bacteria concentration decreases. These data suggest that as the sample is refrigerated there is spatial clumping of the bacteria rather than uniform distribution in the milk sample. In our bulk tank assay we can detect 100 CFU/liter. We have assessed the antibiotic phenotypes of our current banked isolates and have a spectrum of resistant phenotypes from resistant to sensitive. We have also begun genetic characterization of all isolates. We have a complete map of the three county study region. The map includes dairy and other animal enterprise location, streams, industry, other agricultural enterprises, and biosolid and effluent discharge/storage. We have linked this map to our ST isolates and have begun preliminary spatial assessments. The construction of these maps was a collaborative effort between local government, industry, and the university. All work in the preceding year has been to accumulate the databases necessary for the study. All activity is directed at data collection and data management with the aim to characterize the nature of ST in a complex ecosystem.

On-Farm Aspects of Dairy Quality: Quantitative Assessment of Salmonella typhimurium (W.M. Sischo and E.R. Atwill)

There are 3 objectives in the project. 1) Define on-farm dynamics of food borne pathogens with an emphasis on Salmonella. 2) Develop on-farm tools to enhance the use of dairy records. 3) Develop quantitative economic models that describe the mix of on-farm management strategies that minimize economic risk. In the first objective, there are three defined areas. The first is to develop in-vitro cell-based assays to define relative measures of adherence and internalization (invasion) of Salmonella Typhimurium isolates from clinical bovine and human isolates and non-clinical and environmental recovered strains. The current assay is based on Hep-2 cells. The assay has been used to evaluate a small set of Salmonella Typhimurium bovine and human clinical isolates. These isolates have been genotyped and there appear to be distinct differences in internalization between IS types. The second area was focused on assays for detecting the ST on dairies. The primary assay uses conventional salmonella enrichment and plating media but requires 5 separate collections of the same sample in order to detect the pathogen at low levels (1/ml or 0.1/gm). The third area is focused on the dynamics of antimicrobial resistance in calves. The current research includes three bacterial groups: Salmonellae, E. coli, and enterococcus. The work includes assessing the temporal and spatial dynamics and developing quantitative methods to analyze the data. We have followed 7 cohorts of calves on 5 ranches and dairies. We have observed that there is a rapid transition of E. coli from variably resistant on day-0 to highly resistant by day-14. A portion of this change can be ascribed to antimicrobial use on the ranch but a portion does not appear to be related to antibiotic use. These data support a 'selfish gene' model in which traits are conserved as packets of unrelated information that confirm fitness over the lifespan and range of experiences of the bacterial population rather than just at a point in time for the bacterium. In the second objective, the linkage of management to product quality, particularly the reduction of variation in production and quality is emphasized. The research has two directions one to develop practical methods to move dairy data between animal-side to computer record systems. The current work is directed at enabling large dairies and calf ranches to improve their tracking and disease system for calf raising. We are field testing a system on two ranches. An expert system model for diagnosing mastitis was completed. In the third objective, a survey of dairy practitioners and California state veterinarians was performed to assess the perceived opportunity that veterinarians have for implementing food safety related programs on client dairies.

Serologic Detection of Salmonella Dublin Carrier Cows Using ELISA (B.P. Smith)

Identification of salmonella carriers using LPS ELISA serology in a salmonella infected herd requires distinction of chronically infected cattle from convalescent and vaccinated cows. Cows responding to salmonella infection and vaccination produce titers to salmonella LPS that overlap with the lower titers of some salmonella carriers. The objective of this study was to determine if the LPS antigen specificity of the bovine humoral immune response to salmonella LPS antigens differs following vaccination and acute and chronic salmonella infection. The study focused on the "grey zone" of salmonella ELISA serology, specifically, peak titered sera from salmonella bacterin vaccinated and experimentally infected cows and low titered sera from salmonella carriers. The LPS serogroup specificity of the IgG1 and IgG2 response following acute and chronic S. Dublin infection and salmonella bacterin vaccination was evaluated using 5 salmonella serogroup (B, D, E1, C3, and C1) LPS ELISA assays. IgG1 titers of carriers, vaccinated, and acutely infected cows were predominantly O antigen specific. Similarly the IgG2 titers of acutely infected cows were also O antigen specific. In contrast, salmonella carriers produced an IgG2 response to each of the heterologous LPS antigens (B, E1, C3, and C1) examined. The results of this study indicate that the bovine IgG1 isotype response to salmonella LPS is serogroup specific. Conversely, production of IgG2 antibodies to core salmonella LPS antigens shared across salmonella serogroups is a feature of chronic salmonella infections. ***IMPACT***: Identification of cattle chronically infected with salmonella using culture techniques may be compromised by intermittent fecal shedding. Serological identification of salmonella carriers has historically been achieved by demonstration of a persistent serologic response to salmonella antigens. In the current study we were able to demonstrate that the specificity of salmonella serology can be enhanced by specifically measuring the IgG2 response to salmonella antigens. Salmonella carriers were also demonstrated to produce an antibody response to core salmonella LPS antigens allowing them to be distinguished from acutely infected animals.

Reproductive Performance in Domestic Ruminants (B.P. Smith)

Four hundred and fifty cows were enrolled in a clinical trial of 2 salmonella vaccines, an autogenous salmonella bacterin and a commercial porcine Salmonella cholerasuis modified live vaccine. Fecal samples were collected from cows for 10 days following parturition and from calves that received colostrum from vaccinated cows for the first 10 days of life. Ninety eight percent of the cows sampled shed salmonella in feces and 86.5 % of the calves. No significant difference was observed between groups in the proportion of cows or calves shedding salmonella in feces. However, the modified live salmonella vaccine was observed to reduce salmonella fecal shedding by infected cows and calves by approximately 10%. The reduced salmonella fecal shedding of the modified live salmonella vaccine group was statistically significant but appeared to be of limited clinical significance considering the high prevalence of salmonella fecal shedding observed in the population studied. No difference was observed between groups in regard to milk production, culling, or mortality. The high prevalence of salmonella shedding observed in this study indicates the need for the development of better tools to prevent salmonella infections in cattle. ***IMPACT***: In this study the efficacy of 2 salmonella vaccines was evaluated. No effect was observed following salmonella bacterin vaccination. Salmonella bacterins are the most commonly used salmonella vaccines on the market. Vaccination of cattle with a commercial modified live salmonella cholerasuis vaccine (licensed for poultry) reduced shedding of group C1 salmonella by 25 % but had no effect on the shedding of salmonella from other serogroups. The high prevalence of salmonella shedding by dairy cows on this commercial dairy and the limited efficacy of the vaccines used indicates the ongoing need to develop better prophylactic strategies to reduce the prevalence of salmonella in dairy cattle.

Control of Salmonellosis in Cattle (B.P. Smith)

The objective of this study is to develop and evaluate strategies to prevent salmonella in dairy cattle. Four hundred and fifty cows were enrolled in a clinical trial of 2 salmonella vaccines, an autogenous salmonella bacterin and a commercial porcine Salmonella cholerasuis modified live vaccine. Fecal samples were collected from cows for 10 days following parturition and from calves that received colostrum from vaccinated cows for the first 10 days of life. Ninety eight percent of the cows sampled shed salmonella in feces and 86.5 % of the calves. No significant difference was observed between groups in the proportion of cows or calves shedding salmonella in feces. However, the modified live salmonella vaccine was observed to reduce salmonella fecal shedding by infected cows and calves by approximately 10%. The reduced salmonella fecal shedding of the modified live salmonella vaccine group was statistically significant but appeared to be of limited clinical significance considering the high prevalence of salmonella fecal shedding observed in the population studied. No difference was observed between groups in regard to milk production, culling, or mortality. The high prevalence of salmonella shedding observed in this study indicates the need for the development of better tools to prevent salmonella infections in cattle. ***IMPACT***: In this study the efficacy of 2 salmonella vaccines was evaluated. No effect was observed following salmonella bacterin vaccination. salmonella bacterins are the most commonly used salmonella vaccines on the market. Vaccination of cattle with a commercial modified live salmonella cholerasuis vaccine (licensed for poultry) reduced shedding of group C1 salmonella by 25 % but had no effect on the shedding of salmonella from other serogroups. The high prevalence of salmonella shedding by dairy cows on this commercial dairy and the limited efficacy of the vaccines used indicates the ongoing need to develop better prophylactic strategies to reduce the prevalence of salmonella in dairy cattle.

Dairy Food Safety Laboratory (J.S. Cullor)

In collaboration with Crocker Nuclear laboratories the DFSL is continuing to study the effects of radio frequency treatment of dairy waste water on the reduction of coliform bacteria numbers. To date, raw dairy flush water samples have been treated with up to 6 different RF frequencies, and plated for coliform bacteria counts. In all frequencies tested, coliform bacteria counts have been reduced by 99.9% compared to untreated controls. Additional experiments are being performed with dairy manure water which is spiked" with Salmonella and E. coli 0157:H7. The potential impact of these studies is to provide the dairy industry with alternative waste water treatment strategies for pathogen reduction, thus addressing environmental stewardship concerns. Other on going projects include the cold sterilization of milk by laser light, and the quantization of Mycobacteria. To date, inoculums of Mycobacteria and methods of recovery have been established for use in the spiking of broth cultures and milk for treatment to pulsed excimer laser light. ***IMPACT***: These projects serve to answer public concerns of the efficacy of pasteurization and the potential transmission of the Johne's disease agent to humans.

Mastitis and Its Effect on Food Safety and Milk Quality (J.S. Cullor)

To date bovine milk has been spiked with up to seven relevant foodborne pathogenic bacteria and exposed to varying doses of excimer laser light. The results have identified doses capable of completely sterilizing the milk samples. Additional experiments are being designed to assess potential sensory changes of milk exposed to specific doses of laser light. ***IMPACT***: The results of these experiments may help in identifying potential on-farm pasteurization processes aiding in the harvesting of pathogen free milk.

Cloning of Antigenic Genes Expressing Immunogens from Leptospira Interrogans Serovar Hardjo (R.B. LeFebvre)

This project continues to attempt to identify antigens and their respective genes that have potential for providing prophylactic protection against leptospiral infection in dairy cattle. Three genes have recently been identified that are now being characterized as to their sequence and the antigens they express. Should any of these be expressed on the surface of the organisms and expressed in that animal host we will then initiate vaccine trial studies in rodents. ***IMPACT***: An effective vaccine for bovine leptospirosis would be a major breakthrough in preventing bovine morbidity, mortality, and abortion. The whole-cell bacterin now used has been shown to be less than optimal for preventing leptospiral infection particularly for serovar hardjo.

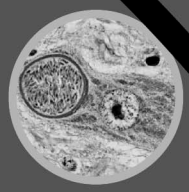
Efficacy of Calf Vocalization Playback as a Method for Inducing Milk Letdown in Dairy Cattle (B. McCowan)

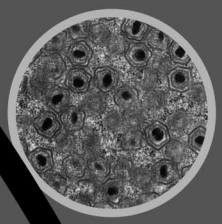
The purpose of this study was to examine the effects of exposing dairy cows to calf vocalizations on milk production. Results showed that cows significantly increased overall daily milk production across the entire herd in response to calf vocalizations during a single milking by approximately 1-2% at two different dairies, which administered bovine somatotropin hormone (bST) to their cows. Thus milk production was increased beyond that of the effects of bST administration. Therefore, the playback of calf vocalizations has the potential to increase daily milk production by at least 2-3% if broadcast during each of the two or three milkings per day. From an economic perspective, even a small 2-3% increase in daily milk production could increase gross profits from $96,798 to $241,995 per year at larger dairies. Actualized net profit would depend on herd size and the fluctuating price of milk at bST and non-bST dairies. However if the playback of calf vocalizations enhances milk production in non-bST dairies equal to or beyond that observed at bST dairies then a new nonchemical and highly economical technique will be available to non-bST dairies for increasing animal productivity.

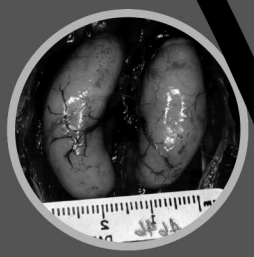
Evaluation of the Pathogenic Potential of Deer Adenovirus Infection in Calves. (L. Woods)

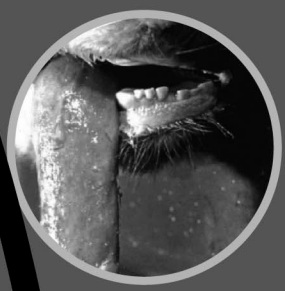
Cows from a local dairy were tested to see if they had antibodies to adenovirus. Greater than fifty percent of the samples tested (N=29) had antibody to adenovirus demonstrating that adenovirus is present locally and half of the animals tested had been exposed to the virus. This also suggested that about 50% of the calves born in this dairy that received colostrum would be free of antibody that would be protective against the deer adenovirus and therefore could be susceptible to infection with the deer adenovirus. The first three newborn calves were tested for antibody and found to have high antibody titers to adenovirus showing that the antibody to adenovirus is passed to the calf through the colostrum. Commercial colostrum was tested for antibody to adenovirus and found to have high levels of antibody showing that the commercial colostrum is protective against adenovirus. Preliminary work suggested we should use colostrum-deprived calves for our inoculation study. To determine if the adenovirus that we will use in the calves is the same as we see in deer in the wild (deadly to deer), we inoculated two deer three times at three week intervals. No clinical signs developed. These two deer did not have any antibody to adenovirus before inoculation, but had very high antibody after three inoculations with adenovirus. This may suggest that the virus may change in cell culture so that it is no longer deadly to deer or these two deer were more resistant to the virus. Since proving the virus used to inoculate the calves is the same virus they would be exposed to in the field when deer and cattle mix (i.e. deadly to deer), and availability of fawns in California is seasonal, the study will be resumed in the summer with orphaned fawns provided by the California Department of Fish and Game. Calves will be inoculated with the same inoculum that produces disease in deer.

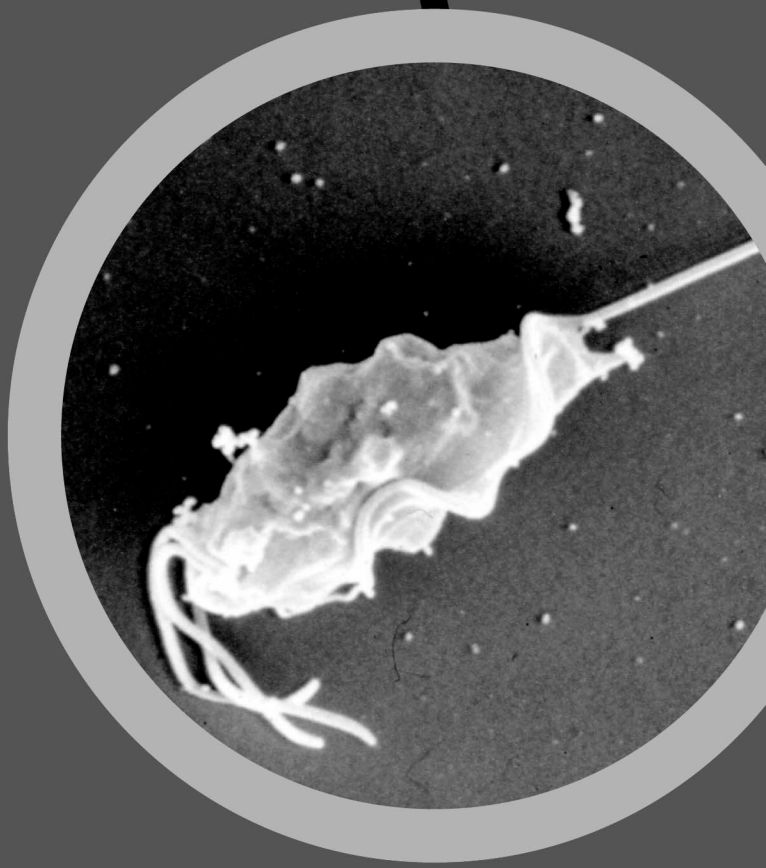
Beef Research











Beef Research Highlights

BEEF RESEARCH HIGHLIGHTS

Beef related research projects account for the second largest area of CFAH research support. Highlights of the beef research programs which focus on issue of high priority to the California cattle industry include:

- **Epizootic Bovine Abortion (EBA)**, characterized by late-term abortion, still birth or birth of weak calves, cause significant economic losses in California. CFAH has established an infectious tissue repository to serve as a predictable EBA challenge and for use in research designed to characterize, propagate (in vitro and animal model) and/or purify the EBA agent.

- **Bovine Trichomoniasis** is a venereal disease which disrupts pregnancy. It is of enormous economic significance in cattle. CFAH researchers are collaborating with local producers and veterinarians to obtain a collection of samples of both "virgin bull" isolates and true T. foetus.

- **Bartonella** are new emerging pathogens that can cause major diseases not only in humans but also in other species. CFAH researchers are studying Bartonella infection as a possible source of reproductive disorders in cattle.

- **Bluetongue Virus (BTV)**, an insect transmitted disease of domestic and wild ruminants, is endemic in California. As a consequence, sectors of the state's thriving livestock industries have been largely excluded from lucrative international trade. CFAH research is aimed at providing science-based criteria for defining standards for international trade.

- **Bovine Respiratory Synctitial Virus (BRSV)** infection causes an acute lower respiratory tract disease in the calf that in severe cases resembles human asthma and can be fatal. CFAH studies focus on the development of therapeutic or prophylactic strategies to ultimately decrease the detrimental effect of bovine respiratory disease on livestock productivity.

- **Cryptosporidium parvum** is a protozoal parasite, capable of waterborne transmission from livestock to humans. The goal is to minimize waterborne transmission of microbial pathogens from animal agriculture to human communities. Practical guidelines are being developed by CFAH researchers which should result in cost-effective management strategies for reducing the risk of microbial contamination of water from animal agriculture.

- **Infectious Bovine Keratoconjunctivitis (IBK) (Pinkeye)** is a serious ophthalmologic disease affecting the cattle industry in California. Recent CFAH research is uncovering novel ways to improve vaccines against this costly disease.

- **Vaccine Studies** brings together CFAH experts working on the molecular biology of human and animal tropical disease agents. Vaccines having both national and international applications have been developed for Rinderpest, Peste des Petits Ruminants, and Food and Mouth Disease. This area of research also includes the development of kits for rapid diagnostic tests.

Population Genetics of Ornithodoros coriaceus (Acari: Argasidae), Vector of the Etiologic Agent of EBA. (W. Boyce)

During the past year over 1,000 Ornithodoros coriaceus ticks were successfully collected from 18 of 43 attempted collection sites located throughout California and southernmost Oregon. So far, three to five ticks from eight of these collection sites have been genetically tested to provide preliminary information which suggests that there are substantial genetic differences among ticks collected from different geographic areas, and that there is relatively low gene flow between populations. This preliminary data was incorporated into an USDA grant proposal submitted this January 2001. We will continue to collect ticks, increasing our range to eastern Nevada and southern Idaho, as well as including sites northward into Oregon. A total of 10 ticks from each successful collection site will be genetically tested to provide significant data. ***IMPACT***: Epizootic bovine abortion (EBA) has a major impact on beef calf production in the western United States. In California, there is an estimated annual loss of 5-10% of the potential calf population due to EBA. The soft tick Ornithodoros coriaceus has been identified as the primary vector of EBA. By studying the population genetics of this tick, we will be able to directly answer whether tick dispersal is a likely mechanism for pathogen dispersal. If not, then the spread of EBA is most likely caused by the movement of infected domestic or wild vertebrate hosts, which has direct relevance to beef cattle management.

Vectors and Vector-borne Diseases of Food Animals (W.B. Boyce, P.R. Crosbie and M.B. Teglas)

Epizootic bovine abortion (EBA) has a major impact on beef calf production in the western United States. In California, there is an estimated annual loss of 5-10% of the potential calf population due to EBA. The soft tick Ornithodoros coriaceus has been identified as the primary vector of EBA. Remarkably little is known about the ecological factors that regulate the distribution and abundance of this tick. This study will identify environmental variables, which will predict the occurrence of this tick, and by extension, the risk of EBA. This information, in turn, will aid farmers and extension agents in developing effective management strategies. In this study, tick collections were attempted at 43 locations throughout California and southernmost Oregon. Over 1,000 Ornithodoros coriaceus ticks (both adult and nymph stages) were collected from 18 of these sites (15 in California, 3 in Oregon). Information on elevation, vegetation type, and whether or not cattle or deer utilized the sites were all recorded. We will continue to collect data, increasing our range to include eastern Nevada and southern Idaho, as well as pushing more northward into Oregon.

Epizootic Bovine Abortion: Geographic Distribution of the Tick Vector and Causative Agent (J. Stott)

While EBA is documented to represent a substantial detriment to the beef industry in California, its role in production-loss in Nevada has only recently been documented. We suspect the infection and disease is much more widespread than the literature suggests and should be recognized as a major deterrent to cattle production in multiple Western U.S. states and Mexico. Furthermore, the ecology of EBA, as regards the tick vector, Ornithodoros coriaceus, is poorly defined. The primary objectives of this project are to better define the geographic distribution of O. coriaceus, develop molecular techniques for identification of the EBA agent in the tick and determine which developmental stage of the tick can harbor the agent and if the agent can be transmitted from an infected female to her eggs. Initial studies directed at furthering our knowledge of vector distribution have resulted in identification of the tick in Nevada and Oregon. Successful development of an EBA-specific PCR has demonstrated the etiologic agent to be present in these tick populations. Preliminary studies directed at better defining the association of

the EBA agent with the tick vector have suggested that the agent is not transmitted from the female to the egg. ***IMPACT***: This research has demonstrated that the tick vector of EBA is present in both Nevada and Oregon and that the etiologic agent of EBA is present. Thus, EBA should be considered a potential cause of reproductive loss in both Oregon and Nevada cattle.

Epizootic Bovine Abortion: Pathogenesis and Establishment of a Tissue Repository (J. Stott)

Epizootic bovine abortion (EBA), often called foothill abortion, has classically been characterized by late-term abortion, still birth or birth of weak calves in susceptible cattle that have been exposed to the coastal range of California and Sierra foothill regions of California and Nevada. Losses typically occur in heifers during their first summer-time exposure to the foothill ranges; animals that have been raised in enzootic areas typically do not experience EBA. EBA is diagnosed pathologically as the etiologic agent has not been identified. The primary, if not only, vector of EBA is the argasid tick, Ornithodoros coriacues. The primary objective of this project is to maintain a collection of EBA-positive fetal tissue and viable tick vectors that can be utilized in multiple projects directed at defining the ecology and causative agent of EBA. A second goal of this project is to determine if transmission of the EBA agent to susceptible heifers at the time of breeding can either interfere with conception and/or cause early embryonic mortality. Pregnant susceptible heifers were inoculated with a variety of tissue homogenates (thymus) derived from fetuses pathologically diagnosed as being EBA. Additional heifers were exposed to the bite of the tick vector. Two tissue pools (cryopreserved in individual aliquots) were identified that contained infectious agent as determined by their ability to cause abortion following inoculation of susceptible heifers; these can be used in future challenge studies and/or efforts directed toward cultivating the agent. One of the three tick-fed heifers aborted. Tissues from all EBA-positive fetuses were both cryopreserved and formalin-fixed for use in future studies. Efforts directed at determining if infection of heifers at the time of breeding could cause reproductive failure were initiated. Preliminary studies (n=20) indicated that heifers inoculated with the EBA agent just prior to breeding had the same reproductive performance as the controls. ***IMPACT***: This project continues to provide invaluable tissues from EBA-infected bovine fetuses and live populations of the EBA tick vector, O coriaceus, for multiple projects directed at defining the ecology, pathogenesis and causative agent of EBA.

Epizootic Bovine Abortion: Identification and Characterization of the Etiologic Agent (J. Stott)

Epizootic bovine abortion (EBA), commonly referred to as foothill abortion, is a major deterrent to beef production in California and Nevada. Although the unusual clinical presentation and unique fetal pathology associated with EBA has been recognized for at least 80 years, the identity of the agent responsible is unknown. A number of detailed studies have isolated diverse viral and bacterial agents from the tissues of affected fetuses and the tick vector, Ornithodoros coriaceus, but all have ultimately failed to be the causative agent of EBA. The primary objective of this project is to define the etiologic agent of EBA through pursuit of both classical and molecular biology techniques. Demonstration of treatment of susceptible pregnant heifers with antibiotics at the time of EBA exposure suggested the pathogen was prokaryotic. While all attempts to cultivate the agent in synthetic media and cell-culture systems has failed, the application of suppression-hybridization polymerase chain reaction (shPCR) was successfully employed to amplify a fragment of the 16S rDNA gene of a novel bacterium present in fetal thymic tissues collected from clinical EBA cases. An EBA-specific PCR was subsequently established and the specificity and sensitivity is currently being defined by application to tissues derived from a variety of aborted fetuses (EBA and non-EBA) and the tick vector. ***IMPACT***: This project has successfully

identified the etiologic agent of EBA and developed the first diagnostic probe for the agent in necropsy tissue and the tick vector.

Immunopathogenesis of Bovine Trichomoniasis (R.H. BonDurant)

This work centers on assessing the in vitro cyctotoxicity of T. foetus for the presumed target cells of this abortion/infertility disease, namely the cells of the early conceptus and the cells of the maternal endometrium. Our previous studies showed that target cells were more vulnerable to lysis when coated with T. foetus shed antigen and specific antibodies. This increased vulnerability was true whether the effector agent was serum complement or the T. foetus itself. We are now repeating these experiments using bovine trophoblast or endometrial epithelial cell cultures as targets. ***IMPACT***: If specific antibody, particularly IgG1, enhances the cycotoxicity of T. foetus for target cells, it is possible that vaccines should be directed away from IgG responses and toward IgA responses.

Reproductive Performance in Domestic Ruminants (R.H. BonDurant)

The objective was to determine whether the venereal bovine pathogen, Tritrichomonas foetus, specifically binds the Fc portion of bovine IgG. The researchers have been unable to identify specific receptors do date, although the trichomonad becomes more cytotoxic to cultured bovine cells in the presence of shed parasite antigen and bovine antibodies to that antigen. In situ studies showed that this shed antigen (so-called TF 1.15 antigen) adheres to the apical surface of the endometrium following experimental infection of heifers. So our hypothesis has been that specific antibody binds to shed antigen, which in turn adheres to bovine target cells. Only a little progress has been attained in elucidating the mechanism of binding. Reagents are only now becoming available that will allow us to directly probe the surface of T. foetus for Fc receptors or molecules that mimic Fc receptors. We plan to continue the search with these new reagents, using funding on-hand. ***IMPACT***: If we can show that the parasite does in fact bind the Fc portion of specific IgG, then we will have elucidated a new pathological mechanism and an immune-evasion mechanism as well. A practical consideration will then be that any vaccine for trichomoniasis should probably NOT be directed towards production of a host IgG response, at least not to the TF 1.15 antigen.

Diagnostic and Immunopathologic Studies of T. Foetus (R. H. BonDurant)

This study characterizes trichomonad isolates from western states, including Montana, South Dakota Wyoming, Nebraska, Colorado, Oregon, and California. We also began a formal solicitation of diagnostic laboratories (Western States Livestock Health Association), in order to receive a significant number of trichomonad isolates from the Western region. To date, we have on-hand more than 30 different isolates of trichomonads (taken from the preputial cavity of intact bulls) that were called Tritrichomonas foetus following the initial culture, but that reacted in a PCR assay as a non T. foetus trichomonad. An additional 30+ isolates reacted as true T. foetus. Two of the non T. foetus trichomonads were from mature breeding bulls, while the remainder were from apparently virgin bulls. In collaboration with the California Animal Health and Food Safety Laboratory, we have discovered through DNA sequencing of the PCR amplicon (which represents genomic DNA surrounding the 5.8s rRNA gene) that the DNA sequence of this segment in all true T. foetus does not differ by >1 base pair over a fragment of >370 bps, whereas the bp pattern of the non-specific trichomonads tend to fall into three general categories. One category seems to be Pentatrichomonas hominis. As a diagnostic, agreement between PCR assessment (using two sets of primers - one common to all trichomonads tested to date and one specific for T. foetus) and an immunoflouroscent assay (using a monoclonal antibody to a conserved surface antigen of T. foetus) has been >98%. One non-T foetus isolate, from a virgin bull, was unable to establish

infection following instillation of 1,000,000 motile cells into the vaginae of 5 estrus-synchronized virgin heifers, i.e., no trichomonads could be cultured from the anterior vagina of any heifers in spite of weekly sampling for 5 weeks. Positive control heifers (infected with a like number of true T. foetus), all yielded positive vaginal cultures weekly through week 5. We conclude that false positive trichomoniasis diagnoses probably occur, that the PCR and/or IFA tests consistently distinguishes between true T. foetus infection and contamination with non-specific trichomonads, and that at least the one non-specific isolate tested is not a pathogen. ***IMPACT***: It is clear that the specificity of the gold standard diagnostic test for bovine trichomoniasis (i.e., preputial culture) is <100%. Since there is no legal treatment for the disease in bulls, nor is vaccination shown to be efficacious, infected bulls are sent to slaughter. It follows that some bulls have been slaughtered when in fact they probably did NOT harbor a venereal pathogen, but rather one or more of the non-specific trichomonads described above. The PCR and IFA tests, with appropriate validation, could provide a second stage confirmatory test to offer for those bulls whose smegma culture is culture-positive.

A New Potentially Zoonotic Tick-borne Infection: Bartonella Infection in Domestic Livestock (B.B. Chomel, R. Atwill and E.R. Sischo)

This research project has identified the presence of Bartonella DNA in several questing adult Ixodes pacificus, including several Bartonella pathogenic for humans and 5 ticks were harboring a strain with a sequence identical to our cattle strain. Furthermore, the researchers have been able to increase the number of cattle and elk herds tested in California. We have been able to bring more preliminary data to support our hypothesis in establishing age prevalence of bacteremia in large French herd. We also did major molecular work to better identify the characteristics of the various domestic and wild ruminants Bartonella isolates. We also have been able to test cows in two more herds, one beef cattle herd from the California coastal range and one dairy cattle herd from southern California, where heifers are sent to the Sierra foothills prior to returning to the dairy farm. A collaborative project with our French colleagues led us to also isolate Bartonella in French cattle herds. We investigated Bartonella bacteremia prevalence in a whole dairy herd and also investigated possible vertical transmission of the agent. ***IMPACT***: Our work has clearly demonstrated the reproductive disorders generated by Bartonella infection in a mouse model, characterized by smaller litters, fetal resorption and bacteremia in live fetuses obtained by caesarean section from bacteremic mice. Similar observations have also been reported in experimentally infected cats. We were not able to demonstrate vertical transmission of the infection in calves born by cesarean section from bacteremic cows, therefore such reproductive disorders could be expected in other species, including cattle. Our leadership in Bartonella research has also led us to investigate infection not only in mammals, but also anthropod vectors. This project is quite original in that it investigates to determine a possible pathogenic role for a new bacterium, only recently identified by our group. It is difficult at this point to quantify the impact of this infection on animal health, however, as a non- negligible part of reproductive disorders in cattle still have an unknown etiology, it is worthwhile to further investigate Bartonella.

A Quantitative Risk Analysis on the Introduction and Transmission of BTV Through Import or Export (T. E. Carpenter)

Bluetongue is 1 of only 16 diseases included in List A by the Office International des Epizooties. The major adverse economic impact of BTV infection in many regions of the world is on international trade and movement of ruminant livestock and germplasm, and not from losses associated with bluetongue disease of ruminants. Central to the development of more rational trade policies pertaining to BTV infection is determination of the risk posed by ruminants previously exposed to the virus. Precise determination of the maximal duration of infectious viremia is

essential to the development of an appropriate quarantine period prior to movement of animals from BTV-infected to BTV-free regions. The objective of this study was to predict the duration of viremia in BTV-infected cattle using a probabilistic modeling analysis of existing data. These results could then be used to facilitate the design of more rational trade policies pertaining to BTV. Data on the duration of viremia in cattle were obtained from previously published studies. Datasets were created from a large field study of naturally infected cattle in Australia as well as from experimental infections of cattle with Australian and U.S. serotypes of BTV. Probability distributions were fitted to the pooled empirical data, and the three probability distributions that provided the best fit to the data were the Gamma, Weibull and Lognormal probability distributions. These asymmetric probability distributions are often well-suited for decay processes, such as the time to termination of viremia. ***IMPACT***: The analyses indicated a greater than ninety-nine percent probability of BTV viremia ceasing in adult cattle within nine weeks after infection, and slightly longer in BTV-infected, colostrum-deprived, newborn calves.

Risk Analysis on the Movement of Animal Pathogens Through the Import/Export of Infected Animals (T.E. Carpenter)

During the past year this study has focused on two areas, viremia and importation. The results obtained are as follows: Bluetongue is 1 of only 16 diseases included in List A by the Office International des Epizooties. The major adverse economic impact of BTV infection in many regions of the world is on international trade and movement of ruminant livestock and germplasm, and not disease associated with bluetongue disease of ruminants. Central to the development of more rational trade policies pertaining to BTV infection is determination of the risk posed by ruminants previously exposed to the virus. Precise determination of the maximal duration of infectious viremia is essential to the development of an appropriate quarantine period prior to movement of animals from BTV-infected to BTV-free regions. The objective of this portion of the overall NRI project was to predict the duration of viremia in BTV-infected cattle using a probabilistic modeling analysis of existing data. This information could then be used to make more accurate decisions about trade policies relative to BTV. Data on the duration of viremia in cattle were gathered from the literature. Datasets were created that consisted of natural infection data from a large field study in Australia, experimental inoculation data from studies performed in Australia with Asian serotypes of BTV, and experimental inoculation data from studies performed in the United States with U.S. serotypes of BTV. Probability density functions were fitted to the pooled empirical data, and the three probability distributions that provided the best fit to the data were the Gamma, Weibull and Lognormal probability distributions. These asymmetric probability density functions are often well-suited for decay processes, such as the time to cessation of viremia. In most cases, we estimated a greater than 99% probability of BTV viremia ceasing in adult cattle within 9 weeks. Information on the number of live ruminants imported to the United States between 1989 and 1998 was obtained from the FATUS (Foreign Agricultural Trade in United States) reports. Complementary data on imports by country was obtained from the USDA database. This data is currently being sorted and analyzed to determine the number of animals shipped per country, number of shipments per country and number of animals per shipment. Preliminary results show that from 1989 to 1998, approximately 11,000,000 bovine; 210,000 ovine and 90,000 other live animals were imported into US. and from 1993 to the present, there has been 14,572 ruminant shipments. Bovine performed approximately 87% of the shipments, followed by ovine, 5%, caprine, 3%, and the rest distributed, in decreasing order, among bison, elk, deer, llama and other camelidea. Approximately, 99% of the shipments came from Canada (87%) and Mexico (12%). From 1994, there appear to be a general increase in ruminant imports to U.S. There were approximately 2,100 shipments in 1994 compared to 2,500 shipments in the subsequent years up to 1998. This increase in shipments could be related with the NAFTA agreement signed in 1994.

IMPACT: The research indicated a greater than ninety nine percent probability of detectable BTV viremia ceasing after nine weeks or less of infection in adult cattle and after a slightly longer interval of BTV-infected, colostrum deprived newborn calves.

Host-virus Interactions in BTV-Infected Cattle (N.J. MacLachlan)

The goal of this project is to better define the pathogenesis of bluetongue virus (BTV) infection of ruminants. During the review period we have completed in vitro studies to define the interaction of BTV with primary cultures of ovine and bovine pulmonary artery and lung microvascular endothelial cells (ECs). These studies established that the kinetics and consequences of BTV infection of the various ECs are distinct, and that differences between the response of ovine and bovine microvascular ECs are consistent with the expression of bluetongue disease in sheep but not cattle. We also have continued studies that confirm that duration of infectious viremia is finite in BTV-infected ruminants and, lastly, we have confirmed the regions of BTV that control virus neutralization. ***IMPACT***: The fact that the duration of viremia is finite in BTV-infected ruminants has considerable relevance to international trade Codes pertaining to bluetongue, the only OIE List A disease that is endemic in the US. Our studies with primary ECs indicate that these cells provide a very convenient and relevant system for the characterization of the pathogenesis of bluetongue disease. Our molecular characterization of the neutralizing determinants of BTV is central to efforts to develop improved vaccines to prevent BTV infection and bluetongue disease.

Bluetongue Virus Infection of Ruminants in California: Pathogenesis and Epizootiology (N.J. MacLachlan)

The goal of this project is to define the pathogenesis of bluetongue virus (BTV) infection of cattle, and to characterize the evolution of BTV. In collaboration with Dr. Brad Mullens at UC Riverside we have investigated the interaction of BTV with its insect vector (Culicoides sonorensis) in the Chino Basin area. Molecular genetic analyses of individual BTV gene segments indicate that different virus strains co-circulate on specific farms, and we now are comparing these strains to those associated with outbreaks of bluetongue disease in ruminants elsewhere in California. We also have shown that the strains of BTV that circulate in California are distinct from those in Asia. ***IMPACT***: Bluetongue remains the only OIE List A disease that is endemic in the US. Accurate determination of the epidemiology of BTV infection in endemic areas such as California is prerequisite to rationalization of current international non-tariff trade barriers pertaining to bluetongue, and to development of improved diagnostic and vaccine technologies for BTV.

Bluetongue Virus Infection of Cattle: Maximal Duration of Infectious Viremia (N.J. MacLachlan)

The objective of this project is to confirm that duration of viremia is finite in bluetongue virus (BTV)-infected ruminants. We have used conventional virus isolation, PCR assay, and the feeding of vector Culicoides sonorensis to precisely determine the duration of viremia in cattle and sheep infected with BTV by the bite of infected insects. We also are evaluating the genetic diversity that is generated in individual BTV gene segments during sequential passage of the virus through its insect and ruminant hosts. These studies now are largely complete and data analysis is well underway. ***IMPACT***: Precise determination of the maximal duration of viremia in BTV-infected ruminants is central to the revised OIE Sanitary Code that is currently pending at OIE. Bluetongue remains the only OIE List A disease that is endemic in the US, and this data is central to efforts to rationalize existing non tariff trade barriers that have been imposed on ruminants and germplasm from the US.

Development and Application of Improved Diagnosis of Bluetongue Virus (B.I. Osburn)

The research on this project during the last year includes the development and application of a multiplex polymerase chain reaction test for the simultaneous detection and differentiation of North American serotypes of bluetongue epizootic hemorrhagic disease viruses. The reason for this assay is that 4 serotypes of bluetongue virus and 2 of epizootic hemorrhagic disease viruses are endemic in California and most of the temperate areas of the U.S. This assay was developed to better determine which of these viruses, or whether both genus and species of the viruses, occur in animals at one time. This is of interest since the insect vector that is associated with the transmission of these viruses may carry both viruses at any one time. If that is the case, it is important to understand if these viruses occur simultaneously in animals and if so do they accentuate the clinical signs of disease. Current methods of detecting the viruses require culturing either in embroynated eggs for bluetongue or in cell culture for epizootic hemorrhage disease viruses. ***IMPACT***: A simplified test that can detect both serotypes of viruses will greatly aid in determining the eventual epidemiology and biology of these viruses in nature. The immediate impact is to develop an improved diagnostic test that will more efficiently and effectively diagnose these viruses in diagnostic samples and it will aid in gaining a better understanding of the epidemiology of these viruses in nature. Rapid diagnostic tests will reduce the time of diagnosing viral infections from 10 days to less than one day. Costs of test will also be reduced.

Bovine Respiratory Diseases: Risk Factors, Pathogens, Diagnosis and Management (L. J. Gershwin)

In collaboration with the Texas station samples of serum and blood cells from calves have been obtained and exposed to dust aerosol prior to shipping from Tennessee to Texas. RNA samples have been prepared from peripheral blood leukocytes and used in RT-PCR to detect IL-2, IL-4, Interferon gamma, and IL-12 message. Serum samples are being tested for IgE antibodies to Aspergillus, a mold we found to be the principle component of the dust. We are in the process of evaluating these samples to see if dust exposure increases a T helper 2 cytokine profile and IgE production against the inhaled particles. ***IMPACT***: Bovine respiratory disease (BRD) is important in feedlot cattle. It is a major source of economic loss to the producer. Our studies focus on the role of the environment on development of allergic sensitization to allergens and the role of hypersensitivity in BRD. Better understanding of the mechanisms of pathogenesis will allow us to develop means to control the disease.

Influence of RSV Infection on Immune Responses to Inhaled Antigen in Bovine Lung (L.J. Gershwin, R.A. Gunther, E.S. Schelegle)

The effect of BRSV infection on inhalation of antigens was studied using Technitium-labeled DTPA and Technitium-labeled ovalbumin (OA). Calves were exposed to aerosolized Tc-DTPA prior to and during infection with BRSV. Clearance of the label was monitored using a gamma camera. With DTPA, a small molecule, the clearance was accelerated during the infection, while with OA, a larger molecule, infection delayed clearance. We studied the uptake of the label in plasma and lymph and demonstrated that it followed the lung clearance. Clearance of labeled OA was most affected during the time of maximal clinical signs of disease. ***IMPACT***: This information is useful in assisting us in defining the development of allergic reactivity to inhaled antigens and the influence of BRSV infection on access of antigent to antigen presenting cells in the lung. Ultimately this information will assist in development of strategies to prevent and reduce bovine respiratory disease.

IGE Responses in Bovine Diseases (L.J. Gershwin)

The major focus has been on examination of IgE responses to vaccines. We tested by ELISA for IgE serum from heifers vaccinated with a combination of commercial vaccines, some of which had shown allergic reactivity. We found that some vaccines (commercial) did elicit IgE responses in some heifers. Components of each vaccine were tested separately to determine which component was inducing the allergic response. In another experiment lung sections from paraffin blocks previously collected from BRSV vaccinated/infected and control calves were analyzed by Taqman RT-PCR for Th1 and Th2 cytokines. Calves that had developed IgE responses to the BRSV proteins by western blot after vaccination and subsequently had developed severe disease after experimental infection showed lower IL-12 and Interferon gamma. We continue to develop reagents and techniques to examine the IgE response to a variety of antigens in this project. ***IMPACT***: Allergic responses to vaccines are extremely problematic. By screening for IgE production we can predict which vaccine formulations are likely to cause development of anaphylaxis in susceptible animals.

Influence of RSV Infection on Immune Responses to Inhaled Antigens in Bovine Lung (L.J. Gershwin, R. Gunther, E. Schelegle and W. Hornof)

The objective of this project is to study the mechanism by which bovine respiratory syncytial virus (BRSV) enhances allergic sensitization to inhaled allergens. The objective reported herein was to study the impact of BRSV infection on clearance of inhaled molecules from the lung This study addresses the hypothesis that one mechanism for increased sensitization is modulation of allergen clearance from the lung. Technetium-diethylenetiraminepentacetate (99mTc-DPTA) was nebulized to calves and clearance was monitored with a gamma camera. This procedure was done before infection and on days 2, 4, 7, 9, 16 and 23 after experimental BRSV infection. In another experiment a larger molecule, ovalbumin (OA), was similarly labeled, administered, and clearance monitored before and on days 7 and 11 post-infection. Clearance of the OA was delayed. Results of 99mTc-DPTA clearance indicated an increase in clearance time on day 4 followed by a marked decrease from day 7 - 9 and 16. Clearance OA was delayed from day 7 through 11, with some variability among calves. Exposure to OA aerosol did not influence clearance on successive days in the absence of virus. Absorption of 99mOA into plasma was also monitored. These studies demonstrate that BRSV infection effects the time that inhaled antigens spend in the lung. The implication of these findings is that BRSV infection may facilitate local immune responses to inhaled allergens thereby increasing likelihood for allergic sensitization. ***IMPACT***: Bovine respiratory disease (BRD) is an important cause of economic loss to the producer. Bovine respiratory syncytial virus is a major component of BRD. Our previous studies show that BRSV stimulates a T helper cell type 2 cytokine profile and IgE production in some calves. This project begins to examine how the allergic sensitization is facilitated. The impact is that knowledge of the mechanism of pathogenesis will enable researchers to develop strategies to prevent this disease.

Immune Response to C-DNA Vaccination for Bovine Respiratory Syncytial Virus (L.J. Gershwin)

The bovine respiratory syncytial virus fusion and nucleoprotein genes have been amplified from the cloned genes and inserted into a plasmid vector. Expression has been documented in cell culture by immunofluorescence and western blotting. Mice have been immunized with the plasmids, separately and together. Currently mice are being followed for development of an immune response to the F and/or the N proteins. ***IMPACT***: A DNA vaccine for BRSV would be likely to induce a strong cellular immune response, not achieved with currently available commercial vaccines.

Assessing Survivability of Bovine-derived C. parvum Oocysts And G. lamblia Cysts on Cow-calf Rangeland (E.R. Atwill)

The research objective is to determine if C. parvum oocysts excreted in the feces of livestock can survive ambient temperatures typical of California rangeland from spring through fall. We acquired profiles of the internal temperatures of bovine fecal patties located on an oak woodland range, approximately 1,400 feet elevation, Madera county, California, from April through September. Fecal pats were located on open range and exposed to solar radiation. Daytime internal fecal pat temperatures exceeded 50 C on a daily basis during much of 7-month monitoring, with temperatures above 70 C not uncommon during the summer months. The difference between the internal fecal patty temperature and air temperature ranged from 10-20 C, such that fecal patties were 40-60% hotter that air from around 11:00 am to 3:00 pm. Using in vitro excystation as a measure of viability and mimicking the 24-hour thermal profiles under experimental conditions, we found that only 2 to 5 days were necessary to inactivate 80 to 90% of oocysts exposed to solar radiation. No significant differences were seen in oocyst inactivation rates for maximum daytime temperatures ranging from 40 to 60 C. ***IMPACT***: The majority of C. parvum oocysts deposited in the fecal material by livestock out on open range appear to be rapidly inactivated by thermal processes during spring through fall in lower elevation regions of California. Beneficial management practices for reducing the risk of waterborne contamination of C. parvum from livestock should focus on management of fresh fecal material, and focus less on aged fecal material.

Epidemiology and Medical Ecology of Waterborne Zoonotic Diseases in Livestock Production Systems (E.R. Atwill)

This project continues to document that the majority of risk of C. parvum contamination from livestock production systems is primarily from the young stock, despite the fact that adult animals produce substantially more fecal material than young stock. This is due to the dramatically reduced shedding intensity of oocysts by adult livestock. Whether these patterns if infection are similar in other geographical regions of the United States is unclear. On-farm risk factors appear to exist for both C. parvum and G. duodenalis infection in farm animals, suggesting that the prevalence of infection can be reduced by livestock owners and animal health professionals if management practices are appropriately modified. Moreover, on-farm beneficial management practices, such as strategic placement of vegetative buffer strips between areas of high fecal deposition and critical source water supplies should further reduce the risk of microbial contamination from livestock production systems, thereby helping our animal agricultural industries move closer to sustainable farming practices despite growing restrictions due to water quality concerns.

Cryptosporidium Parvum And Giardia Lamblia as Potential Causes of Foodborne And Waterborne Disease (D.O. Cliver)

Cryptosporidium parvum and Giardia lamblia often cause foodborne and waterborne disease. We have developed more efficient methods for detecting C. parvum in environmental samples and determining whether the detected agent is probably infectious. We have tested for these agents in droppings from California deer, elk, seals, and sea lions. ***IMPACT***: We have determined that C. parvum survives fermentation and storage in yogurt but is killed by freezing in ice cream mix and by pasteurization in apple juice.

Recombinant Moraxella bovis Cytotoxin for Prevention of Infectious Bovine Keratoconjunctivitis (L. George)

The study identifies the gene and encodes the cytotoxic and hemolytic protein of M. bovis. The cloned protein has been expressed and can be produced in large quantities and purified from whole E. coli with a 4 step procedure. The protein can be used as an inexpensive immunogen and a source for diagnostic reagent, and can be used to compare the toxins from other strains of Moraxella bovis. The project's purpose is to identify, sequence, clone and express the gene encoding the cytotoxin and hemolysin of M.bovis, and to compare the toxins from other strains of Moraxella and Neisseria. ***IMPACT***: We have now successfully isolated the complete M. bovis cytotoxin gene (RTX A gene) and have expressed portions of this gene in E. coli and developed rabbit antisera to these peptides. Rabbit antisera to the carboxy terminus of this protein neutralizes hemolytic and cytolytic activity of native M. bovis cytotoxin.

Enhanced Efficacy and Safety of Recombinant Vaccines (L. Jones, T.D. Yilma, S. Ahmad)

The objective of this project is to increase the safety and effectiveness of live recombinant vaccines. We are using vaccinia virus as a vaccine vector to express the proteins of other viruses that will induce protective immunity to these viruses. Vesicular stomatitis virus causes disease in most mammals so it can be used in mice as a model for a systemic disease and cattle as a model of localized disease. We are also investigating the effects of inactivating several genes in the vaccinia virus itself that are not involved in the growth of the virus but do affect the immune system of animals that are infected with vaccinia virus. We are using naturally occurring proteins that enhance the immune response and the vaccinia virus proteins may interfere with these activities. Development of such vaccines will increase the ability to identify and control livestock diseases.

Development of Safe and Effective Oral Vaccine for Livestock (L. Jones, T. Yilma and S. Ahmad)

The objectives of this project are the development of safe and effective oral recombinant vaccines for livestock. Such vaccines will be simpler and less expensive to use and should greatly benefit agriculture and the economy in the U.S. Although there are many effective livestock vaccines, they are generally administered by injection that is more labor intensive and expensive. Also, since many disease causing organisms enter the body via respiratory or gastrointestinal routes, this method of vaccination should induce a higher level and more specific immunity which will be more effective in controlling infection. Additionally, these vaccines can be used in baits to vaccinate wildlife, as has been done with the recombinant vaccinia virus vaccine for rabies.

Use of a Baculovirus Expression System for Production of Vaccine and Diagnostic Kit Antigens (T. Yilma)

The major objective of this project is to develop diagnostic kits and vaccines for economically important infectious disease agents of national and foreign livestock. We developed rapid and inexpensive indirect ELISA (iELISA) kits for vesicular stomatitis virus (VSV), rinderpest (RPV) and peste-des-petits ruminants virus (PPRV). Now, we have developed the following diagnostic tests to further improve rapidity and specificity. 1. Development of a strip test for VSV: A raid strip test, that utilized recombinant N protein, was developed for field diagnosis of VSV by reverse phase chromatographic technology. The test is performed within 5-10 minutes in the field. The rapid strip test will be produced in large numbers and validated by using multiple anti-VSV serum samples from the field. We plan to develop similiar strip tests for rapid diagnosis of RP and PPR in the future. 2. Development of a rapid cELISA for RP diagnosis: During the FAO/UN Consultation on the Global Eradication of Rinderpest (RP) in October, 1992 and FAO/IAEA RCM meeting in October 2000, it was clearly indicated that both RP and PPR must be

eradicated simultaneously since cattle get subclinical infections with PPRV and small ruminants with RPV. Each serve as a reservoir host for the other and make eradication very difficult. There is a need for developing a rapid and inexpensive test for differential diagnosis of RPV and PPRV; as the nucleocapsid (N) proteins of the RPV and PPRV cross-react to each other. Now, we have developed a competitive ELISA for RP and PPR diagnosis. We are assessing the cross-reactivity of the RP cELISA with anti-PPRV sera under field conditions in Kenya and Senegal. ***IMPACT***: We have developed inexpensive rapid diagnostic iELISAs that use baculovirus-expressed N protein as coating antigens and can distinguish vaccinated from infected animals for export/import purposes. For VSV diagnosis, the kit is adopted by the USDA National Veterinary Services Laboratory at Iowa for routine laboratory diagnosis of VSV infection in cattle, horses, and pigs. The iELISA and cELISA based on recombinant N protein prove to be rapid, sensitive, and inexpensive resource tests for quick monitoring of serosurveillance and disease surveillance programs under the global RP eradication program.

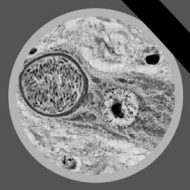
Improving the Immunogenicity of DNA Vaccines for Livestock Diseases (T. Yilma)

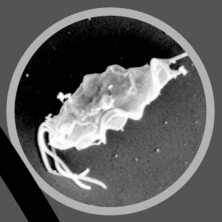
The long-term objective of this project is to increase the safety and efficacy of DNA vaccines. Our inaugural studies, designed to optimize immune responses to DNA vaccines via the co-administration of immune-stimulatory CpG motif oligodeoxynucleotides, indicated that the CpG motifs should be cloned into the backbone of plasmid DNA. Our more recent work has been focused upon the construction of DNA vaccines that express specific cytokines and thus elicit either Th1 or Th2 immune responses. We have incorporated interleukin(IL)-18, cloned through PCR using murine spleen template cDNA, into a DNA vaccine. We have confirmed IL-18 expression in vitro using Western Blotting. Pending further funding, we hope to continue the construction of an IL-6 expressing, a Th2-inducing, DNA vaccine. After which we hope to commence in vivo work. ***IMPACT***: Our laboratory has made significant contributions in the field of vaccine development. We are now broadening our scope and pursuing the field of DNA vaccination. By constructing vaccines that are composed solely of DNA and express immune-stimulating genes, we can induce a tailored yet potent immune response. This type of vaccine is hypothesized to be not only very effective but also very safe, having an endless list of applications.

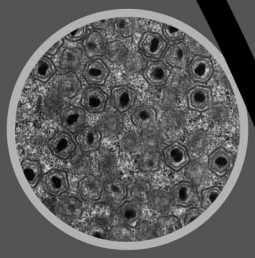
Adjuvant and Attenuating Genes for Live Recombinant Vaccines (T. Yilma)

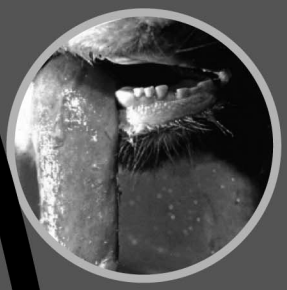
The objective of this project was to develop new methods for producing safer and more effective vaccines through molecular biology techniques. Live recombinant vaccines are produced by inserting isolated genes that elicit protective immunity into an innocuous carrier, such as vaccinia virus. The vaccine used in the global campaign for eradication of smallpox and now widely utilized for research purposes, vaccinia virus has several advantages as a vector since it is a relatively easy virus to "engineer". It can tolerate the insertion of large amounts of foreign DNA, is not associated with any type of cancer, has not been associated with any specific disease, is very stable, replicates rapidly, and is inexpensive to propagate. We are investigating the effects of the deletion of a vaccinia virus gene that binds to interferon-gamma on the safety and efficacy of the virus as a recombinant vaccine. Immunodeficient mice survive longer when injected with vaccinia viruses that have this gene deleted. Thus the gene is important to the virulence of the virus and deletion of this gene increases the safety of the virus as a vaccine. Development of such vaccines will increase the ability to identify and control livestock diseases.

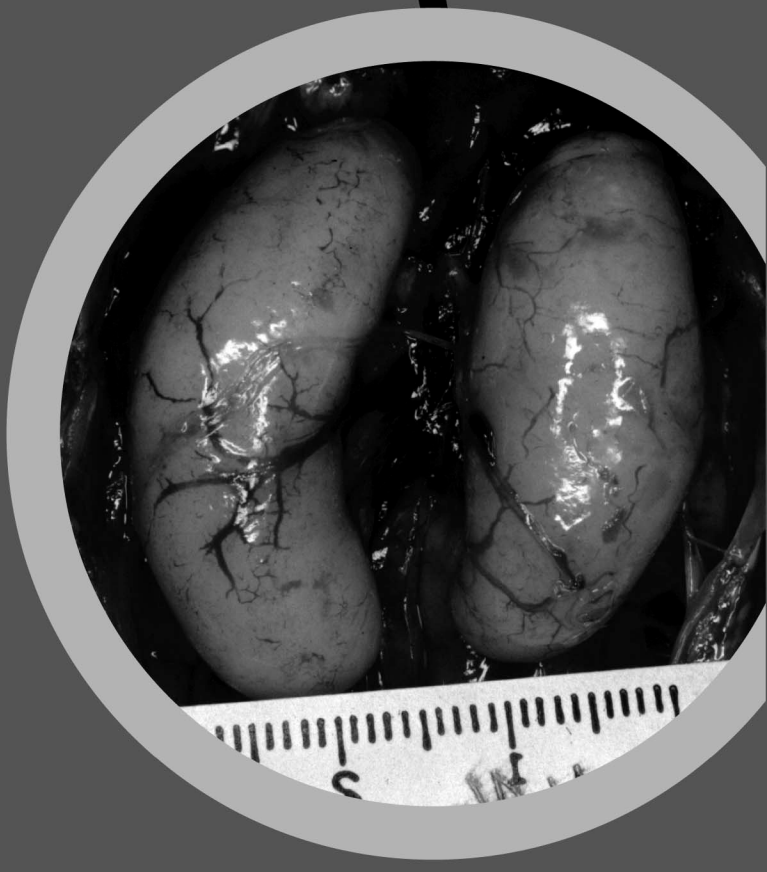
Poultry Research











Poultry Research Highlights

POULTRY RESEARCH HIGHLIGHTS

Poultry researchers in the Center for Food Animal Health are working on a variety of projects addressing priority issues for California producers and industry.

- **Cellulitis**, a poultry disease typified by the presence of subcutaneous lesions and inflammation of the overlying skin, is one of the most economically important diseases affecting broiler chicken production in California. CFAH researchers are using molecular techniques to study the role of Esherichia coli in the virulence and pathogenicity of cellulitis.

- **Food Safety** studies are aimed at identifying the source and incidence of foodborne microbial pathogens such as Salmonella and, Campylobacter. Comprehensive studies take place at various sites on the farm, after transport of birds to the processing plant and during the slaughtering process. These projects provide science-based information necessary to address microbial contamination and food safety issues to be incorporated into a hazard analysis and critical control point program (HACCP).

- **Marek's Disease (MD)** is a ubiquitous virus in the commercial poultry industry worldwide that is primarily controlled through the use of vaccines. Currently, virtually every chicken, laying and broiler, is vaccinated for MD. In recent years, the poultry industry has experienced cycles of vaccine failure followed by identification of increasingly more virulent strains of MD. CFAH research efforts are aimed at identifying and characterizing the genes that are associated with resistance to MD. Incorporating these genes into commercial poultry lines is an increasingly important adjunct to the control of MD by vaccines.

- **Infectious Bronchitis Virus (IBV)** has a worldwide distribution and is considered one of the major health problems of the California poultry industry. This disease causes poor weight gain, reduced feed efficiency, decreased egg production and variations in egg quality. The work of CFAH researchers will contribute significantly to knowledge of the natural history of IBV in the modern commercial broiler flock and will be critical to developing a predictive model for outbreaks and identifying means by which they can be prevented.

- **Lipoprotein Metabolism** and its role in avian reproduction is an ongoing study of CFAH researchers. These studies are designed to better understand the factors that control yolk lipid production and transport from liver to the developing egg yolk. In some cases, genetic and environmental factors may lead to liver disease, production losses and even hen deaths.

Pathogenesis of Cellulitis in Broiler Chickens (J. Jeffrey)

In a series of studies, twelve E. coli isolates were compared for their ability to cause cellulitis lesions in broiler chickens following injection under the skin. We found that some isolates caused mild lesions, while others caused severe disease that spread through the whole body (systemic disease). Isolates also varied in how long it took lesions to form following injection. This indicated that there was variation between isolates for rapidity of lesion formation and for virulence. However, in a second study, when we compared these isolates and others (a total of 50 E. coli) from cellulitis lesions with 50 isolates of E. coli that were isolated from systemic disease in chickens for traits that have been identified as virulence factors for E. coli, we found there was no statistical difference between the two groups. This supports the idea that cellulitis-causing E. coli are the same as other pathogenic E. coli that affect chickens.

Assessment of Immune Responses in Broiler Chickens Following Infection with Cellultis-derived Escherichia Coli Isolates (J. Jeffrey)

We have continued our investigation of the immunologic and pathologic responses to subcutaneous (SQ) E. coli infection in order to elucidate the pathogenesis of cellulitis in broiler chickens. Our previous results had indicated that their was not a relationship between serum antibody response as measured by ELISA, and the resolution of cellulitis lesions following SQ challenge. However, the IgG levels were highly variable within treatment groups. In the current study we use an MHC haplotype defined broiler line in order to reduce individual variability in antibody responses. Twenty birds were divided into 4 treatment groups and received 4 weekly SQ Avaccinations@ with 0, 100, 1000 or 10,000 cfu/ml of an E. coli strain. Chicks were SQ challenged in with the same E. coli with 1000, 10,000 or 100,000 cfu/ml. In the control group, we saw an incremental increase in lesion score as the challenge dose increased. Among the vaccination treated groups, only birds given the 10,000 cfu vaccination treatment showed a reduction in lesion score. This group also had some birds with high anitibody titers, but the antibody levels were not statistically different from the other vaccination treatment groups. As in our previous study we saw no relationship between having high antibody titer and a low lesion score or vice versa. There appears to be a threshold level of prior exposure or vaccination that is required to stimulate a protective response as measured by lesion severity.

Targeting Small Poultry Producers for Health & Management Programs (J. Jeffrey)

A comprehensive survey of farm and management variables and microbiologic testing for Salmonella and Campylobacter was completed on 13 gamebird farms throughout California. Eight pheasant farms, 7 chukar partridge farms and 4 quail farms were visited. Markets for these birds included hunting clubs, live-fowl markets and processing plants (for retail sale). Annual production was highly variable ranging from 60 to 17,500 birds per year. A total of 420 birds were cultured. Three of 13 farms were culture positive for Salmonella and 3/13 different farms were culture positive for Campylobacter. Campylobacter positive cultures were found in 2/140 chukar; 2/120 quail and 0/160 pheasant. Salmonella positive cultures were found in 3/160 chukar, 0/120 quail and 22/140 pheasant. Twenty of the Salmonella positive cultures came from pheasant poults on 1 farm. Statistical analysis of risk factors was not possible due to the sparsity of positive cultures, however, we can conclude this group is not a high risk for food safety. We have completed surveys and microbiologic sampling for 30 chicken farms that specialize as free-range or for the Asian live-markets. Campylobacter positive cultures were identified in 319/600 samples (18 of 20 farms) and 11/600 samples (4 of 30 farms) were positive for Salmonella. Farm and management variables documented by survey are currently being tested for links to the presence of these bacteria.

Farm-to-Fork Food Safety Research For Specialty Poultry Producers (J. Jeffrey)

The overall goal of this proposal is to extend the farm-to-fork concepts of food safety to the producers and processors of specialty poultry products on small to medium-sized farms. We have identified potential collaborators and have set up informational meetings with confirmed collaborators to discuss the details of the project and set up a sampling schedule for spring-summer of 2001. Laboratory and field personnel have been obtained and training is in progress. We are evaluating methods for extending the knowledge gained from this research to producers and associated processors so that it can be incorporated into their hazard analysis and critical control point programs for ensuring food safety. Addressing food safety concerns will enhance the successful marketing of specialty poultry products and the economic viability of small and medium sized farms.

Genetic Diversity of Campylobacter Jejuni Isolated Post-harvest from Skin and Digestive Tract of Commercial Broiler Chicken Carcasses (J. Jeffrey)

A total of 138 isolates of C. jejuni from the intestinal tract or skin of broilers from 5 ranches have been DNA fingerprinted by pulsed-field gel electrophoresis (PFGE). DNA-banding patterns were converted to digital images and analyzed for similarity using the Diversity database software (BioRad). The cluster analysis grouped the isolates into two major clusters with 75% dissimilarity between the two branches. Within each of these two major branches, the similarity between isolates ranged from 40 to 100% for one and 38 to 100% for the other, indicating a great deal of diversity between the isolates. One ranch (AM) had only 2 different DNA banding patterns with 30/32 isolates belonging to a single type, while the other ranches had 8 to 13 types among isolates from the same ranch. On 3 of 5 ranches there were more DNA banding types found among skin isolates than among gut isolates. Within a single broiler up to 4 different DNA fingerprints were observed in the C. jejuni isolated from skin and gut.

Human Salmonella Enteritidis Infection and Shell Egg Consumption (I.A. Gardner)

Generation of a valid prevalence estimate of SALMONELLA ENTERITIDIS(SE) in eggs and an associated confidence interval is of great public health importance because risk modeling indicates that prevalence is the most important factor affecting the magnitude of the overall risk for SE in human beings. Typically, only 1 SE-contaminated egg would be expected in a pool of size 20 because the average risk is about 2 per 10,000. Clusters of contaminated eggs might occur if several birds in a cage were infected over a short time interval. Depending on collection procedures on farm, multiple contaminated eggs could end up in the same batch. We showed that spatial and temporal clustering of SE-contaminated eggs in a pool of 20 eggs can lead to substantial (2-fold to 10-fold) underestimation of the true prevalence. Possible effects of spatial clustering can be avoided by randomly sampling eggs from within sheds and random selection of eggs for pooling. Temporal clustering might be more difficult to avoid especially at the peak of an epidemic within a shed.

Genetic Bases for Immune Resistance Against Marek's Disease in Chickens (P.S. Wakenell)

Most of the important genes are contained in the Major Histocompatibility or "B" complex portion of the genome. Other areas include the "Y", "P", and "L" complexes. We want to produce both a commercial White Leghorn line and a commercial broiler line of chickens that contain the B11 gene (MD resistant gene) without interference of the Ancona chicken background genes that were present in our research chickens. This will allow us to determine whether the B11 gene will confer as strong a resistance to MD virus (MDV) when it is bred into commercial stock as it has with our Ancona X genetic line. Currently, we are continuing backcrossing the Ancona X chickens that contain the B11 haplotype into commercial type White Leghorn chickens and have

approximately 60% of the Ancona background removed. We want to further investigate the influence of the Y system on MD resistance by concentrating on the Y alleles degree of influence in a more moderate B system background than B11. Other researchers have had difficulty demonstrating an effect of the Y system on MD when in less resistant chickens. We have conducted 2 trials using the moderately susceptible Ancona X White Leghorn B2B5 MHC background. Although we have insufficient data at this early phase to evaluate the Y system, the heterozygote background was somewhat more susceptible to the MD challenge than the B11 homozygotes (Trial 1, 26/84 or 31% birds positive; Trial 2 12/56 or 21% birds positive). Finally, we have conducted 6 challenge trials to date in order to do a preliminary search for other potential gene complexes to concentrate on. Two were completed in this past granting year and 4 were reported on last year. Results of the last two trials supported the previous 4 trials.. Overall, the C, D, H and I systems did not show any significant positive or negative effect on development of MD and will not be focused on in future studies . Statistically significant differences were observed only within the P system. Birds having a B19B19P1P1 haplotype had a significantly lower incidence of MD (21%) when compared with birds bearing the B19B19P4P4 haplotype (82%, $P<0.001$). In chicks with the B19B21 background, there was a significantly higher incidence of MD in both the P1P1 and the P4P4 haplotypes (85%) when compared with the P1P4 chicks (34%, $P<0.001$). There were not enough birds in each respective haplotype to evaluate the L system. These preliminary data show that at least the P system influences the incidence of MD after challenge. We want to conduct 2-3 additional trials in order to increase the bird numbers for evaluation of the L system and confirm our findings on the P system. ***IMPACT***: This project will both evaluate the different genes that influence Marek's disease (MD) and help to produce chicken lines that will be resistant to MD.

Genetic Bases for Resistance and Immunity to Avian Diseases (P.S. Wakenell)

Project 1: Currently, we are continuing backcrossing the Ancona X chickens that contain the B11 haplotype into commercial type White Leghorn chickens and have approximately 60% of the Ancona background removed. We have conducted 6 challenge trials to date in order to do a preliminary search for other potential gene complexes to concentrate on. Two were completed in this past granting year and 4 were reported on last year. Results of the last two trials supported the previous 4 trials. Statistically significant differences were observed only within the P system. Birds having a B19B19P1P1 haplotype had a significantly lower incidence of MD (21%) when compared with birds bearing the B19B19P4P4 haplotype (82%, $P<0.001$). In chicks with the B19B21 background, there was a significantly higher incidence of MD in both the P1P1 and the P4P4 haplotypes (85%) when compared with the P1P4 chicks (34%, $P<0.001$). There were not enough birds in each respective haplotype to evaluate the L system. These preliminary data show that at least the P system influences the incidence of MD after challenge. Project 2: Currently there are 2 generations of X-10 birds at UCD. The birds show thickening of the skin on the head (sometimes with comb involvement) and also an abnormal deposition of cottage cheese-like material on the skin with a putrid odor. Finally these birds die in a few days after the onset of the signs. At NIU, from January 1998 to June 1999, a total of 1004 dead birds were reported. Out of these, 53 birds (5.3%) showed the above symptoms and all of them were found to have the Ancona gene/haplotype B8 or B11. So far none of the X-10 birds have exhibited clinical signs similar to these. Gross post mortem findings in the P & F1 generation did not show similar signs seen in UCD 200 birds but showed other interesting lesions. The most striking gross postmortem observation is the presence of fibrosed or thickened mass in the ovaries, oviduct, intestines and liver. These patterns of postmortem lesions were noticed more frequently in the X-10 adult females than the males. Although the birds maintained by Dr. Briles and the X-10 birds come from the same genetic background, they exhibit different clinical and gross postmortem lesions. Although

the UCD 200/206 line of birds is used as animal model for scleroderma, the X-10 birds and the NIU birds show some clinical similarities to the human scleroderma. Unlike UCD 200 birds, which develop the signs at a very young age, the X-10 birds and the NIU birds shows signs in their adult life. UCD 200 birds are better animal models for the diffused form of scleroderma where there is rapid progression of the disease. On the other hand, X-10 birds and NIU birds show a slow and benign course of the disease. These lines of birds can be used as animal models for the limited form of scleroderma, which has a slow progression with limited skin involvement and late visceral involvement. Although both set of birds, X- 10 and NIU birds have the same genetic background (either B8 or B11 haplotype), they exhibit different clinical and postmortem findings. This suggests there might be some environmental factors in play. ***IMPACT***: These projects are designed to both evaluate the different genes that influence Marek's disease (MD) and to investigate an apparently new form of scleroderma in chickens.

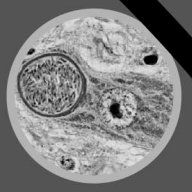
Molecular Epidemiology of Infectious Bronchitis Virus in Vaccinated Flocks (S.K. Hietala)

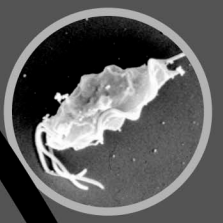
Avian infectious bronchitis virus (IBV) is an economically important pathogen of chickens that is difficult to diagnose and control due to the ability of the virus to mutate. IBV isolated over the past several years from diseased chicken flocks in California include several of these variant strains that cannot be readily identified by traditional laboratory techniques. In efforts to improve diagnosis, virus identification, and the ability to track IBV in flocks, a technique to rapidly sequence or read the virus genome was evaluated. The virus was recovered using standard virus isolation techniques. A small part of the virus genome, known as the surface spike protein, was then genetically sequenced for each of 100 IBV isolates and the pattern of the sequences were compared to IBV vaccine strains, IBV reference strains, and previously published gene sequences of other IBV field variants. AFamily trees@ generated for the IBV isolates identified 10 distinct groups of IBV, with 78% to 100% similarity within the groups. The relationships indicate that IBV variants continue to emerge in well-vaccinated flocks, and that circulation of variants may be limited by where the flocks are found geographically. The technique, direct cycle sequencing, shows promise as a diagnostic tool to support management and biosecurity decisions based on molecular epidemiologic tracking of IBV variants in flocks.

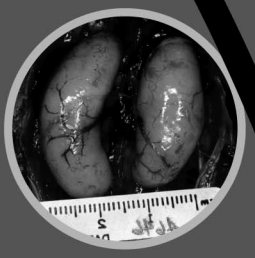
Lipoproteins and Their Metabolism in Avian Reproduction (R.J. Hansen and R.L. Walzem)

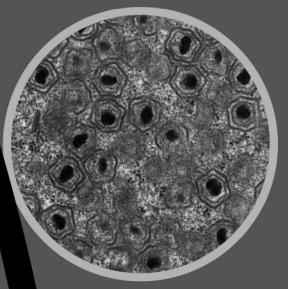
Poultry livers make a unique class of triglyceride-rich, apolipoprotein B-containing lipoproteins specifically for yolk formation called VLDLy. VLDLy are smaller than generic VLDL that provide the hen with energy, and VLDLy triglyceride-energy is less available to the hen's body. This project seeks to understand how VLDLy are made and how the physical differences between generic VLDL and VLDLy cause selective nutrient partitioning towards yolk formation and, ultimately, embryos. We have reported the results of a number of studies related to factors that affect production of VLDLy, such as: age of the hen, molt, and genetic variation. The process of VLDLy assembly is being studied in collaboration with Dr. Robert Hamilton. An exciting discovery of those experiments is that renal proximal tubule cells in birds assemble VLDL with diameters of - 60nm. This process may act to conserve fatty acids, and may support maternal lipid needs during the time that the liver produces VLDLy directed at yolk production. Hens that go out of lay lose the capacity to produce VLDLy in the liver, but this loss is coupled with the appearance of a special lipoprotein, called HDLR, which appears to be produced to reabsorb yolk material from the regressing follicles in the ovary. Attempts to sequence a special protein in HDLR were made, but insufficient protein was available to obtain reliable results. ***IMPACT***: This project sought to understand how VLDLy are made and how the physical differences between generic VLDL and VLDLy cause selective nutrient partitioning towards yolk formation and, ultimately, embryos.

Swine Research









Sheep, Goat & Swine Research Highlights

SHEEP, GOAT AND SWINE RESEARCH HIGHLIGHTS

- **Caprine Arthritis-Encephalitis Virus (CAEV)** in dairy goats is an example of how retroviruses may cause development of a disorder closely resembling human rheumatoid arthritis (RA). CAEV is of major economic importance in goats. CFAH research on CAEV is aimed at identifying the potential risk of infection among parturient does and from doe to kid. The impact of these studies has been to modify recommendations for herd CAEV testing strategies and herd management based on the detection of virus-infected cells in genital secretions.

- **Foot and Mouth Disease (FMD)** is a highly infectious debilitating viral disease affecting pigs, cattle, sheep, goats and deer in addition to wild and domestic cloven hooved animals . FMD is characterized by fever and blister-like lesions followed by erosions on the tongue and lips, in the mouth, on the teats, and between the hooves. Many affected animals recover, but the disease leaves them debilitated. It causes severe losses in the production of meat and milk. Although the United States has been free of FMD since 1929 the threat of an outbreak has severe economic consequences. Biosecurity, the practice of protecting farm animals from disease, has become a major concern with the worldwide threat of FDM. It spreads rapidly throughout animal populations and over long distances on the wind and hence it is difficult and costly to control. Effective biosecurity against FMD and other diseases requires several components including isolation, traffic control, and sanitation that aim to reduce exposure to bacteria, viruses and other organisms. CFAH research is aimed at developing control strategies if an outbreak should occur in California.

- **Mites** are a focus of CFAH research as a source of disease transmission. Wild, free-ranging bighorn sheep have been monitored in an ongoing study that will provide data that will aid wildlife agencies in developing management policies.

- **Swine Reproduction** studies focus on the mechanisms regulating gonadal function which are necessary to sustain reproductive efficiency, animal production and to conserve natural resources. This CFAH research will provide basic information on molecular mechanisms controlling trophoblastic differentiation that is essential for the successful establishment of pregnancy in domestic livestock

- **Digestive Problems** remain important causes of morbidity and mortality in goats, sheep and cattle. One CFAH project examines the use of rumen microbes as an adjuvant therapy for digestive disorders such as displaced abomasum or lactic acidosis. Another CFAH study looks at gossypol, a natural toxicant of cottonseed and its use in feed products.

Caprine Arthritis-Encephalitis Virus Infected Cells in Pre- and Post-partum Dairy Goat Does (J.D. Rowe and N.E. East)

Studies were recently completed which demonstrated CAEV proviral DNA in prebreeding and postpartum genital secretions of infected does. Does with detectable cAEV-infected cells in genital secretions may post greater risk of in-utero or cervico-vaginal CAEV transmission to their kids. During the past year the researchers achieved the goal of characterizing decline of colostral CAEV antibody. We found a mean disappearance of colostral antibodies by age 66 days and that antibodies to CAEV were not detected in 95% of kids by day 93. All kids were seronegative by 108 days, suggesting that serologic testing may begin at 3 to 3 2 months of age instead of the conventional 4 to 6 months. Our current work examining time to PCR reactivity to detect infected PBMC, which has not previously been described, is in progress. We have detected CAEV infection in 23% of study kids to date, with data collection in progress. We identified a cluster of seroconversion/PCR reactivity in kids delivered by the investigators and determined to be PCR negative and seronegative at birth, despite receiving apparently properly processed heat-treated colostrum. We have detected CAEV-infected cells in the tissues of two seronegative precolostral kids and have other samples pending, but additional kids are needed to assess the likelihood of in-utero infection. ***IMPACT***: Our ongoing studies are aimed at identifying means of preventing CAEV infection in young goats and predicting which kids might be a increased risk of perinatal CAEV transmission. The results of this research could impact breeding and culling decisions regarding both the doe and her kids.

Management/Response System for the Risk Analysis of Airborne Disease Transmission (T.E. Carpenter)

A data set containing livestock facility sizes and locations has been compiled from data sets obtained from CDFA, USDA, California Wool Growers Association and other UC researchers. USDA and CDFA are jointly collecting beef, swine, sheep and goat locations in the Fresno, Kings and Tulare county region of California. Data collection began August 2000. Objective 1 will be completed when we obtain and validate these data. Also, research is nearing completion on methods to estimate spatial herd locations with geocoding software, which saves considerable expense by now having to visit each herd individually with a GPS receiver. Objective 2 was completed. A study was conducted in Tulare, Kings and Fresno counties to estimate disease transmission potential among livestock premises, either directly from movement of animals or indirectly via vehicles or persons. Questionnaires and surveys were used to obtain information from beef and dairy producers; artificial inseminators, hoof trimmers and veterinarians; sales yards and a sample of truck routes for creameries, rendering plants and feed companies. These data will provide some basis for developing herd biosecurity strategies, including those necessary if exotic diseases such as foot and mouth disease enter California. The research results were recently accepted for publication in the Am Journal of Veterinary Medicine. Objective 3 was recently completed. Distributions were fit to the direct and indirect contact data collected as part of objective 2. They will now be used in the stochastic simulation model. A new approach is being developed for determining the expected number of effective contacts resulting from indirect and indirect animal contacts. Two surveys have been developed and will be mailed to an international panel of foot and mouth disease experts and to a US panel of foreign animal disease emergency response experts. Results from these surveys will be combined with results from Objective 2 and used in parameters in the simulation model. The simulation model required to test the hypotheses stated in Objectives 4 and 5 is still under development. ***IMPACT***: This project will evaluate a wide range of possible spread and recommend optimal control strategies for an outbreak of foot and mouth disease should it enter California.

Immunology and Molecular Biology of Ectoparasitic Mites (W. Boyce)

Psoroptic scabies is a contagious and debilitating disease of domestic and wild hoofed mammals. Studies suggest that Psoroptes mites do move between wildlife and cattle hosts; although there is some evidence that there is transmission of mites between deer and bighorn sheep. Desert bighorn sheep are now an endangered species. Monitoring of bighorn populations for exposure to Psoroptes mites, and DNA studies of mites from different hosts will provide data that will aid wildlife agencies in developing management policies.

The Epidemiology of Psorptic Scabies and Livestock-Bighorn Sheep Interactions (W. Boyce)

In certain parts of California, domestic cattle are grazed on lands that are also utilized by wild, free-ranging bighorn sheep. We have been monitoring the potential transmission of psoroptic scabies mites between these cattle and bighorn sheep for the last 5 years. During this period, transmission has not been documented to occur between these two groups. Although a high incidence of scabies mite infestations (>50%) has been recorded in populations of bighorn sheep in San Bernardino County, CA, there have been no reports of scabies mites in cattle in that area. We conclude that scabies mites occur in bighorn sheep in California and elsewhere in the western United States, but do not pose a threat to domestic cattle as far as cross-species transmission is concerned. We utilized a sensitive and specific serum antibody test to detect Psoroptes mite infestations in bighorn sheep. This test (developed in our lab) has been used to determine prevalence and distribution of Psoroptes-exposed bighorn sheep. This information in turn has aided Fish and Game departments in California and New Mexico in managing their free-ranging populations of bighorn sheep. We have also developed similar tests for elk (Ziccardi et al. 1996) and Dall sheep (Boyce and Zarnke 1996). Currently, we are collaborating with the California Department of Fish and Game in monitoring the mite infested Cushenbury Pit bighorn population (in San Bernardino County), as they develop mite treatment strategies. We are also continuing to participate with the U.S. Fish and Wildlife Service and the New Mexico Department of Game and Fish in a bighorn sheep reintroduction program in the San Andres Mountains, New Mexico. The population of bighorn sheep in the San Andres Mountains was nearly extinct (except for one ewe) in 1997 due to severe Psoroptes mite infestations. A small group of healthy rams was transplanted to that site in 1999. We are testing these animals twice a year to determine if they are becoming infested with mites. This information will determine if the area will be safe for further restocking. To date, no evidence of Psoroptes mite exposure has been detected in these animals. Progress was also made in determining the genetic relatedness of mites isolated from different animal host species. Initial DNA tests have shown that populations of Psoroptes collected from different animal hosts have enough similar DNA patterns to indicate that these mites may not be host specific, i.e., they may be able to successfully go from one host species to another (Ramey et al. 2000). ***IMPACT***: Psoroptic scabies is a contagious and potentially debilitating disease of domestic and wild ungulates. Domestic livestock are often grazed on land shared by native wild ungulates. The potential of cross-species transmission and subsequent economic losses and/or decimation of wildlife populations has been an ongoing issue between livestock farmers and wildlife agencies. Documentation of the epidemiology of psoroptic scabies will provide data important to the formulation of land and wildlife management policies.

Comparative Study on Limits to Efficient Ovarian Estrogen Synthesis in Pigs and Cattle (A.J. Conley)

Specific enzymatic steps control the steroidogenesis in the gonads: 17 a hydroxylase 17,20 lyase (P450c17), aromatase (P450arom) together with the redox partners cytochrome b5 (b5) and NADPH-cytochrome P450 reductase (reductase) are responsible for the production of androgen and estrogen. A LHRH antagonist (SB75) was used to test the hypothesis that a LHRH is

necessary for the expression and maintenance of these steroidogenic enzymes in the neonatal pig. Sixteen newborn piglets were randomly assigned to treated (SB75 at 50 mg/Kg BW) or control groups per either 7 or 14 days. LH levels were determined on the animal sera by radioimmunoassay. Testes were removed and specific assays to determine total P450 content, as well as enzyme activities for P450c17, P450arom and reductase were performed. Enzyme expression was determined by western blot analysis. LH was decreased in all treated groups. Testicular weight was decreased by 65% of control only after 14 days of treatment. Total P450 content (nmol/mg protein, mean SD) was significantly decreased at 7 days from 0.260.02 to 0.140.04 but at 14 days of treatment. Microsomal enzyme activity was significantly decreased for P450arom (pmol/mg/2h, mean SD) 32432 to 137114 on day 7 and from 30569 to 161115 on day 14. However, P450c17 and reductase enzyme activity at both 7 and 14 days of treatment had a non-significant reduction. All the enzyme activity data has a strong correlation with the enzyme expression determined by western blot. Even in the same group a variation in the enzyme activity is well reflected on the amount expressed protein. We conclude that SB75 inhibited the P450arom expression and activity but their effect on the expression or activity of P450c17 or reductase are less dramatic. Whether SB75 causes these changes directly or indirectly through LHRH-LH/FSH-testosterone flow in the pig testes it can not be determined with this experimental design. ***IMPACT***: These data suggest that in the neonatal pig testes, gonadotropins may play an important role in the regulation of activity and expression of these steroidogenic enzymes.

Unique Promoter Regulating Trophoblastic Differentiation (A.J. Conley)

Expression of CYP17 in the porcine trophectoderm is tightly regulated around day 12 of gestation, coinciding with transient conceptus estrogen production in this species. This lab has recently identified a novel transcriptional start site (-182 bp relative to ATG) utilized in the porcine trophectoderm which is distinct from that used for CYP17 expression in the adrenals and gonads (-48 bp). This distal site is associated with a unique TATA-less promoter region (-189 bp to -133 bp) containing two putative initiator (Inr) sequences and a palindrome spanning the transcriptional start. This minimal promoter is sufficient to provide basal transcriptional activity in luciferase promoter assays. The molecular mechanisms regulating use of this alternative start site in a species- and tissue-specific fashion are not known. The goal of this work was to identify critical areas within the trophoblast promoter region necessary for species-specific expression. Basal and enhancer-driven transcriptional activity of promoter constructs with mutations or deletions was investigated using a luciferase reporter system. Electrophoretic mobility shift assays (EMSA) and Southwestern analyses (SWA) were utilized to test DNA-protein interactions. Nuclear protein extracts from a porcine trophoblast cell line (Jag-1) and the MA10 Leydig cell line were hybridized with 32P-labeled oligonucleotides, separated by PAGE, and the results analyzed by autoradiography. For mutational analysis, the native porcine sequence was changed to the corresponding bovine sequence, a species that does not express CYP17 in the preimplantation trophoblast. Results from EMSAs demonstrated that the porcine but not bovine sequence bound specifically to both MA10 and Jag-1 nuclear proteins. One prominent band was determined to be sequence specific by competition with cold oligonuclotides at 50- and 500-fold molar excess. Mutations of the putative Inr sites and the palindrome from porcine to bovine sequence did not abolish binding activity, suggesting that the species specificity of expression may also involve additional flanking sequences or trans-acting factors. ***IMPACT***: With these results we have demonstrated a clear species-specific pattern of DNA-protein interaction and transcriptional activity that may provide a mechanism for differential control of CYP17 expression in the preimplantation porcine and bovine embryos.

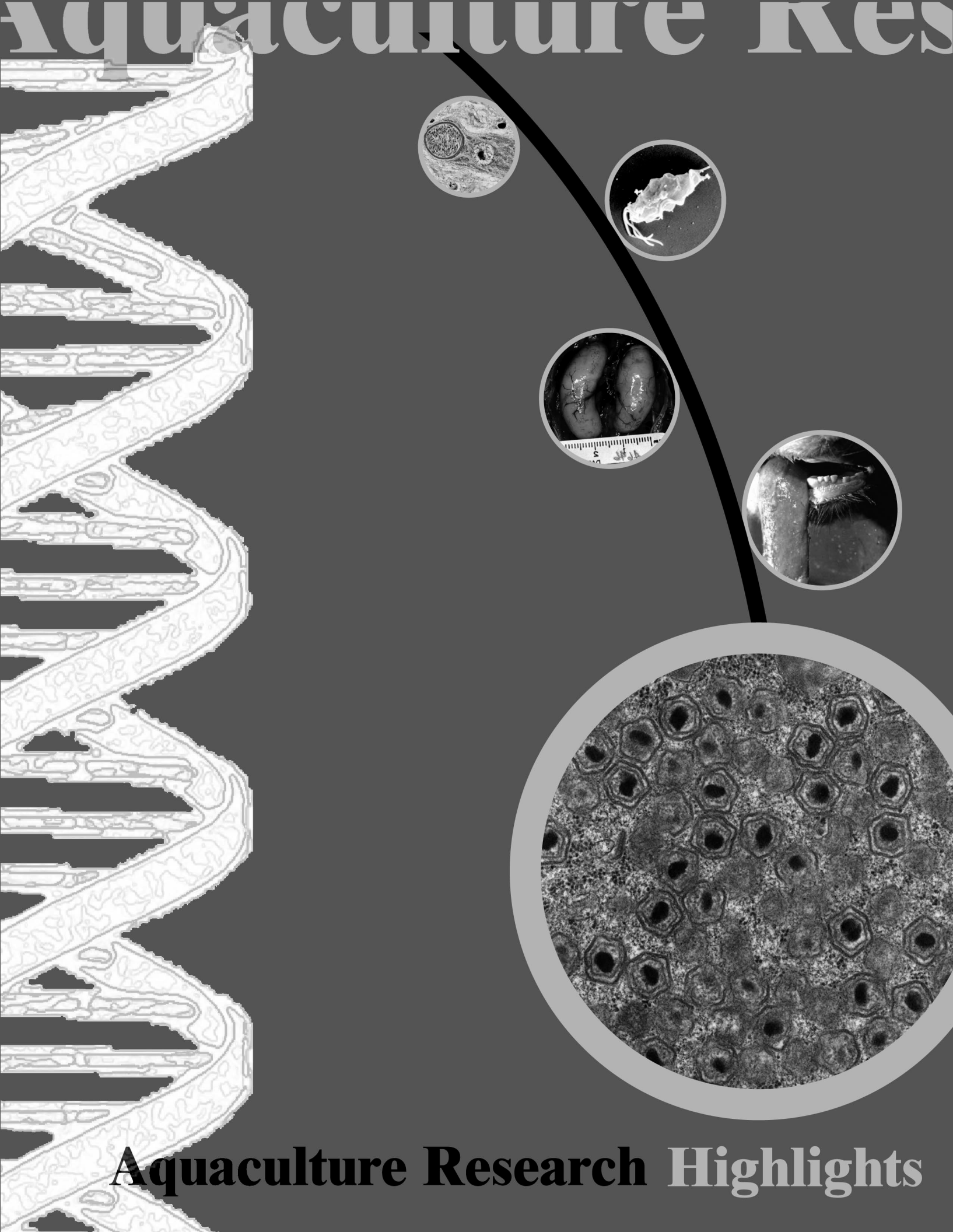
Preservation of Mixed Rumen Microbes (M.L. Bruss)

The main objective of this study was to evaluate freezing techniques for the preservation of rumen microbes in rumen fluid. Rumen fluid from 4 lactating and 4 non-lactating Holstein cows was tested for in vitro total gas production (an indicator of microbe viability)prior to and after 9, 17 and 44 days of being frozen. The rumen fluid had glycerol added to a concentration of 10% and was frozen in 1 L bottles at -20 C. In addition, fresh rumen fluid was tested for total gas production with and without the addition of glycerol. The substrates used for in vitro incubation were starch, cellulose, and starch plus cellulose, and incubation time was 4 hours. The highest rate of gas production occurred with fresh rumen fluid without glycerol, with starch plus cellulose being the highest, followed by starch alone, followed by cellulose alone. Lactation status had no influence except that fresh rumen fluid from non-lactating cows produced more gas from cellulose than that from lactating cows. Addition of glycerol inhibited gas production in fresh rumen fluid by more than 50%. There was a gradual decline of fermentative capacity of frozen glycerol-treated rumen fluid, amounting to approximately 50% over 44 days of being frozen. Although glycerol itself does cause some inhibition of fermentation capacity, it appears to decrease the rate of degradation of mixed rumen microbes in the frozen state since the rate of degradation of fermentative capacity was even greater in samples frozen without glycerol. ***IMPACT***: Rumen microbes in rumen liquor, preserved by freeze-drying and freezing, are ultimately useful as an adjunct therapy for digestive disorders, such as displaced abomasum or lactic acidosis.

Synthesis of Gossypol Derivatives Capable of Coupling to Protein (M. Mount)

A new method has been discovered to couple into gossypol by use of a Gringnard reagent. The coupling of trimethyl 4-bromoorthobutyrate to the aldehyde carbon of gossypol has been demonstrated in the laboratory. This method will compliment the monoclonal system produced by other researchers. Studies currently being performed have shown that cows exposed to 20-28 g gossypol/hd/day do not experience reproductive difficulties. The preliminary data enables researchers to propose dosage levels of gossypol which have a greater likelihood to produce reproductive problems in dairy cows for future toxicological study. ***IMPACT***: This research will help to make it possible to measure gossypol in lactating and dry dairy cows using a more simplified method of detection than currently exists. There is a need to simplify the current approach in order to assess the risk in dairy cows to gossypol intoxication.

Aquaculture Res



Aquaculture Research Highlights

AQUACULTURE PROJECTS

One major line of aquaculture research conducted through the CFAH are studies related to infectious disease in fish species, including an in-depth study of whirling disease (myxobolus cerebralis) in rainbow trout. Viral diseases continue to be some of the most problematic pathogens of fish raised in aquaculture and cause significant losses to the industry each year. Other CFAH research projects examine the effects of environmental stress on aquatic organisms. These studies determine whether fish in local waters have been adversely affected by agricultural and industrial chemicals and aid in making management decisions.

Epidemiology of White Sturgeon Iridovirus and Herpesvirus-2 in Three Commercial Facilities (I.A. Gardner, R. Hedrick, W. Johnson)

A prospective cohort study was done to determine management, fish and environmental risk factors for increased mortality and an increased proportion of runts among white sturgeon exposed to WHITE STURGEON IRIDOVIRUS (WSIV) and WHITE STURGEON HERPESVIRUS-2 (WSHV-2). Even though the 2 viruses were ubiquitous in the grow-out room, the tank-level mortality was very variable. Major determinants of number of dead fish were spawn, source and stocking density. Main predictors of the proportion of runt fish were spawn, mortality incidence and the difference in weight between the largest and smallest non-run fish. Furthermore, additional observations indicated a possible protective effect attributable to previous exposure to the viruses. On the same farm, progeny from 6 different spawns of white sturgeon broodstock were monitored for 20 months for occurrence of outbreaks of WSIV. Signs of WSIV were restricted to tanks from a single spawn each time. Temporal-spatial statistical analysis of outbreaks did not indicate that WSIV-case tanks were clustered in time and space. Furthermore, WSIV was isolated from progeny of all 6 spawns participating in the study, even though occurrence of outbreaks and clinical presentation varied greatly among fish from different spawns. Despite failure to identify virus in samples from broodstock these observations support a hypothesis of vertical transmission of WSIV, with tank-to-tank transmission having a lesser or no role in the spread of the virus. ***IMPACT***: This project will help to determine management, fish and environmental risk factors for increased mortality and morbidity among white sturgeon exposed to two viruses.

Viral Diseases in Aquaculture (R. Hedrick)

Viral diseases cause significant losses to aquaculture each year. Our research focus is to examine viral diseases important to common and fancy carp, sturgeon, salmonids, channel catfish and white seabass. We have developed basic information on the biology of the agents, their physical and biochemical characteristics and have developed new detection procedures involving cell lines isolating the viruses and new DNA-based methods such as the polymerase chain reaction. Cursory attempts to protect fish from these viruses by vaccination has also been attempted. ***IMPACT***: Viral diseases cause significant losses to aquaculture each year.

Molecular Approaches to Stain Differentiation and Virulence Comparisons of Myxobolus Cerebralis (R. Hedrick)

Whirling disease in rainbow trout is caused by a microscopic parasite called Myxobolus cerebralis. We examined parasites obtained from Europe (origin of the parasite) and two locations in the USA, one where whirling disease has caused declines in wild rainbow trout (Colorado) and the other from California where the parasite has not caused any recorded population effects. We compared genes from each parasite and examined if they were more detrimental to rainbow trout in laboratory trials. ***IMPACT***: The studies demonstrated that the parasites all seem to be the same, suggesting as we presumed that the parasite was introduced recently to North America from Europe and that all strains of the parasite are equally capable of causing disease in rainbow trout.

Environmental factors and the abundance of the other host for the parasite, an aquatic worm, are now viewed as determining the severity of the disease in wild rainbow trout.

Aquatic Toxicology and Pathology (Swee Teh)

Work in this area has begun to investigate nutritional and reproductive toxicology due to anthropogenic contaminants in sediments, water column, and food. The objective is to determine whether endangered fish in the Sacramento and San Joaquin Rivers, their delta, and upper San Francisco Bay have been adversely affected by agricultural and industrial chemicals. Protocols have been developed to integrate the aquatic toxicity bioassay with the chronic toxicity endpoints using the biomarker responses in two endangered fish species (Delta Smelt and Sacramento Splittail). The work with the medaka in the area of endocrine disruptors and their effects on reproduction and development has indicated the importance of using this fish as laboratory model in basic science and mechanistic studies. ***IMPACT***: These studies will help to determine whether endangered fish in the Sacramento and San Joaquin Rivers, their delta, and upper San Francisco Bay have been adversely affected by agricultural and industrial chemicals.

OTHER PROJECTS: EPIDEMIOLOGY

The CFAH plays an important role in investigating and evaluating diseases, injuries and production problems in animal populations and recommending actions for prevention or control measures strategies. Many research projects use epidemiological methods which combines concepts and methods of human and veterinary medicine, biology, mathematics, statistics, and economics. CFAH researchers provide a scientific basis for more informed public policy, regulatory and trade decisions and management practices.

Development of Methods in Diagnostic Epidemiology for Food Animal Populations (M. Thurmond)

A method has been developed that provides an estimate of the probability of infection, given an animal's serologic test result value, without the need for estimates of sensitivity and specificity. The method uses probability functions for Neospora caninum ELISA values and for BVDV SN titers, one for known infected animals and one for known uninfected animals, the prevalence of infection and Bayes' formula to derive the probability of infection. The method can be used for any assay, serologic or otherwise, in which the test result is measured as a continuous variable. ***IMPACT***: This approach permits assessment of the probability of infection, rather than simply seropositivity, which has direct application to risk and hazard assessment where estimates of the probability of infection are required. The method permits an assessment of the risk or probability of infection directly from serologic test results that are measured on a continuum.

Development and Application of Quantitative Risk Estimation Methods to Trade of Animals and Animal Products (I.A. Gardner, T.E. Carpenter and W.O. Johnson)

Diagnostic testing is an important component of programs to certify animals free of pathogens prior to export. Testing is also often used as a risk mitigation strategy by importing countries. Typically the dependence (correlation) among results of multiple tests is not considered when the effects of risk mitigation strategies are evaluated. We showed that a positive dependence in test sensitivities results in overestimation of risk reduction. We evaluated serologic test data for bovine paratuberculosis, and brucellosis and toxoplasmosis in pigs to determine the extent of pairwise dependence among these tests. For all 3 diseases, there was a moderate to high positive dependence in test sensitivities. On the other hand, the magnitude of specificity dependence varied by disease ranging from low specificity dependence for tests for paratuberculosis to almost complete dependence for tests for brucellosis. The important practical effect of the dependence is that inclusion of multiple biologically-related tests in assessments of disease introduction risk may not result in predicted reductions in risk assuming independent tests.

Test Dependence Affects Diagnosis and Surveillance of Animal Diseases (I.A. Gardner)

To investigate dependence (correlation) among multiple diagnostic tests, we developed modeling approaches that allowed us to select the optimal number and sequence of tests, that were necessary for serial and parallel testing schemes. For example, of 5 serologic tests for swine toxoplasmosis, only 3 (modified agglutination, ELISA and latex agglutination) were necessary for diagnosis. The method we developed is generalizable to other infectious diseases where there are multiple tests. We have implemented 2 statistical methods (maximum likelihood and Bayesian) for estimation of sensitivity and specificity when there are 2 uncorrelated (independent) tests and 2 populations with different prevalences and the true disease status is unknown. Bayesian methods require the modeling of uncertainty about test accuracy and prevalence. We have shown that Bayesian estimates are more stable but require the extra step of specifying prior distributions. Because this approach is less restrictive than traditional methods that require a gold-standard reference test, it has great potential for more widespread application in animal disease diagnosis

providing the underlying assumptions are correct. If the critical assumption of conditional independence of tests is violated and the tests have a positive dependence, then sensitivity and specificity of both tests will be overestimated.

Quantitative Methods to Certify Freedom from Animal Pathogens (I.A. Gardner)
Improved quantitative methods are needed to allow scientifically-valid statements about the probability of freedom of animals from pathogens that affect animal trade. We developed a Bayesian statistical model that can use herd-level test results from multiple herds in a region or country, adjusting for sensitivity and specificity of tests and the prior probability of disease. The model allows inferences about the probability of disease freedom, the proportion of diseased herds, and the within-herd prevalence. We have compared inferences from our Bayesian approach to those based on traditional statistical methods with published survey data using data for 3 viral diseases of livestock and poultry. The Bayesian model is superior to previous methods because it allows inferences about the proportion of diseased herds and within-herd prevalence which are important inputs into risk assessment models. However, we found that inferences about within-herd prevalence are limited if there are no diseased herds in the sample. Another advantage of the Bayesian model is that outputs are presented as probability distributions which reflect the uncertainty in estimates.

Animal Health Issues Including Cost-Benefit Analysis of Control Issues (T.E. Carpenter, D.C. Hirsh, J. Jeffrey and R.S. Singer)

The objective of this research was to evaluate the application of a vaccine in a population. A spreadsheet simulation model was constructed to estimate the impact of a vaccination program, assuming various population sizes, transmission rates, and vaccine efficacies. Results - Total effectiveness (proportion of cases avoided) increased with the vaccinated proportion of the population. However, with a highly efficacious vaccine, this relationship discontinued after a sufficient vaccination proportion was reached reflecting herd immunity. Evaluation of a case study showed that what might be considered a poor vaccine, based on its low efficacy, may protect a substantial portion of the population if administered to a sufficient number of susceptibles. Further investigation of an equine case study showed that evaluating a vaccine based solely on its efficacy could greatly underestimate its value. In conclusion, it was found that when evaluating a vaccine applied to a population, in addition to the vaccine efficacy, one must also consider the vaccination rate, cost of the vaccine, potential disease transmission rate, and number and cost of cases avoided. Vaccine efficacy may underestimate its value because of the reduction of indirect cases typically avoided when vaccination is applied in a population. ***IMPACT***: This research demonstrated that when evaluating a vaccine applied to a population, in addition to the vaccine efficacy, one must also consider the vaccination rate, cost of the vaccine, potential disease transmission rate, and number and cost of cases avoided. Vaccine efficacy may underestimate its value because of the reduction of indirect cases typically avoided when vaccination is applied in a population.