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JDIP News

Editors

Ian Gardner
(iagardner@ucdavis.edu)

Yrjo Grohn
(ytg1@cornell.edu)

Vivek Kapur
(vkapur@umn.edu)

Ken Olson
(keolson@prodigy.net)

Steen Erikson
(erik0046@umn.edu)

Visit our website at:
http://www.jdip.org

JDIP News is published periodically to enhance intramural communications and ensure that JDIP participants and stakeholders are updated on news of relevance to our community.

Please direct any contributions, suggestions and comments via email to: Steen Erikson at erik0046@umn.edu

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JDIP Year 3 Proposal Status

We've gotten a couple of contracts in the pipeline, and we've received all of the revised budgets and budget justifications. This information has been forwarded on to the USDA, and Year 3 funds should be released by the time you read this. Subcontracts will be processed as soon as the release is received from USDA. Expect a few more weeks before everything is completed. Endless thanks to all of you who have been waiting so patiently for so long.

3rd Annual Conference Update

The audio files from the 3rd Annual Conference are available on the JDIP web site. You can find the link on the News page or in the Investigator's Area/3rd Annual Conference from the menu. You will need to log in to access the conference material.

JDIP: Phase 2 Update

The RFA for Phase 2 will be released soon, along with a timeline that highlights milestones in the proposal review process. This will help keep everyone on track to complete the RFA and review process in time to get contracts executed before the next budget year begins on April 15, 2008.

June 14 is Flag Day
From Wikipedia: In the United States, Flag Day (more formally, National Flag Day), is celebrated on June 14. It commemorates the adoption of the flag of the United States, which happened that day by resolution of the Second Continental Congress in 1777.

In 1916, Woodrow Wilson issued a proclamation that officially established June 14 as Flag Day; in August 1949, National Flag Day was established by an Act of Congress.

June 17 is Father's Day
From Wikipedia: Father's Day is a primarily secular holiday inaugurated in the early 20th century to complement Mother's Day in celebrating fatherhood and parenting by males, and to honor and commemorate fathers and forefathers. Father's Day is celebrated on a variety of dates worldwide, and typically involves gift-giving to fathers and family-oriented activities.

9th ICP Meeting in Tsukuba, Japan

John Bannantine is working with Eiichi Momotani, the local organizer for the ICP meeting, to coordinate an evening symposium about JDIP. Vivek Kapur, John Bannantine, Douwe Bakker, and Frank Griffin will make presentations.

http://wwwsoc.nii.ac.jp/jsp3/9ICP/

News from the Executive Committee

A number of notable developments are being discussed in the weekly Executive Committee meetings:

- John Adams is retiring from the NMPF on July 1, but we're happy to report that he will continue to serve the External Advisory Board in an independent advisory role. We are working to identify a replacement for John as an NMPF representative on the EAB.

- The EAB has identified five members who will play key roles in monitoring the progress of five topic areas. This additional oversight should make the annual RFA process smoother since these members will have first hand knowledge of the proposal review discussions and will therefore be able to share that information with the EAB during their final review of funding proposals. It will also help to ensure that JDIP remains focused on the most important areas of research. The members and their areas of focus are:

    - Epidemiology and Transmission – Larry Hutchinson
    - Diagnostics and Strain Differentiation – Douwe Bakker
    - *Map* Biology and Pathogenesis – Harley Moon
    - *Map* Immunology and Vaccine Development – Greg Pruitt
    - Extension and Communications – John Adams

- In addition to this deeper involvement, the EAB has been added to the distribution list for the Executive Committee meeting minutes and been invited to participate in Executive Committee and Scientific Advisory Board meetings at their discretion.

- John Bannantine proposed a new addition to the JDIP newsletter; each Executive Committee member will submit a brief summary of a journal article in their area of expertise. The summary will be very high-level and will highlight a key finding of the research. Check out the first installment of this feature on page (X) and let us know what you think!

- Discussions are taking place to define the benefits and obligations of JDIP membership. The intent of this effort is to enhance the ability to "market" the JDIP program and to bring focus to the core benefits of participation in JDIP. Once completed, the document will be circulated and placed on the JDIP web site.

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Journal Highlights (Bannantine Bulletins)

The Journal Highlights section will report on articles that members of the Executive Committee believe are important and informative. Articles will cover JDIP-funded and non-JDIP research.

Wu CW, Livesey M, Schmoller SK, Manning EJ, Steinberg H, Davis WC, Hamilton MJ, Talaat AM., Invasion and Persistence of Mycobacterium avium subsp. paratuberculosis during Early Stages of Johne's Disease in Calves., Infect Immun. 2007 May;75(5):2110-9. PMID: 17296749

This study reports on the fate of both M. avium subsp paratuberculosis (MAP) and the host following surgical implantation of MAP bacilli directly within the intestine, thereby bypassing the oral route. The authors include JDIP members Adel Talaat, Elizabeth Manning, Bill Davis, and Mary Jo Hamilton. Although the authors analyzed the initial hours and days following implantation of MAP into the intestine, they were continually tracked for a total of 9-months. This undertaking was complex, carefully planned, and required considerable animal handling expertise. Furthermore, several keen observations were made from these complex studies that have strong implications for future animal studies with MAP. For example, a key finding was that only through this type of surgical model could it be discovered that MAP crosses intestinal tissue and reaches the mesenteric lymph nodes by 1-hr.

Rating: Must Read
Classification: Novel finding

Article

JDIP Core and Project Leaders

Core 1: Epidemiology & Biostatistics
Ian Gardner (co-leader)
530-752-6992
iagardner@ucdavis.edu
Yrjo Grohn (co-leader)
607-253-4394
ytg1@cornell.edu

Core 2: Diagnostics & Strain Differentiation
Janet Payeur
515-663-7676
janet.b.payeur@aphis.usda.gov

Core 3: Genomics, Antibodies & Proteomics
Michael Paustian
515-663-7979
mpaustia@nadc.ars.usda.gov

Core 4: Animal Models & Facilities
Murray Hines II
229-386-3340
mhinesii@uga.edu

Core 5 / Project 5: Extension & Communications
Jeannette McDonald (co-leader)
608-263-5170
mcdonal7@wisc.edu
Ernest Hovingh (co-leader)
814-863-2160
eph1@psu.edu

Core 6: Administration
Vivek Kapur
612-625-7712
vkapur@umn.edu

Project 1: Epidemiology and Transmission
Ynte Schukken
607-255-8202
Yhs2@cornell.edu

Project 2: Diagnostics and Strain Differentiation
Srinand Sreevatsan
612-625-3769
Sreev001@umn.edu

Project 3: MAP Biology and Pathogenesis
Luiz Bermudez
541-737-6538
luiz.bermudez@oregonstate.edu

Project 4: MAP Immunology and Vaccine Development
Paul Coussens
517-353-3158
coussens@msu.edu

Article

ParaTBTools: An EU Update

ParaTBTools: The development of improved tools for detection of paratuberculosis in livestock, M. paratuberculosis in food and for the assessment of the risk of human exposure.

By: Dr. Douwe Bakker, Central Institute for Animal Disease Control – Lelystad, The Netherlands
Dr. Jim McNair,

History of the project

ParaTBTools (aimed at the control of paratuberculosis) is a Specific Targeted Research Project (STREP) of the European Union. After several applications, ParaTBTools was one of the projects to be funded as part of the "Food Quality and Safety" theme in the final year of Framework 6.

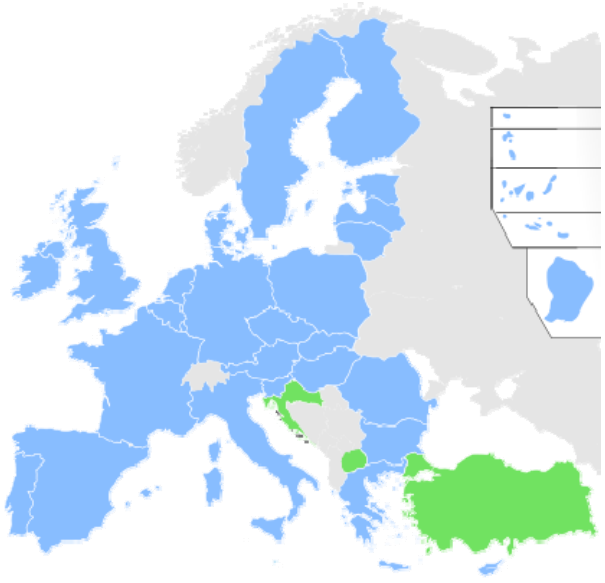
'Framework programmes' (FPs), are the main financial tools through which the European Union supports research and development activities covering almost all scientific disciplines. Within the present FP7, the total amount for European Community financial participation is € 50.5 billion (US$ 66.7 billion) for the period 2007 - 2013. Collaborative research constitutes the bulk and the core of this EU research funding, e.g.: out of this the EU Member States have earmarked more than € 1.9 billion for the theme 'Food, Agriculture and Fisheries, and Biotechnology'. For the preparation of the calls for proposals, the EU consults other EU institutions, in particular the European Parliament, and the EU Member States, as well as by the scientific community, industry and all stakeholders in European research.

An expression of interest submitted by the EU member states Ireland and the UK, as well as questions in the European Parliament relating to the possible relationship between paratuberculosis and Crohn's Disease, resulted in a call for a research proposal on "Mycobacterium avium sub-species paratuberculosis diagnosis and Control, with the purpose to generate new tools for the diagnosis of MAP in animals, for its elimination from animal products and for studying its potential role in Crohn's disease". Three proposals addressing this specific call were submitted, but the consortium of labs that formed ParaTBTools was awarded with the project.

Unfortunately, the consortium lost just prior to the start of the project one of its founders, co-author of the proposal, our dear friend and colleague John Pollock. ParaTBTools will be dedicated to his memory and we hope to be able to deliver work of excellent quality and in good friendship as he would have done.

The project:

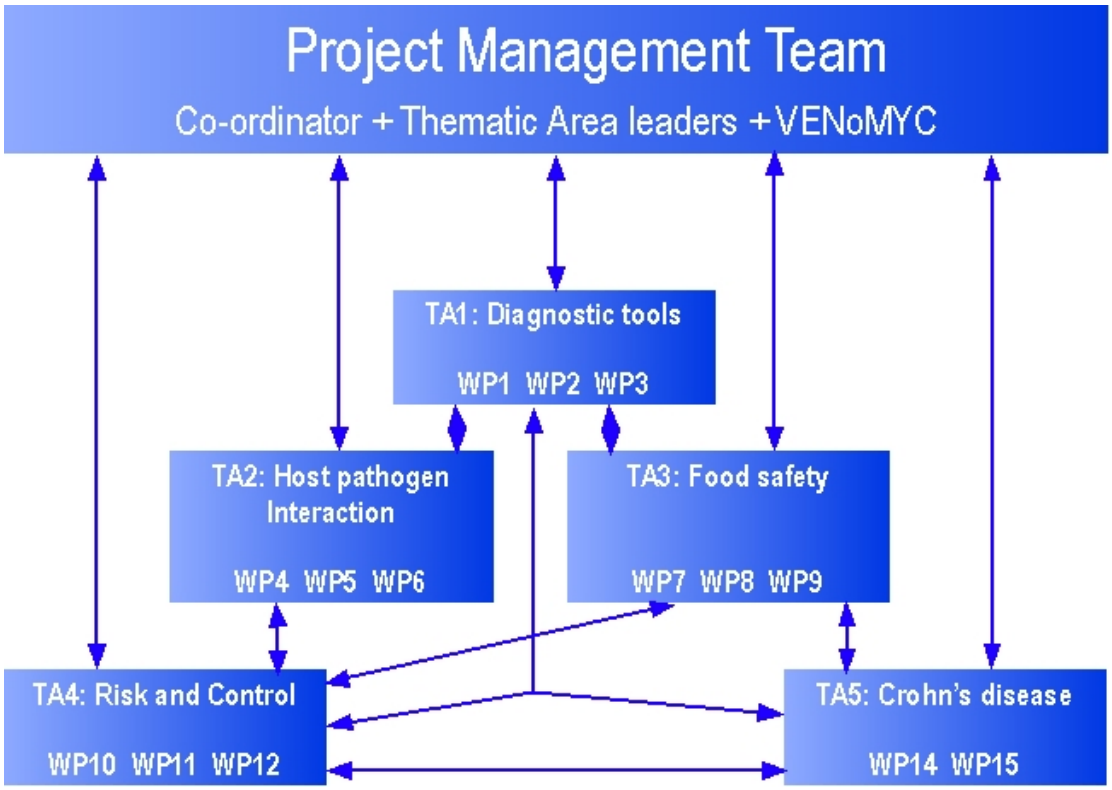
To date, all the studies on paratuberculosis within the EU and elsewhere have been conducted by individual laboratories or small teams of laboratories with limited expertise in the range of disciplines needed to properly study the multifacetted problem of paratuberculosis. Therefore, to be able to address this problem across all aspects of the disease, a multidisciplinary consortium had to be established and which holds expertise in mycobacteriology, immunology, test-development, molecular biology, genetics, epidemiology, risk-assessment, inflammatory bowel disease and foodsafety. The 28 partners forming this consortium originate from 16 countries. Paul Coussens (MSU) is representing JDIP as partner is this consortium.



A highly significant achievement for the project will be the establishment of this multidisciplinary consortium within the EU to study the important and multifaceted problem of paratuberculosis, and the implications for food safety and animal welfare. It is expected that the wide range of disciplines within this consortium will provide a much needed platform of expertise within and beyond the lifetime of this project.

The EU contribution to this project is € 3.9 million for a period of three years, making it a "small collaborative" project. The size of the contribution varies between partners, some of the partners, due to their expertise, perform small, but essential tasks within the consortium. All tasks are assigned at the start of the project for the entire period. However, the management team, responsible for the project can adjust the tasks when needed e.g. when new insights arise or due to scientific developments elsewhere.

The work programme is truly multidisciplinary, and this is reflected in the 16 proposed work packages (WP). Most of these WP's have been formulated using existing knowledge and reagents so they can be initiated at an early time within the project. However, new information as well as new biologicals and technologies generated within the WP's will feed into and influence the design of tasks that are to be initiated in the latter time frame of the project. For example, studies on the survival of *Mptb* in milk and dairy products are planned after the studies on the recovery of *Mptb* from these matrices have been optimized.



To allow for an efficient organisation of this project and this large consortium, 15 WP's have been distributed over 5 Thematic Area's (TA's); TA1 ; Diagnostic Tools, TA2: Host-pathogen interaction, TA3: Foodsafety, TA4; Risk and Control and TA5: Crohn's disease.

Within these Thematic Areas, the WP manager will be responsible to their respective TA leaders and the coordinator for the smooth running and progress of his/her WP. The WP's are combined within a Thematic Area in such a way

that they allow established expertise in disciplines to interact in a "problem solving" and "goal oriented" manner with colleagues from other disciplines, but yet still retain strict management control in respect of milestones and deliverables, this in order to keep a tight control of the progress within the overall work programme. One of the biggest challenges during the project will be to establish and maintain an efficient exchange of information, reagents etc, between the different partners.

Information dissemination

Information dissemination is an important aspect of this project and will be maximised to ensure distribution of information derived from this project, firstly between consortium partners and subsequently for presentations at scientific meetings and publications. Additionally, since this multidisciplinary consortium brings together partners from different backgrounds, including National Reference Laboratories and European Reference Laboratories of the Office International des Epizooties, the dissemination of information and protocols into the wider scientific community as well as to a large number of stakeholders will be secured.

To facilitate information dissemination, an essential role is foreseen for the Veterinary Network of Laboratories Researching into Improved Diagnosis and Epidemiology of Mycobacterial Diseases (**VENoMYC**), EU Co-ordination Action SSPE-CT-2004-501903. The **VENoMYC** coordinator will be responsible for **WP16** and in which network 15 of the 28 partners of ParaTBTools already participate.

In addition, during the preparation of this proposal a number of national, European and international organisations, including Crohn's Disease patient interest groups as well as producer and industrial organisations, expressed their interest to be identified as a stakeholder to this project.

The scientific and technological objectives of this ParaTBTools are addressed within 5 Thematic areas:

Thematic area 1: Standardisation, harmonisation, and improvement of laboratory diagnosis of paratuberculosis in livestock.

The main objective of this **Thematic Area 1**, "Diagnostic tools" is to develop an innovative programme aiming to develop more accurate cultural, molecular and immunodiagnostic laboratory based diagnostic tools for the detection of *Mptb* (in faeces, blood, tissues, meat and milk). Development of such tests is considered invaluable for any control or eradication programme for paratuberculosis in ruminants and to guarantee the foodsupplies to be free of the bacterium. In addition, such tools would be essential for research efforts aiming to elucidate the potential role of *Mptb* in the etiology of Crohn's disease.

WP 1 will be focussed on the standardisation and harmonisation of the available reagents and diagnostic tests, aimed either at the detection of the bacterium (faeces, blood, tissues, milk or dairy products), or the immune response in infected animals. Using different approaches **WP 2** is aimed at developing improved antigens needed for immunological testing, Improvement of antigens will be obtained by quality controlled crude *Mptb*-antigen production using a micro-array approach and a search for novel and more specific antigens using a proteomics and expression libraries in E. coli.

In addition, potentially very useful, glyco-antigens will be further analysed for use as specific antigens in immunodiagnostic testing.

In **WP 3**, the aim is to improve the available routine microbiological diagnostic tests. The molecular detection tools (PCR) will be improved using capture and extraction methods. Improvement of cultural methods will be explored using semi-automated culture systems (MGIT and ESP-TREK) in combination with PCR detection as a rapid detection technique. Improvement of immuno-histological methods by categorisation of lesion types, establishment of image analysis technique, use of different *Mptb* specific monoclonal antibodies and newly developed DNA-probes.
A novel LAM-based ELISA will be further optimised and further enhanced with regard to specificity by the selection of high-affinity Llama antibodies for usage in a blocking ELISA.

Thematic area 2: The Interaction between Host and Pathogen in Ruminants Infected with *Mycobacterium paratuberculosis*: Development of Improved Diagnostic Tests.

There remains a lack of knowledge concerning the host immune responses to the *Mptb*. This knowledge is crucial to the development of improved diagnostic tests. **Theme 2** sets out to address this by assessing new diagnostic tools developed within **Theme 1** in experimentally infected cattle (**WP 4**); comparing immune parameters with disease outcome and therefore infectivity (**WP 5**); and optimising current blood-based tests for the early diagnosis of paratuberculosis (**WP 6**).

However, matters are complicated by the antigenic similarity of mycobacterial species and the need to differentiate immune responses to *Mptb* from other environmental and pathogenic mycobacteria. In order to address this, **WP 4** will compare immune repertoires in cattle infected with *Mptb* and environmental mycobacteria.

Diagnostic assays must not only diagnose infection or exposure to *Mptb* organism but also indicate the likelihood of clinical disease. This will be addressed in **WP 5** where local and systemic immune responses will be compared in goats, sheep and deer. This work should lead to a better prediction of pathology in infected animals and therefore their infectivity to others.

The IFN-g test has the advantage of speed and can be easily assessed against any group of *Mptb* antigens. However, the test is dependent upon the active presentation of antigen to T cells and so is sensitive to the time delay between sampling and testing. Recently, workers at DFVF., Copenhagen, have found that by adding the antigen presentation co-stimulatory cytokine IL-12 along with the antigens used in the assay, the specific IFN-g production can be enhanced. As the IFN-g test is currently one of the best candidates for the diagnosis of paratuberculosis in pre-clinical animals **WP 6** will investigate the optimisation of current IFN-g assays. This WP will be used to validate this test using deliberately infected cattle and goats, as well as in naturally infected animals (**WPs 4**, **5** and **6**).

Thematic area 3: The Inactivation of *Mycobacterium paratuberculosis* (*Mptb*) in milk and dairy products.

Many studies investigating the heat resistance of **Mycobacterium paratuberculosis** (*Mptb*), have demonstrated the ineffectiveness of standard pasteurisation regimes including HTST to totally kill *Mptb* in artificially and naturally infected raw milk.

WP's 7 & **8** will address the lack of uniformity, with respect to both repeatability and reproducibility, in culture and molecular detection methods and develop 'gold standards' in culture and molecular protocols for dairy products. **WP7** will focus on improvement of the techniques used and in **WP8** a milk ringtrial will be organised for members of both ParaTBTools as well as for members of the EU-wide **VENoMYC** network.

A number of recent efforts have concentrated on modifying the time temperature parameters of pasteurisation to improve its lethality for *Mptb* in liquid milk with differing degrees of success. There is also unpublished data indicating that homogenisation and centrifugation, practised regularly in the processing of liquid milk, can enhance

the lethality of the heating process based on the dispersal and elimination of cell clumps respectively. WP9 will determine the efficacy of both processing parameters to eliminate/inactivate *Mptb* from raw milk in conjunction with pasteurisation. Further tasks in WP9 will investigate the inactivation of *Mptb* during the manufacture and post-production storage/ripening of cheese and yogurt products.

Thematic area 4: Risk and control:

Deficiencies in the available diagnostic tests result in us knowing little about the true dynamics of the spread of *Mptb* through animal populations and consequently little about the potential for contamination of the environment and food products with this agent. Thematic area 4 of this programme sets about objectively and systematically examining what we do know about risk and control of paratuberculosis in livestock (WP10) and evaluating the qualitative and quantitative risks for animals (WP11), farm businesses (WP12) and human populations (WP13). This theme will also provide a big picture context in which to frame the other work packages within the proposal. For example the modelling will inform the laboratories developing new diagnostics as to the levels of improvement on the current tests (e.g. sensitivity and specificity) needed if control is to be achieved and thus the modelling may be seen as setting targets for the diagnostic developers (WP10 & WP11). Through WP10, Theme 4 initially sets about examining the properties of the current tests and reassessing their limitations in the light of our current knowledge about use of tests without a gold standard. Existing test strategies and prevalence estimates for control of paratuberculosis will be evaluated and these will be revised. To achieve this, the team will sift through the published information about these tests and also examine surveillance data from several different European countries and carry out a metanalysis.

WP10 will also function to channel information from elsewhere in the project to WP's 11, 12 & 13. A major constraint of work on paratuberculosis to date is that it focuses at farm level. However in reality for most European countries there is essential trade in livestock and consequently trade of paratuberculosis. It is essential that this project examines the bigger picture and WP11 will examine this higher level using a modelling approach (there is no other viable alternative). The quantification of the abilities of current and future paratuberculosis-tools needed to achieve effective disease control in EU livestock systems will form the major part of WP11. The outputs from both WP10 and WP11 are essential for the successful completion of WP12 where we will develop, test and deploy an economic framework to determine the relative economic value of paratuberculosis control.

Ultimately to succeed most control schemes depend upon the compliance of the farmers not government directives, which in turn depends upon farmers' awareness of their relative benefit from compliance or non-compliance. A constant cycle of iterative interaction between WPs 10, 11, and 12 is envisaged. The resource allocation decision relating to control paratuberculosis if selected by the farmer will ultimately impact on the human exposure to *Mptb*. WP13 will therefore utilise the output from all three of the other TA4 workpackages, the output from WP9 and other sources of information including published data and expert opinion to develop disease-risk frameworks for paratuberculosis and to estimate the risk to humans from the food chain. In this way this theme will develop three different epidemiological/economic tools that will not only answer the immediate objectives of the proposal but will also be constructed in such a way that they can form tools to aid future research following the establishment of improved tests.

Thematic area 5: Characterise the interaction between humans with Crohn's disease (CD) and *M. paratuberculosis* to establish whether a causal relationship is present

Contemporary research from several independent centres suggests that the majority of people with CD are infected with *Mptb*. *Mptb* persists during human infection, in very low numbers and in a non-acid fast form which requires reversion to a vegetative phenotype to culture. Current culture media either fail to promote this conversion, or allow *Mptb* growth but at an extremely low rate, thereby prohibiting effective study. In other mycobacterial species,

promotion of growth from low inocula has been achieved by the addition of specific quorum sensing peptides or resuscitation promoting factors (RPF). RPF homologues are present within the *Mptb* genome. WP 15 proposes to study the culture of *Mptb* from human samples by determining the optimal conditions for human *Mptb* growth with the benefit of additional *Mptb* specific recombinant RPFs.

The most current view is that CD is caused by defective interaction between the bacterial flora of the gut and that the innate immune system plays a key role in the pathogenesis of the disease. *Mptb*'s ability to grow and survive in the intestine of many species together with the strong immunoreactive properties of mycobacteria makes *Mptb* a potential key player in the development of CD in predisposed patients. To address this question, it is important to investigate both the immunological consequences of human interaction with *Mptb* and the influences a human host will have upon the *Mptb* phenotype. The first will be investigated in WP 14 studying the host reactivity of T-cells in CD granulomas against *Mptb* antigens. Knowledge of the specificity of the T-cells found in CD lesions will suggest ways in which *Mptb* may contribute in CD pathogenesis. The second will require establishment of a *Mptb* genotype (WP 15).

Few studies have established the genotypes of *Mptb* detected in humans and compared them with *Mptb* from animals or the environment. WP 15 proposes to construct a total *Mptb* genome microarray and will use this array to generate DNA total genome profiles of a representative panel of virulent and avirulent *Mptb* isolates from a wide variety of hosts, including humans. This will provide a comprehensive database of *Mptb* marker genes, that more accurately than current systems, reflect the strain phenotype, and each strains potential for host virulence. The demonstration of highly specific antibody responses against *Mptb* in patients with CD would support *Mptb* as a cause of CD. A problem with many previous immunological studies is that crude antigens like Purified Protein Derivative (PPD) or cell sonicates from *Mptb* have been used, containing antigens that are shared between mycobacteria. The availability of the *Mptb* microarray will allow characterization of new antigen candidates and the logical design of novel *Mptb* diagnostics. Transcriptomic RNA profiling of *Mptb* expression using the *Mptb* microarray will be studied in collaborative and interwoven studies involving *Mptb* models of growth with *in vitro* culture (WP 2) and in bovine and human infection models (WP 15).

By studying the specificities of antibodies in CD disease it is possible to determine whether they are generated preferentially against a single pathogen (*Mptb*). The comparison of human sera against specific *Mptb* antigens in WP 14 from areas with a high and low prevalence of *Mptb* infection in domestic livestock will give further insight into this question.

AMSC Manuscript

The AMSC Manuscript is on the JDIP web site! You can find it at the bottom of the "Core 4" section or, if you're an AMSC member, you can find it in the JDIP Documents/AMSC section of the Investigator's Area. You'll need to log in to access the document, but it is available to all JDIP members for non-commercial research or educational use. Please do not distribute this document!

Article

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Article continued...

Upcoming Meetings and Conferences

- Joint Meeting of the American Dairy Association (ADSA) and the American Society of Animal Science (ASAS)
  Henry B. Gonzalez Convention Center, San Antonio, TX
  Sunday, July 8 – Thursday, July 12, 2007
  http://adsa.psa.ampa.asas.org/meetings/2007/index.asp

- 40th Annual Convention of the American Association of Bovine Practitioners
  Vancouver, British Columbia, Canada.
  Sept. 20 - 22, 2007
  http://www.aabp.org/meeting/default.asp

- World Dairy Expo
  Alliant Energy Center of Dane County, Madison, WI
  October 2-6, 2007
  http://www.world-dairy-expo.com/gen.home.cfm

- USAHA/AAVLD 111th annual meeting
  John Ascuaga's Nugget Casino Resort in Reno, Nevada
  October 18 - 24, 2007
  http://www.usaha.org/meetings/

- 9th Annual ICP Meeting, International Association for Paratuberculosis
  Tsukuba International Congress Center, Tsukuba, Japan
  Monday October 29 - Friday November 2, 2007
  http://wwwsoc.nii.ac.jp/jsp3/9ICP

- NMPF/NDB/UDIA Annual Meeting
  Lake Buena Vista, FL
  November 12-14, 2007
  http://www.nmpf.org

JD ON THE WEB

Johne's Disease-related Websites

Organization	URL
American Association of Bovine Practitioners	http://www.aabp.org
American Dairy Science Association	http://www.adsa.org
American Society for Microbiology	http://asm.org/
American Veterinary Medical Association	http://avma.org
Conference of Research Workers in Animal Diseases	http://www.cvmbs.colostate.edu/microbiology/crwad/index.htm
Infectious Diseases Society of America	http://www.idsociety.org
International Association for Paratuberculosis	http://paratuberculosis.org/
JDIP: Johne's Disease Integrated Program	http://www.jdip.org
JDIP Sample Shop	http://seeker.doit.wisc.edu/jdip/Default.aspx
National Agricultural Library	http://www.nal.usda.gov/awic/pubs/johnes/johnes.htm
National Johne's Education Initiative	http://johnesdisease.org/
National Veterinary Services Laboratory	Laboratory Certification Site: http://www.aphis.usda.gov/vs/nvsl/labcertification.html
United States Animal Health Association	http://www.usaha.org
University of Wisconsin Johne's Information Center	http://www.johnes.org
University of Wisconsin Johne's Disease Veterinary Certificate Programs	http://vetmedce.vetmed.wisc.edu/jdvcp/
USDA Johne's disease website	http://www.aphis.usda.gov/vs/nahps/johnes/
USDA-APHIS-VS–National Center for Animal Health Surveillance	http://www.aphis.usda.gov/vs/ceah/ncahs/index.htm

JD IN PRINT

Johne's Disease-related Articles in Producer Publications – March - May, 2007

Hoard's Dairyman:
"Industry gains confidence in Johne's milk ELISA test," by Robert H. Whitlock, March 25, 2007, page 226.

Dairy Today
Dairy Herd Management
Western Dairy Business
Midwest Dairy Business
Northeast Dairy Business
Holstein World
Feedstuffs
Angus Journal
Beef Today
Drover's Journal
Successful Farming
Southeast Farm Press

Hot New Titles in Bovine Journalism:

Mrs. O'Leary's Barn News:

Pastures of Madison County:

Bovine Beat:

Horns and Udders:

Feeding Trough Digest:

Cow Tipping News:

New in the Marketplace:

Available from Amazon.com:

Slingshot Flying Cow With Moo Sound

List Price: ~~$8.99~~
Price: **$3.75**
You Save: $5.24 (58%)

Product Features

- Slingshot Cow flies with a MOOOOOO screeeam! Just pull him back and let him fly!
- Slingshot Flying Cow Ages 4 & up
- The Slingshot Flying Cow is nothing short of outrageous!
- The Cow can be shot long distances using his elastic hands.
- Put one finger in the each of the hands' pockets, pull back and let go.

As an added bonus, every time you shoot it, it lets out three loud MOOOOO calls.

JD IN PRINT

Johne's Disease-related Publications – March - May, 2007

- **Abubakar I, Myhill DJ, Hart AR, Lake IR, Harvey I, Rhodes JM, Robinson R, Lobo AJ, Probert CS, Hunter PR.**, A case-control study of drinking water and dairy products in Crohn's Disease–further investigation of the possible role of Mycobacterium avium paratuberculosis., Am J Epidemiol. 2007 Apr 1;165(7):776-83. Epub 2007 Jan 19., PMID: 17237136 [PubMed - indexed for MEDLINE]
- **Al Hajri SM, Alluwaimi AM.**, ELISA and PCR for evaluation of subclinical paratuberculosis in the Saudi dairy herds., Vet Microbiol. 2007 Apr 15;121(3-4):384-5. Epub 2007 Feb 4. No abstract available., PMID: 17339085 [PubMed - indexed for MEDLINE]
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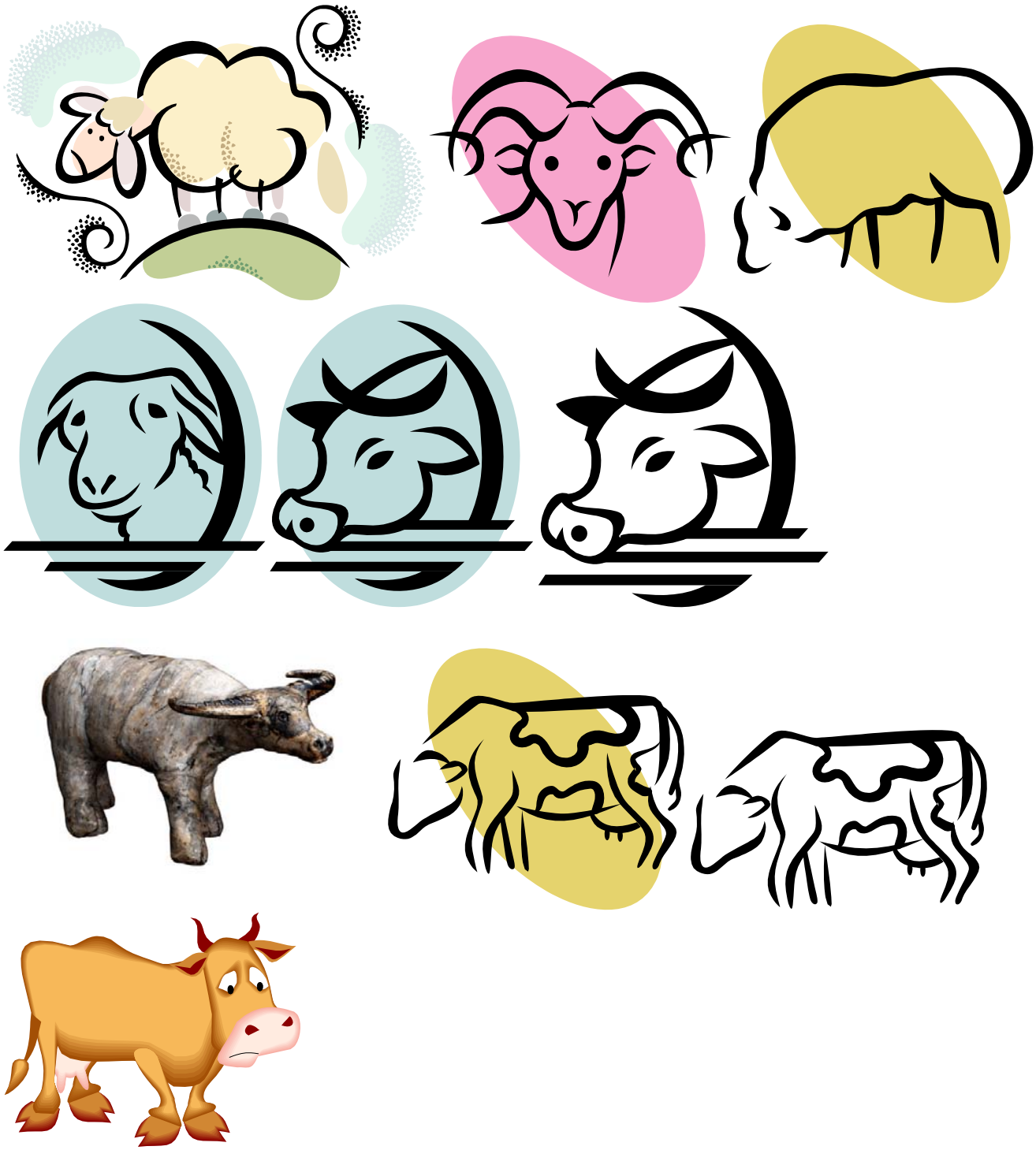


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