The Paratuberculosis Newsletter

March 2009

An official publication of the
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Notes from the Editor

2009 is well on it's way and it's with great pleasure that I present the first Paratuberculosis Newsletter for this year.

In this edition (p.4), you will find the call for abstracts for 10ICP which will be held in Minnesota in August 2009. Please note that the abstract deadline is 1st of May. It will be great to see all the Paratuberculosis enthusiasts again. Abstract submissions will be online, details can be found at http://www.cvm.umn.edu/outreach/events/icp/icpabstract/home.html. The abstract system will be ready soon, but the requirements for abstracts are already available. Please check the above website regularly for updates.

I kindly request that once your 10ICP abstracts are written and submitted to the 10ICP organising committee, you consider writing a short contribution (e.g. 10-20 lines) about your research to the next newsletter. I am quite sure that you have something interesting to share.

Søren Saxmose Nielsen
Editor

DEADLINE FOR NEXT ISSUE: May 15, 2009.

All contributions should be sent to ssn@life.ku.dk

1. IAP Business

International Association for Paratuberculosis

Financial Report-- 2008 (1/1/08 – 12/31/08)

	Checking	Savings	CD	Total
Opening balance (1/1/08)	$10,705.70	$35,772.26	$52,599.91	$99,077.87
Q1 Closing balance (3/31/08)	$14,621.31	$35,818.16	$53,191.74	$103,631.21
Q2 Closing balance (6/30/08)	$15,332.76	$35,860.27	$53,751.45	$104,944.48
Q3 Closing balance(9/30/08)	$ 3,921.73	$30,899.19	$54,135.16	$88,956.08
2008 Closing balance (12/31/08)	$4,2639.29	$30,932.81	$54,524.53	$89,693.63

Receipts

	Dues	Book Sales	Interest	Total Receipts
Q1	$4100.00	$50.00	$677.73	$4827.73
Q2	$1600.00		$601.82	$2201.82
Q3	$ 200.00		$422.63	$ 622.63
Q4	$ 350.00		$422.99	$ 772.99
Year total	$6250.00	$50.00	$2125.17	$8425.17

Expenses

	Credit card processing fees		Other	Total Expenses
Q1	$274.39			$274.39
Q2	$101.05	Open Journ Sys	$787.50	$888.55
Q3	$ 43.03	10ICP advance	$16,568.00	$16,611.03
Q4	$ 35.44			$35.44
Year total	$453.91		$17,355.50	$17,809.41

Net income

Q1	$ 4553.34		
Q2	$ 1313.27		
Q3	$ -15,988.40		
Q4	$ 737.55	Year total	$ - 9,384.24

-submitted 1/5/09, Raymond W. Sweeney, VMD; Secretary-Treasurer

10th International Colloquium on Paratuberculosis

The 10th International Colloquium (10ICP) of the International Association for Paratuberculosis will take place on the University of Minnesota campus in Minneapolis, Minnesota, Sunday, August 9, through Friday, August 14, 2009. More details, including a tentative schedule, are available at the web site
http://www.cvm.umn.edu/outreach/events/icp/home.html

Call for Abstracts – 10ICP

Instructions for 10th ICP abstracts.

Abstract preparation

Abstracts which will be considered by the Scientific Committee should meet the following requirements:
- there shall be no more than 300 words (excluding title, names of authors and affiliations);
- the abstract should contain a clear objective, materials and methods used to describe the objective, results and conclusion.

Do NOT include introductory statements in the abstract that describe the bacterium itself or its importance. Examples of these types of statements are below:
- "MAP is a gram positive acid fast bacterium the causes Johne's disease."
- "MAP may cause Crohn's disease, but this is still controversial."
- "MAP is responsible for 1.5 billion dollar losses to the dairy industry."

At the time of abstract submission, please indicate whether you prefer a poster or oral presentation.

Among submitted abstracts a limited number will be selected for a 10 minute oral presentation by the Scientific Committee for each of the 7 sessions listed below.

Abstracts that are not selected for oral presentations will be invited to give a poster presentation. It is the policy of the International Association for Paratuberculosis that no submitted abstracts for the 10ICP will be turned down for presentation, hence the term Colloquium.

You should also indicate which theme you consider the most appropriate for your presentation. The Scientific Committee can determine that the presentation is more appropriate in a different theme. The themes are:

1. Host Response and Immunology
2. Control Programs
3. Public health
4. Pathogenomics and MAP Biology
5. Diagnostics and Genotyping
6. Epidemiology
7. Johne's disease Initiatives

Abstracts are due May 1, 2009. Specific information on how to submit your abstract will be available on this web site soon.
http://www.cvm.umn.edu/outreach/events/icp/icpabstract/home.html

2. Short scientific reports

A procedure to assist in the identification of slow growing *Mycobacterium* from slant cultures

Joseph Elliot Williams and Gilles R.G. Monif

Despite the growing use of automated culture systems, many diagnostic facilities continue to utilize Herrold's egg yolk medium (HEYM) for the isolation and identification of potentially pathogenic mycobacterium. USDA-NVSL standardized procedure advocates the use of HEYM for culture isolation (Whipple et al., 1991).

The absence of detectable growth to the naked eye on agar slant does not necessarily mean that colony forming units (CFU) are not present. The following technique has been incorporated into the Infectious Disease Incorporated Laboratory Manual (Anon.) to retrieve here to "not detectable" CFU's from a slant.

This technique is presented in the hopes of assisting diagnostic laboratories which have yet to obtain automated culture systems.

1. Add 1.5 ml of phosphate buffered saline (PBS) to the assumed negative slant.
2. Using a transfer loop, gently scrape the surface of the agar. Vortex the tube for 30 seconds.
3. Remove the liquid component (some of the starting volume of PBS may be lost due to absorption by the agar). Centrifuge at high speed for 10-15 minutes.
4.
    a. For staining and identification by light microscopy, suspend the pellet in 50ul of PBS and spot onto a glass slide. Staining confirmation identifies the presence of a slow growing mycobacterium, but does not confirm which mycobacterium species is present.
    b. For DNA amplification, suspend the pellet in lysing buffer.

If the specimen is from a pig, horse, sheep or bird, perform direct polymerase chain reaction (PCR) test using IS*1311* primers that identify *Mycobacterium avium* subsp. *paratuberculosis*, *M. avium*, and some mycobacterium between the two.

If positive or if the original source of the material is from a cow, go directly to testing with IS*900* based primers.

References

Whipple DL, Callihan DP, Jarnagin JL, 1991. Cultivation of *Mycobacterium avium* subsp. paratuberculosis from bovine fecal specimens and a suggested standardized procedure. J Vet Diag Invest. 3: 365-373.

Anon., Infectious Diseases Incorporated, Bellevue, Nebraska 68123

4. Comments & Opinions

The difference between an "A" and a "The"

Gilles R. G. Monif

Some times a truth becomes over extended by inference, and is so doing loses its status as a truth. When the distinction between an "a" and a "the" becomes blurred, the potential for interpretative errors is created.

Mycobacterium avium subspecies *paratuberculosis* (Map) is a cause of mycobacterium infection in herbivores. Map is not the cause of mycobacterium infection or in herbivores. *Mycobacterium avium* and *Mycobacterium bovis* are causes comparable disease in herbivores.

USDA and the Food and Drug Administration have required that Map diagnostic test have their specificity based on the IS*900* insertion sequence. USDA's recommendations relative to herd management assume that Map is the cause of Johne's disease. Map isolates identified by the IS*900* insertion sequence is a cause of Johne's disease in dairy cows, but are they the cause of Johne's disease in dairy cows?

The specificity implied by the IS*900* insertion sequence for mycobacteria causing Johne-like disease may not be all inclusive or specific. The literature contains a number of papers which question the specificity of the IS*900* insertion sequence. Coffin et al. (1992) argued that on the basis of restriction fragment length polymorphism analysis, some M. avium subspecies paratuberculosis are more M. avium-like, while others were not. Cousins et al. isolated mycobacteria distinct from Map from the feces of ruminants possessing IS*900*-like sequences detectable by IS*900* polymerase chain reaction (PCR) (Cousins et al., 1999). Whittington et al. (1998) identified a polymorphism specific to a Map cow as well as others common to both the "C" and "S' types of Map compared. Englund et al. (2002) described recovery from a healthy cow a mycobacterium harboring one copy of a sequence with 94% identity to IS*900* at the nucleic acid level. The isolate was shown to be related to *Mycobacterium cookie*, as assessed by 16S rRNA sequencing. Other authors have been able to achieve strong amplification with several PCR primers described for the detection of IS*900*. *M. terrae*, *M .xenopi*, *M. scrofulaceum*, *M. chelonae* and strains related to *M. cookii* have been shown to cross-react with IS*900* primers used to detect Map (Englund et al., 2002; Tasara et al., 2005). In an analysis of 23 Map isolates, Semret et al. (2006) demonstrated that IS*900* is highly conserved with only two sequevars distinguishing sheep and cattle lineages. They have contended that amplification of IS*900*-like sequences is not sufficient as a proxy for Map.

Frothingham (1999) and Turenne et al. (2007) has contended that the pathogenic mycobacteria emanated from within an evolutionary bottle neck in which Map appears to have evolved from *M. avium*. Given the fact that both Map and *M. avium* share a comparable demographic profile and produce a comparable disease entity, it is only logical to postulate that between the two species pathogenic genomic variants exist.

Mycobacterium avium is a well documented pathogen in swine, domestic fowl and migratory birds. A growing body of evidence is emerging that *M. avium* is the primary cause of Johne's disease in horses. Since our initial reports of equine Johne's disease in horses, we have identified three additional cases (Sheppard et al., 2008; Monif et al., 2008). In addition, Dr. C. C. Wu (Purdue University) has identified a sixth horse with Johne's disease due to *M. avium*. The identity of the pathogenic mycobacterium in horses has been confirmed by three different diagnostic facilities as being *M. avium* and not Map.

If the existence of genomic polymorphism among pathogenic mycobacteria is confirmed, the finding will weaken one of the major buttresses against the postulate that Map is the (rather than a) causative agent of Crohn's disease in humans. Previously, mycobacteria other than Map have been demonstrated by DNA amplification to be present in the tissues of individuals with Crohn's disease. If the 1311 primers and probes were to

demonstrate that the gastrointestinal tissues of an even greater number of individuals with Crohn's disease contain the DNA of pathogenic mycobacteria, another buttress denying mycobacterial causality of Crohn's disease would be compromised.

References

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4. Paratuberculosis Calendar

Please report to Søren Nielsen (ssn@life.ku.dk) should you have knowledge of any events that you find relevant to include in the calendar.

2009
July

August 9-13, 2009. 10th International Colloquium on Paratuberculosis, St. Paul/ Minneapolis, Minnesota, USA (http://www.cvm.umn.edu/outreach/events/icp/icpabstract/home.html).

August 10-14, 2009. 12th International Symposium on Veterinary Epidemiology and Economics. Durban, South Africa (http://www.isvee12.co.za).

August 25-28, 2009. *M. bovis* V Conference, Wellington, New Zealand (http://www.mbovisconference.org/)

5. List of Recent Publications

Alexander DC, Turenne CY, Behr MA, 2009. Insertion and deletion events that define the pathogen *Mycobacterium avium* subsp. *paratuberculosis*. J Bacteriol. 191:1018-1025.

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