

The Paratuberculosis Newsletter

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Notes from the Editor

There are many people involved in paratuberculosis research, diagnosis, control and surveillance activities around the world. The Paratuberculosis Association would greatly benefit from the sharing this information. I would like to thank contributors to the current issue, but sadly the number of contributions to this issue has been few. I urge you to provide information on events, studies or the like that you consider of relevance to us. Lastly, a reminder that the deadline for the next issue is August 15, 2008.

Søren Saxmose Nielsen
Editor

DEADLINE FOR NEXT ISSUE: August 15, 2008.

A reminder will be sent in July.
If your contribution is > 250 words, please ask for guidelines on how to format the contribution prior to submission
All contributions should be sent to ssn@life.ku.dk
If you have not received a receipt within a few days, please re-send!

1. IAP Business

Emeritus members of International Associations for Paratuberculosis

The International Association for Paratuberculosis (IAP) can grant emeritus membership in recognition of individuals, who have been members of the Association for a considerable period of time, have had paratuberculosis as an important focus of their research or service work, and have made significant contributions to the field and/or the Association.

Drs. Finn Saxegaard of Norway and Marie Françoise Thorel of France were awarded the emeritus status at the 7th International Colloquium on Paratuberculosis in Bilbao, Spain in 2002 and at the 8th ICP in Copenhagen, Denmark in 2005, respectively. There were no candidates to the awards proposed for the 9 ICP.

Dr. Thorel has had a long career (30 years) in the paratuberculosis research field, being one of the participants in the first International Colloquium on Paratuberculosis held in Ames, Iowa in 1983. She was the organizer of the second Colloquium in Paris in 1988 from which the International Association for Paratuberculosis started. She also was a co-founder of the IAP and Director representing France for several years.

She has written 90 scientific papers on paratuberculosis dating back to the first reports on the bacteriological characterization of *Mycobacterium avium* subsp. *paratuberculosis*, as well as many articles on the isolation and culture of these bacteria. Her most important contribution was, probably, the numerical taxonomy study that, following adscription of Map to the *M. avium* species by Dr. Saxegaard, established the relevant differences that prompted its classification as a subspecies.

She has also written highly relevant reference works on the diagnosis of paratuberculosis like the French reference protocol for Map isolation, and the OIE Manual section on paratuberculosis. She has taken part in different advisory committees like the European Commission ad hoc committee on possible links between Crohn's disease and paratuberculosis.

Dr. Thorel has finished her professional career in 2004 as Senior Research Officer and Deputy Director of the AFSSA at Maisons-Alfort (France), where she also was Head of the National Reference Centre for Mycobacteria, Head of the National and OIE Reference for Bovine Tuberculosis and Paratuberculosis, and Head of the FAO Collaborating Centre on Mycobacteria.

Dr. Saxegaard has also contributed substantially to research in paratuberculosis and of his 68 journal publications, very few have not been on mycobacteria. He was also among the few pioneers attending that first colloquium in Ames.

Dr. Saxegaard was in charge of diagnostics of tuberculosis and paratuberculosis at Dept. of Tuberculosis at the National Veterinary Institute, where he worked from 1979 to 1999. His first research project at this department was investigations on whether wild animals could serve as a reservoir for mycobacterial infections. Sero-typing of mycobacterial isolates was used in this project, and the results led to an interest in differentiation and thereby classification and nomenclature wood pigeon mycobacteria, as distinct from what were later known as *Mycobacterium avium* subsp. *paratuberculosis*.

Paratuberculosis has primarily been a problem in goats in Norway, and not in cattle, although paratuberculosis was described in Norwegian cattle already in 1908. Therefore, it



was hypothesised that an apparently goat-specific strain existed, and studies were carried out. The results showed that goats developed clinical disease following experimental inoculation, whereas cattle did not. These and other results were compiled in his thesis “Research into mycobacterial isolates from wild animals and related known species of mycobacteria”, which was published from the National Veterinary Institute in Oslo, Norway in 1989. Dr. Saxegaard was an enthusiastic supporter of vaccination and thanks to him paratuberculosis ceased to be a problem in goats in Norway for many years.

Dr. Saxegaard retired in 1999.



Dr. Marie Françoise Thorel



Dr. Finn Saxegaard

Currently, Drs. Thorel and Saxegaard are the only emeritus members of the Association. Longstanding members who have retired may be given the membership status “Emeritus”. Nominations can be made from all members, but since these people keep giving advice and actively participating in the research on paratuberculosis in spite of having officially “retired” from most of their institutional positions they also seem to prefer to keep their links to research in their favourite subject through their full membership in the IAP. This might make the Emeritus awards go vacant for some time.

2. Communications from members

Dutch Dairy industry initiates action on ParaTB and Salmonella

This spring 2008 the Dutch dairy industry starts a nationwide action on ParaTB and Salmonella in dairy cows.

Dairy farmers are invited to test their dairy cows individually on ParaTB.

In 2008 the dairy sector invests in total ca. 5 million euro in the reduction of ParaTB and Salmonella.

Both projects are a joint initiative of the Dutch Dairy Organisation (NZO), the Dutch Farmers organization (LTO) and the Product Board for Dairy (PZ).

The ParaTB testing is in 2008 free of charge for the dairy farmers.

Tests are done by the Animal Health Service in Deventer in individual milk samples with a high specific antibody ELISA.

P. Franken, PhD, DVM.

3. Short scientific reports

Detection of *Mycobacterium avium* subsp. *paratuberculosis* by Acid-fast Staining and nested PCR in the Placentome of a Pregnant Dairy Cow with Johne's disease

Claus D. Buergelt, J. Elliot Williams

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Background

Johne's disease, an insidious infectious disease of ruminants, occurs worldwide. The disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), a facultative intracellular pathogen. The principal pathway of transmission for Map is thought to be the fecal-oral route, and the calf is the most susceptible to such transmission. The isolation of Map from sites distant of the intestinal tract such as udder, fetus, liver, male reproductive tract and peripheral blood has suggested active dissemination of Map and alternate transmission pathways such as milk, semen, and transplacental infection of fetuses (McQueen et al., 1979; Seitz et al., 1989; Sweeney et al., 1992). Such observed dissemination of Map should broaden the epidemiologic concepts of Johne's disease.

Culture studies have shown that between 20% and 40% of fetuses from cows with clinical Johne's disease were infected in utero, compared with 9% of fetuses being culture positive from subclinically infected animals (Sweeney et al., 1992).

This communication describes the visualization of Map bacilli in a placentome of a pregnant Holstein cow that was removed from the herd because of clinical signs of Johne's disease and subsequently necropsied and microscopically examined for evidence of the disease.

Case Report

A 7-year old Holstein dairy cow from a Johne's disease proven herd, 8 months pregnant, developed signs of diarrhea, and weight loss. The cow was subjected to antemortem serologic analysis of Johne's disease through ELISA and AGID. She reacted low positively on ELISA and was negative on AGID after 48 hours. A nested PCR (nPCR) performed on blood and antemortemly obtained allantoic fluid through allantoiscentesis on the standing animal was negative for Map DNA. With a presumed diagnosis of Johne's disease, the animal was euthanized and submitted for a complete necropsy. Necropsy performed on the dam provided the pathologic diagnosis of a granulomatous enteritis and lymphadenitis. Only few acid-fast bacilli were demonstrated with special stain in the target sites confirming the case as paucibacillary Johne's disease. The microscopic examination of various organs from the fetus was negative for evidence of microscopic changes. Likewise, tissue examination via nPCR was negative as was recollected allantoic fluid, amniotic and fetal abomasal fluids.

While collecting one randomly selected cotyledon during the necropsy procedure, a tissue imprint was made from this tissue and subjected to acid-fast stain and nPCR. The acid-fast stain revealed one cluster of bacilli likely in a macrophage (Fig. 1) and when subjecting the tissue to nPCR, amplicons were visualized on gel electrophoresis at the expected locations with the primers used (Fig.2). Fetal liver, spleen, brain, allantoic fluid as well as amniotic fluid were negative with nPCR.

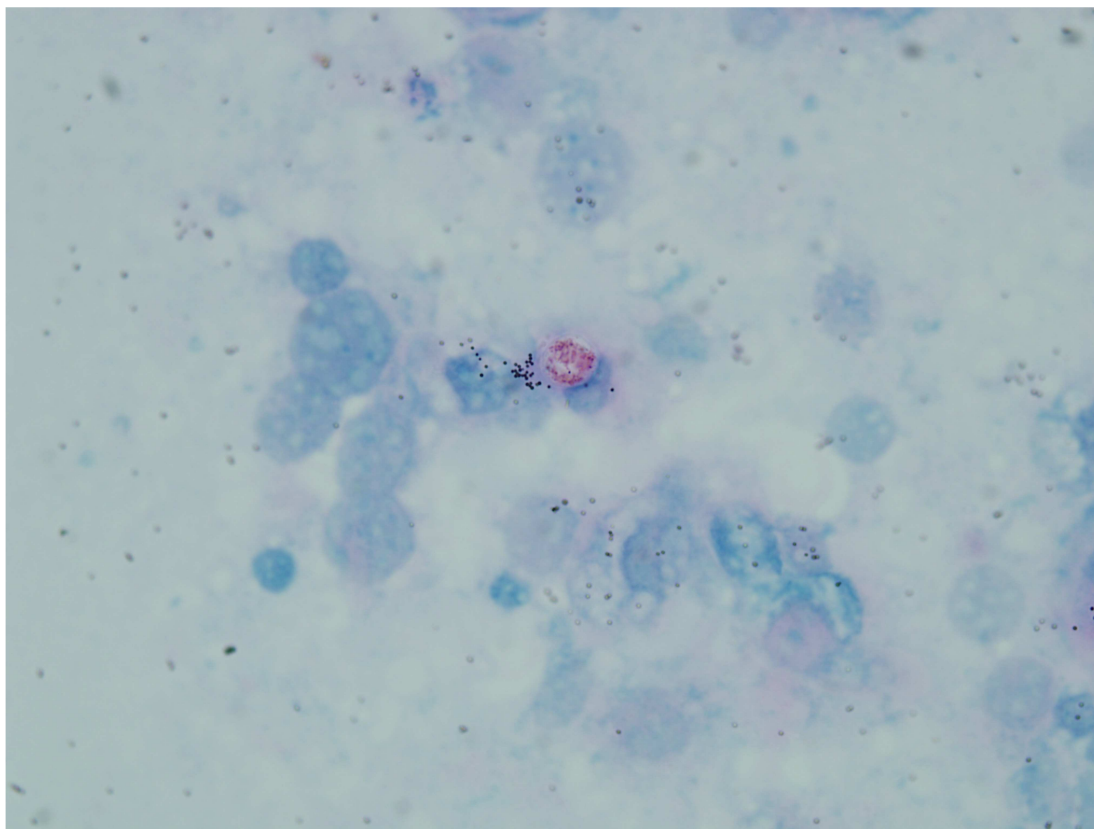


Fig. 1. Cluster of acid-fast positive rods in the cytoplasm of a cotyledonal resident cell. Ziehl-Neelsen, x100.

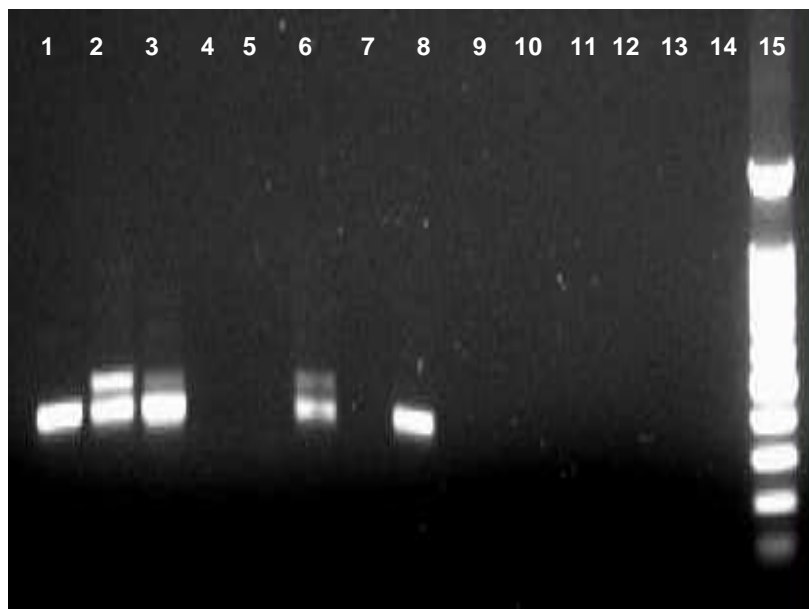


Fig. 2. Gel electrophoresis of IS900 amplification products of acid-fast positive bacilli after tissue imprint subjected to nPCR. Single bands are between 400 and 300 bp. Lanes 1,2 pos controls from lab strain # 295 ; lane 3 (pos.) ileo-cecal lymph node from JD positive dam; lane 4 liver from dam, lane 5 supramammary lymph node from dam, lane 6 (pos.) mesenteric lymph node from dam, lane 7 fetal spleen, lane 8 (pos.) placentome, lane 9 fetal brain, lane 10 fetal liver, lane 11 abomasal fluid fetus, lane 12 allantoic fluid, lane 13 amniotic fluid, lane 14 neg. control (dH₂O), lane 15 molecular markers

Conclusion

The visual tissue demonstration of Map in the placentome of a Johne's disease cow and the subsequent verification of its specific Map DNA, is proof of a bacteremic phase and in-utero transmission of the bacilli, interestingly in a paucibacillary animal in this case. Microscopic examination of the cotyledon did not show granulomatous inflammation and the fetal tissues also were free from granulomatous inflammation. This appears to be the first description of Map bacilli in the bovine placentome and visual local demonstration of a transplacental potential of transmission.

Ruminant placentation is grossly classified as cotyledonary with caruncles and cotyledons forming the maternal-fetal interphase. Ruminant placentation microscopically is classified as syndosmochorial (epitheliochorial) not allowing for transfer of maternal immunoglobulins to the fetus. It also appears that the fetus itself is immunotolerant/immunoincompetent towards Map regardless of gestation as fetal inflammatory responses as seen in the dam are not expressed. Specific antibody and CMI studies in Map infected fetuses are missing to support this hypothesis.

We have utilized a percutaneous collection technique for allantoic placental fluid on the standing animal in the third trimester of pregnancy to analyze for transplacental infection with Map as a tool for managerial decision of culling dam and fetus should both be detected as positive to infection with Map (Callan et al., 2002).

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Persistent Bovine Fecal Map Shedding in First Month

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Much of the information relative to the fecal shedding of *Mycobacterium avium* subspecies *paratuberculosis* (Map) in calves is derived from experimentally induced infection (Sweeney et al., 2006; Wu et al., 2007). In the calf model system, after 48 hours following oral administration of an infectious dose, Map can no longer be identified in fecal samples for the next nine or more months (Waters et al., 2003). Little or no data is available concerning early fecal shedding by congenitally or neonatally infected calves. Serial fecal cultures, and fecal direct and nested polymerase chain reaction determinations of Map DNA (FecaMap®, Bellevue, Nebraska) were available on a female Holstein calf born on October 26, 2006 to a cow with clinical Johne's disease (Table 1).

Table1. Serial fecal cultures for *Mycobacterium avium* subspecies *paratuberculosis* (Map) and PCR determinations of Map DNA

Date	Culture	Fecal PCR	
		Direct	Nested
Nov. 6, 2006	Positive		
Nov. 16, 2006		Negative	Positive
Nov. 22, 2006		Negative	Positive
Nov. 30, 2006	Positive*	Negative	Positive
Dec. 6, 2006		Negative	Positive
Dec. 11, 2006		Positive	Positive
Jan. 17, 2007		Negative	Positive
Feb. 8, 2007		Negative	Positive
Feb. 22, 2007	Positive*	Negative	Positive

* 20-40 colonies per slant

Whether the calf's Map infection was congenitally or postnatally acquired cannot be determined. Nevertheless, the quantitative assessments of the organism and the persistence of detectable DNA within its fecal samples make simple passive shedding pass-through unlikely (Sweeney et al., 1992). What this case demonstrates is that in naturally occurring Map infection, most probably congenital infection, fecal shedding can be demonstrated within the first month of life.

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Duration of Bovine Maternally Acquired Antibodies to *Mycobacterium avium* subsp. *paratuberculosis*: A Case Study

J. Elliot Williams, Kristina Steinfieldt, Gilles R.G. Monif

CASE REPORT

Cow YL710 was a seven year old *Mycobacterium avium* subsp. *paratuberculosis* (Map) AGID positive, pregnant Holstein cow with an initial body condition score of 3.25. The diagnosis of Map had been established by recovery of Map from fecal samples and the repeated identification of Map DNA as determined by nested polymerase chain reaction (FecaMap®, Bellevue Nebraska) test within fecal samples. Repeated sampling of cow YL710's milk prior to, after parturition, and during the nursing period were negative for the presence of Map DNA as determined by both direct and nested PCR.

The calf was an apparently health term female calf on October 20, 2006. At birth, she weighed an estimated 85 pounds. She was kept with and feed from her mother for approximately four months. Based upon serial fecal direct and nested polymerase chain reaction tests and fecal cultures, the calf was deemed to have been infected *in utero*. The calf's development through age nine months has been normal.

The comparative calf/mother AGID and ELISA titers are listed in Table 1. When first tested on February 11, 2006, the calf's AGID and Map ELISA tests results were positive and 2.76 respectively. The mother's corresponding AGID and Map ELISA test results were positive and 3.32. By December 6, 2006, the calf's Map ELISA titer was below the cut-off used to determine a positive test (ELISA greater than 2.0). The maternal Map ELISA titer on May 10, 2007 was 3.4. The calf's AGID test remained positive until approximately February 1, 2007 (77 days). The next 14 AGID tests were negative.

Little information has been published specifically demonstrating how long organism specific antibodies to a derived from maternal bovine colostrum can give a false positive diagnostic test result for Map. The calf's ELISA test became non-diagnostic within approximately 21 days (three weeks). The significant change in ELISA titer appears to indicate that a significant reduction of passively transferred immunoglobulins had occurred by that date. In the additional 56 days of observation, the calf's Map ELISA titer continued to progressively diminished. A positive Map AGID test was demonstrable for approximately an additional 56 days (8 weeks). The differences in duration of detectable ELISA and AGID suggest that in cows with Johne's disease, these tests appear to measure different key antigenic determinants.

With respect to the overall elimination of maternally acquired IgG immunoglobulins in other herbivores, when tested by single radial immunodiffusion to quantify specific IgG immunoglobulin levels in newborn foals, maternally acquired immunoglobulins appeared to be effectively cleared between the 10th and 12th week of life (Tizard, 2004).

Reference

Tizard IR (ed), 2004. Immunity in the Fetus and Newborn in Veterinary Immunology. Seventh Edition. Saunders, Philadelphia, Pennsylvania, p. 228-229.

Table 1. Comparison of ELISA and AGID test results on cow #YL710 and her calf

Date	YL710 ELISA [#]	Calf ELISA [#]	YL710 AGID	Calf AGID
Aug. 6/2006	NA		pos	
Aug. 15/2006	2.39		neg	
Aug. 18/2006	1.59		pos	
Aug. 22/2006	1.53		pos	
Aug. 31/2006	1.62		pos	
Sept. 7/2006	1.03		pos	
Sept. 14/2006	0.57		pos	
Sept. 21/2006	1.71		pos	
Sept. 28/2006	1.06		pos	
Oct. 5/2006	1.2		pos	
Oct. 12/2006	1.24		pos	
Oct. 19/2006	3.5		pos	
Oct. 26/2006	3.32	2.76	pos	pos
Nov. 2/2006	2.9	2.38	pos	pos
Nov. 9/2006	2.3	2.37	pos	neg
Nov. 16/2006	2.31	2.24	pos	pos
Nov. 22/2006	2.61	2.33	pos	pos
Nov. 30/2006	2.66	1.96	pos	pos
Dec. 6/2006	2.4	1	pos	pos
Dec. 14/2006	2.8	1.26	pos	pos
Dec. 22/2006	2.4	1.4	pos	pos
Jan. 4/2007	1.2	-	pos	pos
Jan. 11/2007	2.06	1.52	pos	pos
Jan. 17/2007	2.52	0.89	pos	pos
Jan. 25/2007	2.62	1.05	pos	pos
Feb. 1/2007	2.4	0.44	pos	neg
Feb. 8/2007	2.5	1.04	pos	neg
Feb. 15/2007	2.11	0.98	pos	neg
Feb. 22/2007	2.73	1.24	pos	neg
Mar. 1/2007	0.69	0.76	pos	neg
Mar. 8/2007	3.5	1.5	pos	neg
Mar. 15/2007	3.3	0.75	pos	neg
Mar. 22/2007	3.1	0.61	pos	neg
Mar. 29/2007	2.8	0.78	pos	neg
Apr. 5/2007	3.2	0.47	pos	neg
Apr. 12/2007	3.8	0.59	pos	neg
Apr. 19/2007	3.4	0.42	pos	neg
Apr. 26/2007	2.6	0.44	pos	neg
May 3/2007	3.2	0.46	pos	neg
May 10/2007	3.4	0.38	pos	neg
May 24/2007	2.8	na	pos	na

[#] ELISA interpretation: 0.1 - 1.5: negative; 1.6-1.99: suspicious; 2.0-2.5: low positive; ≥2.6 - positive.

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 or August, 2009 (dates not final). 10th International Colloquium on Paratuberculosis, St. Paul/ Minneapolis, Minnesota, USA.

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

5. List of Recent Publications

- Balseiro A, García Marín JF, Solano P, Garrido JM, Prieto JM. Histopathological classification of lesions observed in natural cases of paratuberculosis in free-ranging fallow deer (*Dama dama*). *J Comp Pathol*. 2008, 138:180-8.
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