Paratuberculosis

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

<table>
<thead>
<tr>
<th>Test (Total)</th>
<th>For</th>
<th>Specificity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement fixation test (81)</td>
<td>Antibody</td>
<td>Paratuberculosis</td>
<td>81 wild ruminants</td>
</tr>
<tr>
<td>Immune diffusion test (275)</td>
<td>Antibody</td>
<td>Paratuberculosis</td>
<td>275 wild ruminants</td>
</tr>
<tr>
<td>Culture HPC-sedimentation (15 888)</td>
<td>\textit{M. avium} subsp. \textit{paratuberculosis}</td>
<td>Paratuberculosis</td>
<td>12 812 cattle faeces, 376 cattle tissues, 113 milk filters (cattle), 1 901 wild ruminant faeces, 406 wild ruminant tissues, 30 Crohn’s patient faeces, 250 cow’s milk samples</td>
</tr>
<tr>
<td>Quantitative real-time PCR (42)</td>
<td>\textit{M. avium} subsp. \textit{paratuberculosis}</td>
<td>F57 and IS900 \textsuperscript{1}</td>
<td>42 milk samples</td>
</tr>
<tr>
<td>PCR (156)</td>
<td>Isolates</td>
<td>Quadruplex system for \textit{M. avium} species\textsuperscript{2} and \textit{M. avium} complex members identification</td>
<td>144 Mycobactin J dependent: 107 cattle isolates, 37 deer isolates, 12 Mycobactin J. non-dependent</td>
</tr>
<tr>
<td>IS900 RFLP (246)</td>
<td>Isolates</td>
<td>Standardized method\textsuperscript{3}</td>
<td>169 cattle origin, 61 wild ruminants origin, 5 goat, 7 rabbit, 2 sheep, 2 environment</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Specific fragments for paratuberculosis; home made technique, paper under preparation.
\textsuperscript{2} Home made technique for the identification of members of \textit{M. avium} species and \textit{M. avium} complex; paper under preparation.

2. Production and distribution of diagnostic reagents

Mycobactin J for \textit{Mycobacterium avium} subsp. \textit{paratuberculosis} Mycobactin dependent strains isolation.

A total of 1 000 ml was used in our laboratory and a total of 485 ml were supplied to the laboratories in the Czech Republic: State Veterinary Diagnostic Institute, Prague (280 ml), Olomouc (175 ml) and Jihlava (30 ml).

A total of 170 ml were supplied to the EC countries: (50 ml) to The Netherlands, (20 ml) to Cyprus and (100 ml) to the Slovak Republic.
Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

None

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

None

5. Research and development of new procedures for diagnosis and control

Conventional “quadruplex” PCR system for the identification of members of *M. avium* species (including *M. avium* subsp. *paratuberculosis*) and *M. avium* complex was routinely used and paper is under preparation.

Our laboratory developed the technique for isolation of the total ultra pure DNA (including DNA of *M. avium* subsp. *paratuberculosis*) from up to 50 ml of milk. Subsequently, the presence of *M. avium* subsp. *paratuberculosis* could be detected by two independent real time PCR systems with internal amplification control based on two specific independent loci: the multiple copy insertion sequence IS900 and a single copy fragment F57. The developed technique allows, on the assumption that 50 ml of the milk is analysed, to detect at least 3 *M. avium* subsp. *paratuberculosis* cells in 1 ml of the starting amount of milk using F57 fragment as a target; whereas, IS900 real time PCR system is more sensitive and is able to detect approximately 1 cell in 3 ml of the milk. Paper is under preparation.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

RFLP analysis was carried out on the following *M. avium* subsp. *paratuberculosis* isolates from Argentina (23 cattle and 5 deer isolates), Austria (18 cattle isolates), Slovenia (9 cattle isolates), Scotland (2 deer isolates) and the Czech Republic (119 cattle, 54 deer, 5 goat 7 domestic rabbit, 2 sheep and 2 environmental isolates).

7. Provision of consultant expertise to OIE or to OIE Member Countries

Consultations and discussion about paratuberculosis control and/or certification program during the business trips in Europe (Slovakia, Austria, Germany, Spain, Ireland, Denmark, Belgium, The Netherlands, United Kingdom, Sweden, Norway, France, Greece and Cyprus) and in America (Argentina, Brazil, USA, Uruguay).

8. Provision of scientific and technical training to personnel from other OIE Member Countries


**Dr. Fernando Paolicchi**, Animal Health Group, INTA and Faculty of Agrarian Sciences, University of Mar del Plata, Argentina. Topic: Detection of *Mycobacterium avium* subsp. *paratuberculosis* in different matrices and typing of isolates by different molecular laboratory techniques. 17.5.-11.8.2006.

**Dr. Johannes Lorenz Khol**, University of Veterinary Medicine, Vienna, 2nd Medical Clinic for Ruminants and Swine, Austria. Topic: Detection of *Mycobacterium avium* subsp. *paratuberculosis* in different matrices and typing of isolates by different molecular laboratory techniques. 8.-26.5.2006 and 8.9.2006.
Dr. Louis Fischer, Geschäftsführer, Labovet GmbH, Labor für Veterinärdiagnostik und Hygiene, Vienna, Austria. Topic: Detection of Mycobacterium avium subsp. paratuberculosis in different matrices and typing of isolates by different molecular laboratory techniques. 8.9.2006.

Univ. Prof. Dr. Martin Wagner, Dipl. ECVPH, Coordinator Molecular Microbiology Group at the Institute for Milk Hygiene, & Technology and Food Science Coordinator Research Focus Food Safety and Risk Assessment, Department for Veterinary Public Health and Food Science, Vienna, Austria. Topic: Detection of Mycobacterium avium subsp. paratuberculosis in different matrices and typing of isolates by different molecular laboratory techniques. 8.9.2006.


9. Provision of diagnostic testing facilities to other OIE Member Countries

We have examined by culture cattle tissue samples and faeces from Austria, faeces from The Netherlands and Slovakia.

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

None

11. Participation in international scientific collaborative studies

Our laboratory was involved in the following national and international projects dealing with the development of different the techniques (liquid culture, quantitative real time PCR etc.) for the detection of M. avium subsp. paratuberculosis in different matrices for the developing the certification program for paratuberculosis:

- **National grants**


- **European Union Grants:**


  Study on paratuberculosis in correlation with the food, the animal health and the environment. Research promotion foundation's framework programme for research and technological development 2003-2005. No. RPFS FP 2003-2005; AEIFO 1104/02. Co-ordinator: Dr. Liapi Maria, Veterinary Officer at Cyprus Veterinary Services, Nicosia, Cyprus.

12. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

- **Presentations at international conferences and meetings**


- **Scientific publications in peer-reviewed journals**


- **Other communications**


- **Other publications related to mycobacteriology**


