Lymphadenitis in children is caused by *Mycobacterium avium hominissuis* and not related to ‘bird tuberculosis’

L. E. S. Bruijnesteijn van Coppenraet · P. E. W. de Haas · J. A. Lindeboom · E. J. Kuijper · D. van Soolingen

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**Abstract** *Mycobacterium avium* is the most commonly encountered *mycobacterium* species among non-*Mycobacterium tuberculosis* complex (nontuberculous mycobacteria) isolates worldwide and frequently causes lymphadenitis in children. During a multi-centre study in The Netherlands that was performed to determine the optimal treatment for mycobacterial lymphadenitis, concern was expressed in the media about the possible role of birds as sources of these *M. avium* infections, referred to as ‘bird tuberculosis.’ To examine the involvement of birds in mycobacterial lymphadenitis, 34 *M. avium* isolates from lymphadenitis cases were subjected to IS1245 restriction fragment length polymorphism (RFLP) typing. This genotyping method enables the distinction of the subspecies *M. avium* subsp. *hominissuis* and the ‘bird-type’ *M. avium* spp. *avium*. Highly variable RFLP patterns were found among the lymphadenitis *M. avium* isolates, and all belonged to the *M. avium hominissuis* subspecies. A relation to pet birds in the etiology of mycobacterial lymphadenitis could not be established, and the source of the infections may be environmental.

**Introduction**

While the absolute number of clinical isolates of nontuberculous mycobacteria (NTM) has worldwide increased gradually with the upcoming of the acquired immunodeficiency syndrome (AIDS), the number of isolates in The Netherlands remained stable in the last decade. In the period from 1996 to 2005, 4,640 NTM isolates were collected at the national tuberculosis reference laboratory (Dutch National Institute for Public Health and the Environment, RIVM), of which 1,484 (32%) belonged to the *M. avium* complex. *Mycobacterium avium* is not only a well-known cause of pulmonary disease, especially in immunocompromised patients, but is also the most commonly encountered species in mycobacterial lymphadenitis in children. The exact incidence of *M. avium* lymphadenitis is difficult to assess but is estimated at 1.15 per 100,000 children (1–18 years) in The Netherlands [1].

From 2001 to 2004, a nationwide multi-centre study to examine the optimal treatment of NTM mycobacterial cervicofacial lymphadenitis (surgery vs medical therapy) was performed [2]. In this study, it was found that 94 (70%) of 135 diagnosed *Mycobacterium* infections were caused by *M. avium* [3]. As a consequence of this study, numerous articles appeared in regional and national newspapers in The Netherlands in which the concern was expressed that mycobacterial lymphadenitis in children is a form of ‘bird tuberculosis’ (selection of newspaper articles: [4–8]). This is based on the conventional but still widely accepted dogma that *M. avium* infections in humans are derived from birds. Moreover, many patients included in the
treatment study and diagnosed with *M. avium*-associated lymphadenitis appeared to keep pet birds in the household or had an otherwise frequent contact with birds, as was demonstrated in a questionnaire for epidemiological purposes [3]. However, no demographic information on the presence of pet birds in the general population in The Netherlands is available, and it therefore remains unclear whether there is any correlation between household pets and lymphadenitis in children.

With the improved possibilities for the diagnosis of mycobacterial infections, new (sub-)species have been identified, and new insights in taxonomy are being developed. Previously, in the *M. avium–M. intracellulare* complex, 28 serotypes were distinguished [9]. The species *M. avium* consists of the subspecies *M. avium* ssp. *avium*, *M. avium* ssp. *silvaticum* and *M. avium* ssp. *paratuberculosis* [10]. Restriction fragment length polymorphism (RFLP) typing, using insertion sequence IS1245 as a probe, is a standardised epidemiological tool for the molecular typing of *M. avium* [11, 12]. The targeted insertion element IS1245 is specific for the species *M. avium* and is present in a highly variable number and location in the genome of these bacteria.

Application of this typing method led to the recognition that *M. avium* isolates from birds of a wide variety of species exhibited one identical pattern [12–15]. This three band pattern was designated the ‘bird-type’ RFLP pattern. To exclude the raised confusion in the epidemiology of *M. avium* infections, the ‘bird-type’ *M. avium* strains were further characterised, and this subspecies was named *M. avium avium* [14]. The *M. avium* isolates from human and porcine sources showed highly polymorphic IS1245 RFLP patterns and were designated ‘*M. avium hominissuis*’ [14].

In this study, the contribution of the bird-type *M. avium avium* to lymphadenitis in children in The Netherlands is examined to confirm or exclude the involvement of birds in the aetiology of this disease.

**Materials and methods**

**Strains and culturing methods**

*M. avium* strains were isolated from children included in the lymphadenitis treatment study. Inclusion criteria for this study were an enlarged cervicofacial lymphadenitis for a period longer than 3 weeks, with negative serology for other infectious causes of chronic lymphadenitis. Excluded were patients with an immunosuppressive therapy or disease [3]. In addition, skin testing with *M. tuberculosis* complex and three NTM sensitins was performed [16]. Diagnosis was confirmed by culture and/or specific real-time polymerase chain reaction (PCR), performed on lymph node aspirates or surgically obtained tissue biopsies [3, 17]. Culturing was performed at 35°C in liquid *Mycobacteria* growth indicator tube medium enriched with polymyxin B–ampicillin B–nalidixic acid–trimethoprim–azlocillin mixture and oleic acid–albumin–dextrose complex or on solid *Löwenstein Jensen* medium (Becton Dickinson, Alphen a/d Rijn, The Netherlands). Strains were sent to the RIVM in Bilthoven and processed in IS1245 RFLP typing (Table 1).

**Species identification**

Real-time PCR was performed as described previously [17]. A genus-specific and a *M. avium*-specific PCR were performed on all isolated strains as well as on deoxyribonucleic acid (DNA) extracts of bird materials. When a positive signal was found in exclusively the genus-specific real-time PCR, the amplicon was purified using a gel-extraction kit (Qiagen, Venlo, The Netherlands), and the PCR product was sequenced to determine the species the respective bacteria belonged to. Sequencing was performed on basis of the PCR primers using the Big Dye Terminator ready reaction mix (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and analysed on an ABI 3100 automatic Sequencer (Applied Biosystems). A second identification was performed at the RIVM, either by 16S sequencing [18] or by a reverse line blot (InnoLipa V2, Innogenetics, Gent, Belgium), to confirm the identity of the *M. avium* strains.

**Molecular typing**

A database has previously been assembled by the RIVM of RFLP patterns from *M. avium* isolates from humans in The Netherlands in the period 1996–1997 or from animals and soil in a period of decades. IS1245 RFLP typing was performed as described previously [11, 15]. RFLP patterns of 34 *M. avium* isolates from lymphadenitis cases were analysed and compared with known patterns in the database using the Bionumerics software (Applied Maths BVBA, Sint-Martens-Latem, Belgium).

**Bird materials**

Materials of pet birds from two patients diagnosed with *M. avium*-associated lymphadenitis were collected. One patient owned two parakeets and kept them inside the house. Another owned eight parakeets, and they were kept in an aviary in the yard. Considering the age of the included children, it is not expected that the children were the direct caretakers of the birds. The parakeets had different ages, gender and breed. The birds did not show any sign of illness. Swabs were collected from the cloacae of the parakeets, and faeces were scraped from the bottom of the cages (as fresh as possible). All samples were divided equally in two portions and examined by two different institutes (National Institute
Table 1  Characteristics of patient strains

| Strain number | Onset of disease | County/region | Age (month) | Pets | Swimming | Sandpit | Children’s farm | Season | Ethnic background | Typing detailsa |
|---------------|------------------|---------------|-------------|------|----------|---------|-----------------|--------|------------------|----------------|
| NLA000102014  | June 2001        | FL            | 35          | –    | –        | +       | +               | Summer | NE               |                |
| NLA000200875  | Feb 2002         | OI            | 20          | +    | –        | –       | –               | Spring | NE               |                |
| NLA000201583  | June 2002        | NH            | 25          | –    | –        | +       | +               | Summer | NE               |                |
| NLA000201584  | July 2002        | NH            | 19          | –    | –        | –       | –               | Summer | NE               |                |
| NLA000201871  | July 2002        | NH            | 21          | –    | –        | –       | –               | Autumn | NE               |                |
| NLA000300211  | Sept 2002        | GL            | 27          | –    | –        | +       | +               | Autumn | NE               |                |
| NLA000300212  | Oct 2002         | FL            | 34          | –    | –        | +       | –               | Autumn | Afghanistan      | Clade 7502     |
| NLA000300987  | Oct 2002         | ZH            | 148         | +    | +        | –       | –               | Winter | NE               | β               |
| NLA000300601  | Jan 2003         | FL            | 38          | +    | –        | –       | +               | Winter | NE               | Clade 7509     |
| NLA000400124  | May 2003         | ZH            | 31          | +    | –        | –       | –               | Winter | NE               | δ               |
| NLA000300985  | Feb 2003         | GL            | 67          | +    | +        | –       | –               | Spring | NE               | β               |
| NLA000300986  | Feb 2003         | NH            | 18          | +    | –        | –       | –               | Winter | NE               | Clade 7501     |
| NLA000302015  | July 2003        | NH            | 45          | –    | –        | +       | –               | Summer | NE               |                |
| NLA000301141  | Mar 2003         | LB            | 57          | +    | +        | +       | –               | Spring | NE               | Clade 7509     |
| NLA000301142  | Mar 2003         | Dr            | 17          | +    | –        | +       | +               | Spring | NE               | Clade 7501     |
| NLA000302014  | July 2003        | NB            | 15          | +    | –        | –       | –               | Spring | NE               | Clade 7509     |
| NLA000302016  | May 2003         | NH            | 50          | –    | +        | +       | +               | Spring | NE               | γ               |
| NLA000302013  | June 2003        | FL            | 73          | –    | +        | –       | +               | Summer | Morocco (half)   | γ               |
| NLA000400892  | May 2003         | NB            | 47          | +    | +        | +       | –               | Autumn | NE               | α               |
| NLA000302203  | July 2003        | NB            | 21          | –    | –        | –       | –               | Autumn | NE               | Clade 7509     |
| NLA000302205  | Sept 2003        | ZL            | 58          | +    | +        | +       | –               | Autumn | NE               | δ               |
| NLA000302204  | May 2003         | ZL            | 36          | +    | +        | +       | –               | Spring | NE               | Clade 7502     |
| NLA000302300  | Aug 2003         | UR            | 25          | +    | –        | +       | –               | Autumn | NE               |                |
| NLA000400059  | Sept 2003        | GL            | 47          | –    | –        | +       | +               | Autumn | NE               | Clade 7502     |
| NLA000400274  | Dec 2003         | GL            | 20          | –    | –        | +       | –               | Winter  | NE               | Clade 7501     |
| NLA000400819  | Feb 2004         | NB            | 104         | +    | +        | –       | –               | Autumn | NE               | Clade 7502     |
| NLA000400398  | Nov 2003         | ZH            | 44          | –    | +        | +       | +               | Autumn | NE               | Clade 7502     |
| NLA000400620  | Dec 2003         | NH            | 27          | +    | –        | +       | –               | Winter  | NE               | Clade 7501     |
| NLA000400619  | Feb 2004         | NH            | 26          | –    | –        | –       | –               | Winter  | NE               | α               |
| NLA000400821  | Feb 2004         | NH            | 52          | +    | +        | +       | +               | Winter  | NE               | α               |
| NLA000400895  | Mar 2004         | NH            | 18          | –    | –        | –       | –               | Spring  | NE               | α               |
| NLA000401217  | Feb 2004         | NB            | 56          | –    | +        | +       | –               | Spring  | NE               | Clade II/ɛ      |
| NLA000401218  | Feb 2004         | LB            | 19          | +    | –        | +       | –               | Spring  | NE               | Clade I        |
| NLA000300854  | Feb 2004         | FL            | 32          | +    | +        | +       | +               | Spring  | NE               | Clade II/ɛ      |

a Typing details include clade designation according to Komijn et al. [19] and similarity (95–100%) designation between strains of this collection: α, β, γ, δ or ε.
The RFLP patterns of several *M. avium* strains were 95–100% similar to that of another strain while no correlation was observed with any patient factor (Table 1: designation α–ε).

**Bird materials**

Culture and real-time PCR performed on the bird materials revealed two faeces specimens positive for mycobacteria. One was only detected by culture and was identified as *M. terrae*. The other one was concordantly detected by culture and real-time PCR and identified as *M. malmoense*. Both identifications were done by sequencing the internal transcribed spacer region and by comparison in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov).

**Discussion**

Atypical mycobacteria are ubiquitously present in our environment, but few clinical cases have so far been directly linked to their natural reservoirs [20–22]. Traditionally, lymphadenitis in children caused by *M. avium* is indicated as ‘bird tuberculosis’ because historically *M. avium* bacteria were thought to have an association with birds. Because a striking percentage of the lymphadenitis patients in The Netherlands were in contact with birds, the concern was expressed in the media that birds were the source of infection for these lymphadenitis cases. This was not only driven by the fact that children naturally play outside in sand and soil but also by the fact that 8 of 34 children were in households where pet birds were present. Therefore, in this study, *M. avium* isolates from the lymph nodes of 34 children with lymphadenitis were subjected to IS*1245* RFLP typing and compared. The main objective in this comparison was to verify the occurrence of the bird-type *M. avium* within this group of patients. Although the patient strains revealed a high degree of variation in the IS*1245* RFLP patterns, the bird-type was not found in any of the 34 *M. avium* isolates, which excludes the possibility that bird-type strains are the source of infection. Moreover, no *M. avium* could be isolated from bird materials collected from the patients’ pet birds.

In several studies, this DNA typing method has been applied for epidemiological purposes [12, 13, 19, 23–25], and almost all *M. avium* isolates from birds revealed a characteristic bird-type three-banded IS*1245* RFLP pattern, while multi-banded RFLP types were very rarely encountered in bird isolates. The method has been investigated for its stability of patterns [26], and its specificity for the IS*1245* has been discussed in several papers [12, 27].

Earlier studies (partially published: [19]) conducted by the RIVM demonstrated a high degree of similarity
between human and porcine *M. avium* isolates. IS1245 RFLP typing of 146 isolates from humans in The Netherlands—the total number of *M. avium* strains collected in the year 1996 and 1997—yielded highly variable DNA fingerprint patterns, and a large part of the isolates could be assigned to nine different clades with a high degree of similarity among the RFLP patterns. A large part of the isolates both from human and porcine sources were grouped in the same clades.

The *M. avium* isolates of this study were scattered over several clades as was similarly found for the lymphadenitis isolates from the period 1996 to 1997. Six strains from this
study belonged to clade 7502 in which in previous studies the majority of strains from human immunodeficiency virus-infected patients clustered (unpublished thesis, Schneider, Bilthoven, The Netherlands). The high variability among the RFLP patterns may demonstrate the presence of high numbers of M. avium hominissuis bacteria in environmental sources and/or variability in source of infection.

While a high degree of variation was generally observed between the typed M. avium strains of the lymphadenitis cases, several patient strains were identical to one other strain. Comparison of the strains to the IS1245 RFLP database showed one strain from 1996 to be identical to one strain from 2004. This indicates the long-term genomic stability of the representative strains and the ability of these bacteria to prevail in a stable niche.

The bird-type M. avium has been isolated from birds [12, 28] and proven to be virulent in birds [29]. The birds kept in the patients’ home showed no detectable signs of illness. However, while mycobacteriosis is cause of mortality in birds, infected birds often show no signs of previous disease [30]. Pet birds might be carriers of M. avium subsp. hominissuis strains and could therefore transmit these M. avium bacteria to children causing lymphadenitis in a significant number of them. While mycobacteriosis in birds can best be diagnosed using biopsies from liver or spleen [31], successful isolation of mycobacteria has been described from faeces and cloacal swabs [31, 32] from infected birds. In this study, however, we were not able to grow M. avium from bird faeces and cloaca swabs. Therefore, this hypothetical carriernesship of birds could not be confirmed. Because the pilot experiment with bird materials was very small in size and the materials were not collected at time of onset of the disease, the possibility that pet birds are carriers of M. avium hominissuis cannot be completely excluded. The pet birds can also be an indirect cause; because M. avium is ubiquitous in soil, water, animals, food and sawdust [19, 20, 24, 33], these bacteria may well spread directly from such sources.

No other contact with farm animals than an occasional trip to the children’s farm (33%) was apparent in these patients as shown in Table 1. Therefore, pigs or other farm animals are not likely to have served as sources of infection [24].

Reed et al. [34] described in a random household survey conducted in Florida that the only apparent risk factor for M. avium infection is prolonged exposure to soil. Von Reyn et al. [35] described that while some clinical M. avium cases in AIDS patients could be linked to the household water supply by molecular typing, colonisation of the water supply with M. avium does not increase the risk of infection.

No correlation could be found in strain distribution with any kind of behaviour or geographical residence of the patient [3]. We therefore conclude that the sources of M. avium causing lymphadenitis in The Netherlands are variable and ubiquitous and no relation to pet birds has been determined.

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