Monaldi Arch Chest Dis
2007; 67: 3, 169-172

Mycobacterium Bovis infection in children in the same family: transmission through inhalation

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ABSTRACT: ***Mycobacterium Bovis Infection in children in the same family: transmission through inhalation. A.A. Velayati, P. Farnia, M.R. Boloorsaze, M.F. Sheikholslami, S. Khalilzadeh, S.S. Hakeeme, M.R. Masjedi.***

Two children in the same family were infected with *Mycobacterium bovis ("M. bovis")*. The molecular typing showed an identical source of infection. Althoughon school of thought was that the route of transmission was by ingestion of contaminated dairy milk, in other it was thought to be by air-borne transmission. The presentation highlighted the possibility *of M. bovis* infection in the pediatrics populations through aerosols.

Monaldi Arch Chest Dis 2007; 67: 3, 169-172.

Keywords: Mycobacterium bovis, inhalation, transmission.

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Work performed: National Research Institute Of Tuberculosis and Lung Disease (NRITLD).

Financial support: This work was supported by a grant from NRITLD 11/5/005.

Case report

A brother and sister presented in April 2004 to the Department of Pediatrics in the "National Research Institute of Tuberculosis and Lung Diseases" Tehran, Iran. The boy was 5 and the girl was 1 years old. Both of them had history of productive cough, nocturnal sweats and weight loss. They gave a medical history of previous illness, which was misdiagnosed as disseminated B.C.G. infection in the boy and pulmonary tuberculosis in the girl by smear microscopy and clinical symptoms. A physical examination of the boy showed disseminated lymphodenopathy in both sides of neck and underarms (fig. 1). The sizes of lymph node varied (from 1x5 to 4x4 cm) and some of them showed fistula formation with localised drainage. The chest CT-scan showed hilar and mediastinal lymphodenopathy, but there was no active infiltration or consolidation. Lymphodenopathy and hepathosplenomegaly were the only abnormal findings in the CT-scans of the abdomen. The manifestation of diseases in his younger sister (who did not receive B.C.G. vaccination) started when she was three months old. In her physical examination fine crackles in both lungs were the only abnormal finding. Her chest X-ray showed collapse of the right upper lobe and her chest CT-scan showed compensatory hyperaeration in the right middle and lower lobe (fig. 2). Both of children had negative tuberculin skin test (PPD-S) results. The microscopic and Loewenstein-Jensen culture results of sputum, gastric washing and biopsy specimens from lymph nodes were reported to be positive for acid-fast bacilli. The biochemical and drug susceptibility patterns of strains are summarised in tables 1 and 2. Immunological studies (flow cytometer; FACS Calibur), software simul SET v 3.1; Becton Dickinnson on their blood CD4,CD8 and CD4/CD8 was reported to be normal. Pathological examination of lymph nodes biopsies in the boy found inflammation "Chronic Granulomatous inflammation suggestive of mycobacterial infection". Before being admitted to the hospital, they were treated with the locally recommended short course regimen consisting of three -times weekly isoniazid (10 mg/kg), rifampicin (15 mg/kg), ethambutol (20 mg/ kg) and pyrazinamide. However, after the laboratory identified the organism as *M. bovis* resistant to all first line anti tuberculosis drugs, the patients switched to dapsone (50 mg/day),clofazimine (100 mg/ml), ofloxacin (100 mg/day), prothionamide (125 mg/day) with multi-vitamins for 18 months. In ad-

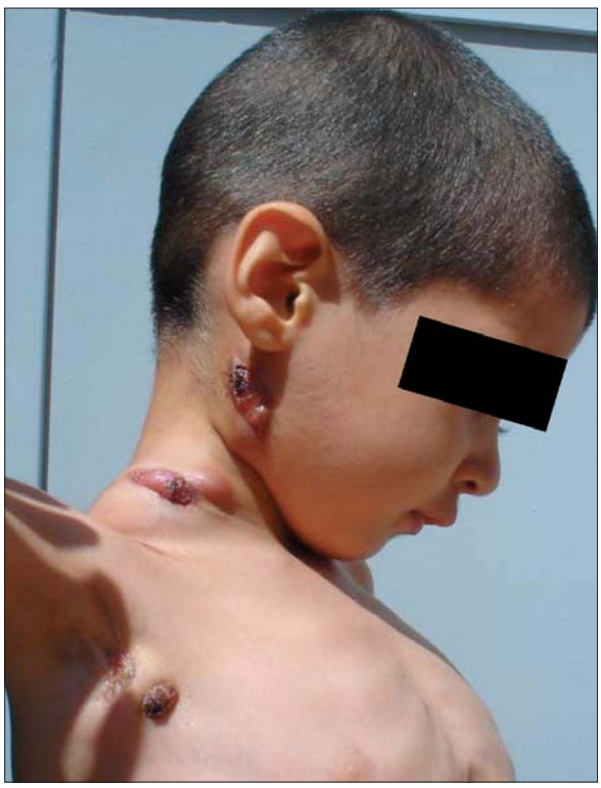

Fig. 1. - At the time of hospitalszation, the disseminated lymphoadenopathy seen in both sides of neck and under arms. The size of lymph node were from 1x5 to 4x4 cm.

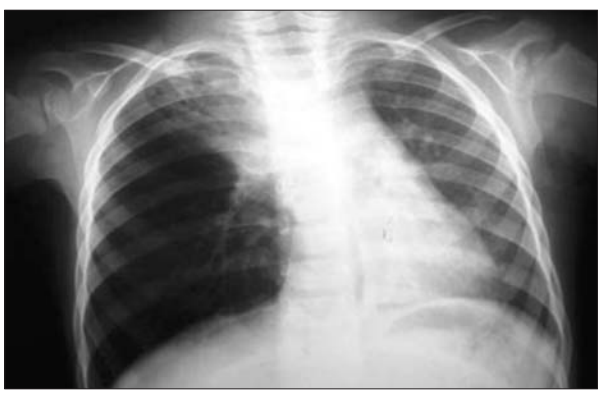

Fig. 2. - Chest-X-ray taken from the girl revealed collapse of right upper lob (at this time she has already taken one course of first line anti-TB regimens).

dition, the boy was given clarithromycin (3 cc /twice per day) and the girl had co-amoxiclave (3.5 cc/thrice per day). Although, due to severe GI distress in the boy, we had to discontinue the medicine for one full month. The girls chest X-ray became clear in the end of 18 months therapy, whereas, the boy had to take therapy for another 6 months (24 months of therapy). Neither of them showed any sign of recurrent infection after completion of their therapy.

DNA-fingerprinting and Spoligotyping

Extraction of DNA from Mycobacterium strains and DNA fingerprinting with IS6110 as a probe were performed by standard protocols. For spoligotyping, the DR region was amplified by PCR using primers derived from a DR sequence [1]. The amplified DNA hybridised to a set of 43 immobilised oligonucleotides derived from the spacer sequences of *M. tuberculosis* H37RV and *M. bovis* BCG P3 by reverse line blotting.

Discussion

Mycobacterium bovis, the causative agent of bovine tuberculosis, is known to infect a wide rang of domestic and wild animals, including humans. The human form of *M. bovis* infection has similar clinical forms as that caused by *M.tuberculosis.* However, the extra-pulmonary form is more prevalent and is often seen as lymph gland infections of the neck region, urinary or reproductive tract lesions [2-5]. The pulmonary form occurs less frequently and is usually occupationally related. It is seen most often in adults who work closely with cattle or their carcasses. The respiratory transmission of this organism, in pediatric-aged populations have not been documented until now. Children are accidental hosts for *M. bovis* infection and they are not efficient transmitters of *M. bovis* to others due to the low numbers of bacteria that they shed in the sputum. In children, the only documented risk factor for *M. bovis* infection is ingestion of dairy products, likely to have derived from raw and unpasteurised milk. In this report we demonstrate the

Table 1. - Biochemical testing results for M. tuberculosis complex group and the collected clinical specimens

Oxygen performance	**Cylcoserine 20 µg/ml in L.J**	**Thiosemicarbazon**	**Urease**	**Nitrate Reductase**	**Niacin production**	**Species**
Aerobic	Sensitive	Sensitive	Positive	Positive	Positive	**M. tuberculosis H37 RV**
Microaerophilic	Sensitive	Sensitive	Negative	Negative	Negative	**M. Bovis**
Aerobic	Resistant	Resistant	Positive	Negative	Negative	**M. Bovis B.C.G**
Microaerophilic	Sensitive	Sensitive	Negative	Negative	Negative	**Culture specimen from the boy**
Microaerophilic	Sensitive	Sensitive	Negative	Negative	Negative	**Culture specimen from the girl**

Table 2. -The results of susceptibility testing against first and second-line drugs regimens by proportional method in M. Bovis isolated from brother and sister in the same family

Susceptibility results by L.J culture Media		**Drugs/ ml**
Brother specimins	**Sister specimins**	
Resistant	Resistant	Isonizid (0.2 µg/ml)
Resistant	Resistant	Rifampin (40 µg/ml)
Resistant	Resistant	Streptomycin (5 µg/ml)
Resistant	Resistant	Ethambutol (2 µg/ml)
Resistant	Resistant	Pyrazinamide (1200 µg/ml)
Susceptible	Susceptible	Capreomycin (10 µg/ml)
Susceptible	Susceptible	Ciprofloxacin (2 µg/ml)
Susceptible	Susceptible	Cycloserine (30 µg/ml)
Susceptible	Susceptible	Ethionamide (20 µg/ml)
Susceptible	Susceptible	Kanamycin (20 µg/ml)
Susceptible	Susceptible	Ofloxacin (2 µg/ml)

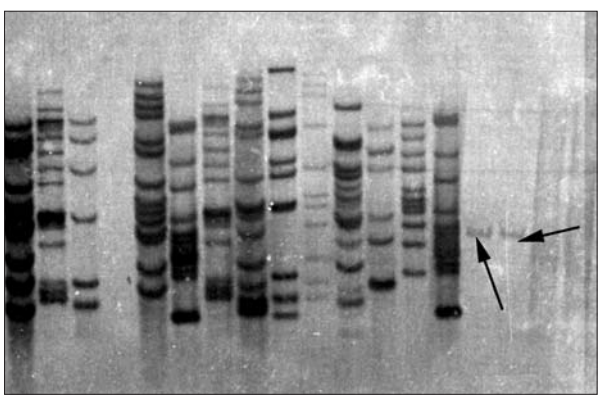

Fig. 3. - The RFLP of Mycobacterium isolates; the isolates 15 & 16 belongs to brother & sister.

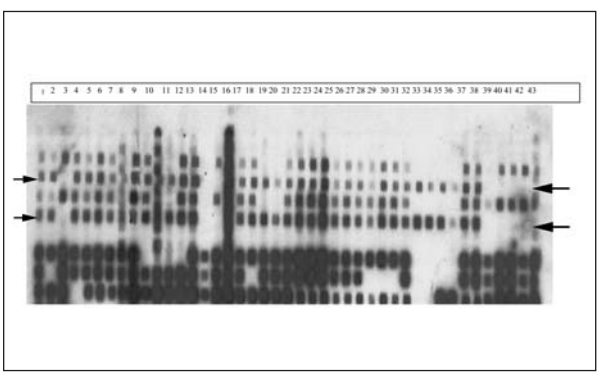

Fig. 4. - Spoligotyping patterns of specimens marked by arrows belongs to a brother and sister infected with *M. Bovis*. Usually the last 5 DR spacer (39-43) is absent in *M. Bovis*.

possibility *of M. bovis* infection through the aerosol route in pediatric cases. Fingerprinting using IS6110 and DR regions as probes indicated that both cases were infected with the identical strains (fig. 3, 4). Retrospective studies of cases showed that their father was working in the milk industry and the whole family habitually drank and / or ate unpasteurised milk or its products. Both children were fed with a commercial infant formula until 12 months of age, although their mother has already admitted occasionally giving unpasturized milk to the boy after he was one year old. Disease manifestation with extra-pulmonary symptoms started when he was one and half years old. His previous medical history showed three incomplete periods of treatment with first line drug regimens and he presented to the hospital with pulmonary and extra-pulmonary symptoms. His younger sister showed pulmonary symptoms when she was three months old. She had been fed with a commercial infant food formula. Therefore, we propose that the first child was infected through ingestion of contaminated dairy milk and the second child was infected by the aerosol route from her brother. In this context, both father and mother had negative PPD tests and were smear and culture negative.

In conclusion *M. bovis* infection can cause different clinical symptoms and the correct diagnosis is only possible through proper laboratory investigation.

Acknowledgements: We thank patients and their family for cooperating with our laboratory team. We appreciate the help of Mycobacteriology Dept, National Institute of Public and The Environment, The Netherlands for gifting the standard Mycobacterium strains.

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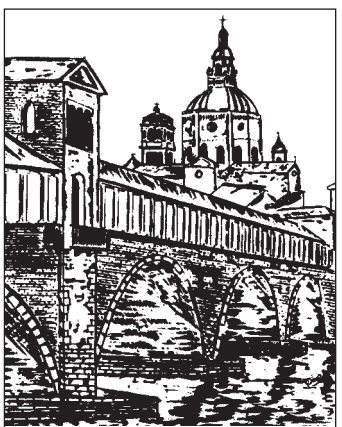

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