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► **To cite this version:**

Maroua Fliss, Nedra Meftahi, Neira Dekhil, Bisma Mhenni, Mohamed Ferjaoui, et al.. Epidemiological, clinical, and bacteriological findings among tunisian patients with tuberculous cervical lymphadenitis. International Journal of Clinical and Experimental Pathology, E-Century Pub., 2016. <pasteur-01446692>

**HAL Id: pasteur-01446692**

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Submitted on 26 Jan 2017

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## Original Article

# Epidemiological, clinical, and bacteriological findings among tunisian patients with tuberculous cervical lymphadenitis

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Received January 24, 2016; Accepted April 22, 2016; Epub September 1, 2016; Published September 15, 2016

**Abstract:** The incidence of tuberculous cervical lymphadenitis (TCL) is likely to be on the rise in Tunisia over the last two decades. However, this pathological condition remains poorly characterized, in regard to involved mycobacterial species. Purpose: To study the etiology and treatment outcome of TCL among Tunisian patients; to indicate the mycobacteria responsible for the majority of TCL cases. This prospective study has involved 114 patients, clinically diagnosed as TCL, presenting to a National referral hospital in Tunis, from November 2011 to January 2014. Results: 69 patients displayed typical cytological signs of TCL, whose mycobacterial etiology was confirmed in 23 cases. 4 cases may be a possible disseminated TB. Mycobacterial species assignment could be established for 15 culture-positive specimens, 11 of which were found to be *Mycobacterium bovis*, while the remaining were identified as *tuberculosis*. 6 of *M. bovis* isolates belonged to the BOVIS1 spoligopattern, and 3 of the *M. tuberculosis* isolates to the Haarlem3, one of the most prevalent genotype associated with pulmonary tuberculosis in Tunisia. Although all subjects lived in an urban area, the majority declared having consumed raw milk and derived products. The cure rate was low, as among patients that completed an anti-tubercular chemotherapy of at least 8 months, only 55.5% were cured. Conclusion: Our results are consistent with literature since positive cases demonstrated by AFB smear test don't exceed 37.4% and varied by culture between 19 and 71%. This is the first indication that *M. bovis* is a significant cause of TCL in Tunisia. Consumption of unpasteurized dairy products is the most likely source of transmission. The low cure rates among TCL cases should call health authorities for improved management and therapeutic schemes.

**Keywords:** Tuberculosis, tuberculous cervical lymphadenitis, *M. bovis*, *M. tuberculosis*, molecular typing

## Introduction

Tuberculosis (TB) is one of the most deadly infectious diseases known to mankind [1]. According to the latest global estimates, 9.0 million people developed TB and 1.5 million died from the disease in 2013 [2]. Human TB is primarily caused by *Mycobacterium tuberculosis* and, to a lesser extent, by the other members of the *Mycobacterium tuberculosis* complex (MTBC), which aside from *M. tuberculosis*, includes *M. bovis*, *M. africanum*, *M. microti*, *M. canettii*, *M. pinnipedii*, and *M. caprae* [3-6]. Although primarily considered to be a pulmo-

nary disease, TB can affect almost any organ of the body. Hence, we refer to extrapulmonary TB (EPTB) when any site other than the lung is affected [7]. EPTB has become a major concern worldwide, whose prevalence is rising especially among HIV co-infected patients [8, 9]. The most common sites of EPTB are lymph nodes, genitourinary tract, bones and joints, meninges and the central nervous system, peritoneum and other abdominal organs [10, 11].

Tuberculous lymphadenitis is the commonest form of EPTB worldwide, and most statistics show that cervical lymph nodes are the most

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commonly affected a manifestation termed tuberculous cervical lymphadenitis (TCL) [12]. The tuberculous bacilli that cause TCL in humans are usually *M. tuberculosis* and *M. bovis*. The latter species, and to a lesser extent *M. caprae*, accounted as the major etiologic agent of TCL before the widespread pasteurization of milk and the implementation of effective TB control measures in cattle [13, 14]. This situation prevails currently in most countries where bovine TB is endemic, with no effective control measures, such as detection and elimination of infected animals, regular slaughterhouse meat inspection, and milk pasteurization. If, in addition, HIV infection is prevalent in these countries, the risk for zoonotic infection due to *M. bovis* becomes exacerbated among HIV-co-infected TB patients compared with HIV-negative TB patients [15-17]. By contrast, in high-income countries where bovine TB is well controlled or eliminated, zoonotic cases of TCL are rarely seen [18, 19].

Apart from MTBC strains, an increasing number of TCL cases is attributed to nontuberculous mycobacteria (atypical mycobacteria), and this number appears to be on the rise in many developed countries [20].

In Tunisia, a middle-income country of 11 million inhabitants, TB burden has been steadily declining since the implementation of a national TB program in 1959. TB incidence rates varying from 23/100 000 (in 2005) to 31/100 000 (in 2012) were reported [21]. In the last two decades, Tunisia, like many other countries, experienced a substantial increase in EPTB cases, reaching 56.9% of all new TB cases registered in 2012 [22]. It is estimated that 50% of all EPTB cases affect lymph nodes, the majority of which (70% to 90%) are located to the cervical region [23]. To our knowledge, the relative contribution of *M. tuberculosis*, *M. bovis*, and atypical mycobacteria in the high prevalence of TCL cases in Tunisia has not been addressed. In an attempt to address this question, the present study was carried out.

### Materials and methods

#### *Clinical specimens*

This prospective study has involved 114 of the 197 patients seen in our referral hospital, clinically diagnosed as TCL from November 2011 to January 2014. Samples were collected in the

context of the routine diagnostic activity from consenting individuals presenting with tuberculous cervical lymphadenitis. Information about their age, site of residence, geographic origin, history of TB and risk factors, notably with regard to zoonotic *M. bovis* infection, were collected. Data on human immunodeficiency virus (HIV) infection status were also recorded. To safeguard patient confidentiality, the database was password protected. In addition, patients' identifiers in this publication do not reflect medical record numbers.

All patients received an histological examination of the affected lymph node, after a nodal Fine Needle Aspiration (FNA), when the technical means were available, or after biopsy. Each sample originated from a different patient.

In total, 69 lymph node specimens, displaying typical cytological signs of TCL, consisting of 42 fine needle aspirations (FNA) and 27 excisional biopsies were collected. The FNA volume samples varied from 1 to 3 ml and the size of biopsies from 1.5 to 4 cm. All specimens were forwarded to the Unit of Typing and Genetics of Mycobacteria at the "Institut Pasteur de Tunis" for bacteriological and molecular analyses.

#### *Sample processing, mycobacterial species assignment, and drug susceptibility testing*

Samples were decontaminated using the sodium hydroxide-Nacetyl-L-cysteine (NaOH-NALC) method [24], and subjected to acid fast bacilli (AFB) smear examination. Cultures were performed by simultaneously inoculating BBL MGIT (Mycobacterial Growth Indicator tubes) (Becton Dickinson, USA) and Löwenstein-Jensen (LJ) culture tubes with 500 µl and 200 µl of the decontaminated samples, respectively. All isolates were identified as MTBC strains using biochemical tests (production of niacin, catalase activity, nitrate reduction), and accurately assigned to *M. tuberculosis* or *M. bovis* species using the genomic deletion analysis approach previously described by Parsons *et al* [25]. This PCR-based approach differentiates MTBC strains based on the presence or absence of six genomic regions of difference (RD) (RD1, RD3, RD5, RD9, RD10, and RD11). To further confirm *M. bovis* isolates, we searched by nucleotide sequencing for the pyrazinamide resistance-conferring mutation G169C in the *pncA* gene sequence, as described previously [26].

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**Table 1.** Baseline characteristics of the patients with confirmed TCL

Patient N°	Date of admittance (month/year)	Sex/ Age	Residence	Specimen type	History of cervical lymphadenitis among close contacts	Consumption of raw milk and/or its derived products	Other risks for TCL	
							Job (Contact with zoonosis...)	PTB Antecedent
7	11/2011	F/59	Tunis	Biopsy	No	Yes	No	No
14	01/2012	F/36	Tabarka	FNA	Yes	Yes	No	Yes
16	01/2012	M/43	Beja	FNA	No	Yes	No	No
22	02/2012	F/08	AinDrahem	Biopsy	No	Yes	No	No
24	03/2012	F/25	Kef	FNA	No	Yes	No	No
26	02/2012	F/64	Kef	Biopsy	Yes	Yes	No	Yes (sister)
28	02/2012	M/05	Kef	FNA	-	-	No	No
39	01/2012	F/25	Siliana	Biopsy	No	Yes	No	No
42	05/2012	F/32	Siliana	Biopsy	No	No	No	No
44	04/2012	F/40	Tunis	FNA	No	Yes	No	No
46	03/2012	F/32	Tunis	FNA	Yes	No	No	Yes (mother)
56	05/2012	F/14	Siliana	Biopsy	No	Yes	No	No
60	05/2012	F/08	Tunis	FNA	No	Yes	No	No
62	06/2012	F/30	Beja	Biopsy	No	Yes	No	No
65	10/2011	F/28	Tunis	Biopsy	No	No	No	No
69	11/2011	F/26	Siliana	Biopsy	No	Yes	No	Yes
74	12/2012	F/45	Beja	Biopsy	No	Yes	No	Yes
77	01/2013	M/15	Tunis	FNA	Yes	Yes	No	No
85	06/2013	F/09	Kef	FNA	No	Yes	No	No
94	09/2012	F/16	Beja	FNA	No	No	No	Yes
103	11/2013	F/23	-	FNA	-	-	-	No
110	11/2012	F/63	Tunis	FNA	-	No	No	Yes
116	01/2014	F/60	Medjez El Bab	FNA	No	Yes	Yes	No

FNA: Fine needle aspiration. -: no data.

Drug susceptibility testing (DST) against isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide was performed by the proportional method on LJ media at a concentration of 0.2, 40, 4.0, 2.0 and 200  $\mu\text{g ml}^{-1}$ , respectively [27].

### Molecular typing of MTBC isolates

The spoligotype patterns were obtained as described by Kamerbeek et al [28] using an in-house prepared membrane. The specificity and reproducibility of the hybridization signals obtained with this membrane were assessed by testing a number of MTBC strains with known spoligotype profiles (*M. tuberculosis* H37Rv, Haarlem, Beijing, LAM and CAS, and *Mycobacterium bovis*, *Mycobacterium africanum* and *Mycobacterium canettii*).

MIRU-VNTR typing was performed by the method of Supply et al and was used to complement spoligotyping for optimal discriminatory power. Seven loci (MIRU 04, MIRU 26, MIRU 31, ETR-A, ETR-B, QUB 26, QUB 11b) were targeted. Briefly,

bacteria were first suspended in 50  $\mu\text{l}$  ultrapure nuclease-free water, boiled for 10 min, and immediately cooled in an ice bath for 5 min. A 20  $\mu\text{l}$  volume of the bacterial lysate was added to the PCR mixture of each MIRU-PCR reaction mix to a final volume of 50  $\mu\text{l}$ . This mixture contains 0.1  $\mu\text{l}$  HotStarTaq DNA polymerase (0.5 U) (Qiagen) with 10  $\mu\text{l}$  Q-solution, 0.5 mM each dATP, dCTP, dGTP and dTTP, 5  $\mu\text{l}$  PCR buffer and varying concentrations of  $\text{MgCl}_2$  (1.5 mM, 2 mM, 2.5 mM) in 16 PCR buffer. PCR products were run in a DNA thermal-cycler (model 9600; Perkin-Elmer) under the following conditions: 95°C for 15 min, followed by 40 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1.5 min, with a final extension at 72°C for 10 min. PCR products were analyzed on a 3% Metaphor agarose gel (BioWhittaker Molecular Application).

### Results

69 (67 adults + 2 children) of 114 patients, admitted to the referral hospital for tuberculous cervical lymphadenitis, displayed typical cytological signs of TCL. Diagnosis was based

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**Table 2.** Bacteriological, biochemical, and molecular identification of acid fast bacilli

Isolate from patient N°	AFB	Culture		Biochemical tests			RD						<i>pncA</i> G169C mutation	Identification
		LJ	MGIT	Niacin	NR	Catalase	RD1	RD3	RD5	RD9	RD10	RD11		
7	-	-	+	+/-	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
14	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
16	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
22	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
24	+	-	+	+	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
26	+	-	+	+	+	+	+	-	+	+	+	-	-	<i>M. tuberculosis</i>
28	+	-	+	+	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
39	+	-	+	-	-	+	+	-	-	-	-	-	+	<i>M. bovis</i>
42	-	+	+	+	+	+	+	+	+	+	+	-	-	<i>M. tuberculosis</i>
44	-	+	+	+	+	+	+	-	-	-	-	-	+	<i>M. bovis</i>
46	+	+	+	+	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
56	-	+	+	-	-	+	+	-	-	-	-	-	+	<i>M. bovis</i>
60	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
62	++	+	+	+	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
65	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
69	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
74	+	+	+	+	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
77	+	+	+	-	-	+	+	+	-	-	-	-	+	<i>M. bovis</i>
85	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
94	-	-	+	-	-	+	+	-	-	-	-	-	+	<i>M. bovis</i>
103	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-
110	-	+	+	-	+	+	+	+	+	+	+	+	-	<i>M. tuberculosis</i>
116	-	+	-	-	+	+	+	+	+	+	+	+	-	<i>M. tuberculosis</i>

AFB: acid fast bacilli smear staining; LJ: Löwenstein-Jensen; NR: nitrate reductase. +: positive result. -: negative result. NA: not applicable.

on cytohistological exams (presence of granuloma and necrosis). TCL was confirmed in 23 cases, as mycobacteria could be directly demonstrated by AFB smear test (33.3%) and/or culture (21.7%). 4 cases may be a possible disseminated TB (one of the family members were affected by pulmonary TB).

TCL patients were all HIV-negative with a mean age of 30.6 years (range, 5 to 64) (**Table 1**). TCL was significantly associated with female patients (86.9% vs 13.0%;  $P < 0.001$ ). The majority of TCL patients (15/22; 68.2%) originated from the north west of Tunisia, while the remaining resided in Tunis. We could demonstrate no apparent epidemiological links between them. Four patients declared that, at least, one of their close contacts had experienced cervical lymphadenitis, and most of the patients (16/21; 76.1%) declared having consumed raw milk and/or unpasteurized milk products, **Table 1**.

AFB smear test was positive in 18 samples among the 23 TCL cases, 8 of which proved culture-negative. Of the 15 culture-positive

samples, 7 were AFB smear-negative. The liquid culture MGIT was more sensitive than the solid LJ medium (93.3% vs 60.0%). In only one case, the culture was positive in LJ medium and negative in MGIT, **Table 2**.

Mycobacterial species assignment could be established unequivocally for 15 specimens using biochemical tests, the PCR-based RD deletion analysis, and detection of the *pncA* G169C mutation. 11 (73.3%) specimens were found to be *Mycobacterium bovis*, while the remaining 4 (26.6%) isolates proved *Mycobacterium tuberculosis*.

Molecular typing was performed on the 15 cultured mycobacterial isolates by targeting the DR region (spoligotyping) and the seven MIRU-VNTR loci (MIRU 04, MIRU 26, MIRU 31, ETR-A, ETR-B, QUB 26, QUB 11b). The BOVIS1 spoligopattern was the most prevalent among *M. bovis* isolates, representing 54.5%, **Table 3**. Of the 6 BOVIS1 isolates, 2 (from patients 7 and 24) displayed the BOVIS1-BCG spoligopattern, **Table 3**. Since these two strains harbored the RD1 region, which is deleted from all BCG



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**Table 4.** Drug susceptibility testing results and treatment outcome

Isolate from Patient N°	Species	Drug susceptibility testing					Medications used (treatment period) <sup>1</sup>	Treatment Outcome <sup>2</sup>
		H	S	R	E	Z		
7	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RH (2M*)	Persistence
14	-	-	-	-	-	-	RHZE (9M)	Cured
16	-	-	-	-	-	-	RHZE (2M*)	Persistence
22	-	-	-	-	-	-	RHZE (2M)/RH (12M)	Cured
24	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (9M)	PR <sup>3</sup>
26	<i>M. tuberculosis</i>	Sen	Sen	Sen	Sen	Sen	RHZE (8M)	Cured
28	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (9M)	-
39	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (9M)	Persistence
42	<i>M. tuberculosis</i>	Sen	Sen	Res	Sen	Sen	RHZE (3M*)	Persistence
44	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (11M)	Cured
46	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (8M)	Cured
56	<i>M. bovis</i>	Sen	Sen	Res	Sen	Res	RHZE (9M)	Persistence
60	-	-	-	-	-	-	RHZE (9M)	Cured
62	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (9M)	Persistence
65	-	-	-	-	-	-	RHZE (1M)/RH (9M)	Persistence
69	-	-	-	-	-	-	RHZE (6M)/RH (8M)	Persistence
74	<i>M. bovis</i>	Sen	Sen	Sen	Res	Res	RH (10M)	Cured
77	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (3M)/RH (8M)	Cured
85	-	-	-	-	-	-	RHZE (18M)	Cured
94	<i>M. bovis</i>	Sen	Sen	Sen	Sen	Res	RHZE (8M)	Persistence
103	-	-	-	-	-	-	-	-
110	<i>M. tuberculosis</i>	-	-	-	-	-	RHZE (12M)	Persistence
116	<i>M. tuberculosis</i>	Sen	Sen	Sen	Sen	Sen	RHZE (3M)/RH (5M)	Cured

-: no data available. Sen: sensitive; R: resistant; H: isoniazid; S: streptomycin; R: rifampicin; E: ethambutol; Z: pyrazinamide. M: month. <sup>1</sup>Drugs were taken on a daily basis. <sup>2</sup>Outcome at the end of the treatment period. <sup>3</sup>PR: paradoxical deterioration (appearance of new nodes). \*7, 16, 42: Loss of follow up.

strains tested thus far, they were unambiguously classified as *M. bovis*, **Table 2**. The remaining *M. bovis* isolates had no homolog in the SITVITWEB database. When all the spoligo-types were queried against the *M. bovis* database (www.mbovis.org), they all have been assigned a shared type (ST), **Table 2**. The 11 *M. bovis* isolates could be grouped into one of the 6 identified STs. MIRU-VNTR analysis detected 8 different allelic profiles (samples with different number of copies in at least one locus of the ones analyzed). Aside from allelic profiles D and I which grouped 3 and 2 isolates, respectively, the remaining 6 isolates had unique allelic profiles, **Table 3**. Of the 4 *M. tuberculosis* isolates, 3 showed the Haarlem3 (H3) spoligo-signature, while the remaining strain belonged to the "U" spoligotype family.

Susceptibility to anti-tubercular drugs was tested for 14 of the 15 cultured strains. The major-

ity of isolates was susceptible to all first-line drugs, with the exception of two isolates that showed monoresistance to rifampicin and ethambutol, **Table 4**. As expected, all *M. bovis* isolates proved resistant to pyrazinamide.

Basically, most of the patients (19/22; 86.36%) received at least 8 months of chemotherapy consisting of various combinations of the four drugs rifampicin, isoniazid, pyrazinamide, and ethambutol, **Table 4**. Of these 19 cases, 10 (52.6%) have been cured. The remaining patients showed either persistent lymphadenitis (42.1%) or deterioration appearance of new nodes (5.2%).

### Discussion

In Tunisia, TCL is the most frequent EPTB with 30-50% of the TB cases, but no studies regarding the mycobacterial species involved in TCL in Tunisia were reported in literature.

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Diagnosis of TCL is mainly based on cytohistological examination (presence of granuloma and necrosis) and AFB staining. In most cases, the treatment is administered without bacteriological confirmation, and hence, the real contribution of mycobacteria to cervical lymphadenopathy cases is as yet unknown. This study was carried out in order to determine the extent of TCL cases among 114 patients presenting with cervical lymphadenitis in Tunisia, and to unambiguously identify the involved mycobacterial species (the remaining patients were not included in the present study due to unwillingness to be biopsied or aspirated).

Our findings indicate that mycobacteria accounts for approximately 33% of all our cytologically and histopathologically diagnosed lymph node tuberculosis. These results are consistent with literature since positive cases demonstrated by AFB smear test don't exceed 37.4% and varied by culture between 19 and 71% [29, 30]. A recent Tunisian study, report 6.4% positive cases for AFB smear and 3.2% for culture from 31 samples collected in the center of Tunisia [31].

The culture failure may be due to an inadequate transport and storage conditions. All tests were done in the same laboratory and any possible issues with procedures have been excluded.

Our rate is likely to represent the real contribution of mycobacteria in cervical enlargement cases. Indeed, aside from using the classic detection means (AFB smear test and culture on LJ medium slants), mycobacterial growth detection was also performed on MGIT, one of the most sensitive liquid medium available to date. Equivalent or higher TCL incidence rates (37%, 43% and 63.8%) were reported among patients with cervical lymphadenopathy in other countries, and these variations may be due to differences in diagnostic procedures and/or regional specificities [10, 32, 33]. We do not dismiss the possibility that TCL rates could also vary from one region to another in Tunisia, which should prompt a nationwide study. With regard to the clinical pattern, TCL in Tunisia was similar to what has previously been reported in other geographical settings, in that it is more common in females and in young age groups [7, 12].

Our study indicates that *M. bovis* is likely to be the most important cause of TCL in Tunisia

(73.3%). This finding is consistent with the fact that most of the patients (80%) declared having consumed raw milk and/or unpasteurized dairy products. Indeed, a previous study aimed at evaluating the frequency of *M. bovis* isolation from raw milk in Tunisia, demonstrated that consumers of unpasteurized milk or derivatives are at high risk of zoonotic infection with *M. bovis* [12]. Furthermore, most of the patients originated from the North West of the country, which is an agricultural region with high cattle breeding densities. In these regions, and most likely in the rest of the country, consumption of raw milk is a cultural tradition in certain communities, since it is generally believed that fresh, unprocessed, whole milk is more natural than packaged UHT-treated milk, has a better taste, and provides more health benefits. Aside from contaminated foods, zoonotic TB due to *M. bovis*, could exceptionally be due to aerosols inhalation following close contact with infectious cattle, or accidental inoculation from contaminated materials, particularly in slaughters. Our epidemiological data support the premise that consumption of raw milk or its products is the most likely cause leading to TCL in Tunisia.

Four TCL cases were associated with *M. tuberculosis* isolates, three of which were assigned to the Haarlem3 genotype (ST50). This result is in line with the preponderance of this genotype in pulmonary tuberculosis in Tunisia [34, 35].

The finding that 8 smear-positive samples were culture-negative is worthy of consideration. Six of these cases could have been contacted and interviewed. They confirmed having previously been treated in the private sector before being admitted to the hospital, and that they had most likely received antibiotics. According to the prevailing practices, these patients could have been given fluoroquinolones, penicillin, amoxicillin, and/or other antibiotics, which may therefore explain why their samples were culture-negative (antibiotics do indeed turn a smear positive patient into smear negative). AFB-positive and culture-negative samples from TCL patients have already been described and were attributed to previous antimicrobial therapy [36]. The occurrence of these cases in Tunisia highlights the pressing need for a better coordination between the private sector and the national TB program.

Despite the demonstrated efficacy of combined 6-month chemotherapy in treating tuberculous

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lymphadenopathy [37, 38], the cure rate of 55.5% among the Tunisian patients that received at least 8-month chemotherapy is relatively low. Given the absence of multidrug resistance, this low cure rate is likely to be due to the lack of adherence to treatment. For 3 cases (7, 16 and 42) persistent lymphadenopathy is not unexpected since these patients received inadequate treatment, **Table 4**. This was due to the loss of follow-up. For the remaining patients, the majority hadn't a combined chemotherapy as recommended by OMS this may explain the low cure rate observed. In fact, Tunisia usual pattern is an adaptation of the WHO diagram (association: RIF- INH- PZA-ETH) and the average treatment time processing for TCL without alternative location is 10.9 months. Despite recommendations, the period of 6 months is only slightly followed: 7/10 physicians reported treating nine months or more. The intermittent use of chemotherapy can also explain the low rate since all our cases were outpatients and DOT couldn't be considered.

Hence, educational campaigns should not only focus on the risk of consuming raw milk, but should also insist upon the patients regarding adherence to treatment. Furthermore, since the majority of TCL in Tunisia is caused by *M. bovis*, involvement of PZA in the treatment regimen should be reconsidered (no nation wide-data for pyrazinamide resistance rate is available). In fact, to further confirm *M. bovis* isolates implication, we searched by nucleotide sequencing for the pyrazinamide resistance-conferring mutation G169C in the *pncA* gene sequence and all our samples presented the mutation.

In conclusion, and to our knowledge, this is the first study describing in detail the etiology and treatment outcome of TCL among north Tunisian patients. The findings indicate that *M. bovis* is responsible for the majority of TCL cases, and those individuals who consume raw milk and its derived products are at high risk. This study also pinpoints the pressing need to intensify educational campaigns on the risk of zoonotic tuberculosis, and should call for a better management of TCL cases in order to reach higher cure rates.

### Acknowledgements

This study received financial support from the Tunisian Ministry of Higher Education, Scientific

Research and Information and Communication Technologies and ethical agreement.

### Disclosure of conflict of interest

None.

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