Mycobacterium leprae on Palatine Tonsils and Adenoids of Asymptomatic Patients, Brazil

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We investigated palatine tonsil and adenoid specimens excised from otorhinolaryngological patients in a leprosy-endemic region of Brazil. Fite-Faraco staining identified *Mycobacterium* spp. in 9 of 397 specimen blocks. Immunohistochemistry and molecular analysis confirmed the presence of *Mycobacterium leprae*, indicating that these organs can house *M. leprae* in persons inhabiting a leprosy-endemic region.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that especially affects skin and peripheral nerves (*1*). In 2018, the registered global prevalence in the 6 World Health Organization regions was 184,238 cases (0.24/10,000 population), showing a decrease of 8,475 cases over the previous year (*2*). Although its incidence in Brazil has declined during 2009–2018, leprosy continues to be a major public health problem at the national level (*1*). Reports of *M. leprae* resistance against antimicrobial drugs used in multidrug therapy raise concern about the future of leprosy treatment (*2*). Therefore, not only does leprosy persist, but the emergence of multidrug-resistant *M. leprae* is a potential threat to global public health (*3*,*4*).

Although the exact mode of leprosy transmission is not known, it is thought that the upper respiratory tract, in particular the nasal mucosa, is the usual site of primary infection (*3*). Because we have previously identified *M. leprae* in oral mucosa of leprosy patients (*5*), we aimed to investigate other anatomic sites that could host this microorganism to clarify the epidemiology and transmission mechanisms of leprosy.

In this study, we hypothesized that *M. leprae*, after penetration through the airway mucosa, could infect the palatine tonsils and adenoids, because these organs represent the first immune defense line against inhaled or ingested antigens (*6*). We also theorized that if leprosy is a highly contagious disease (*1*), a considerable part of the population in endemic regions might be infected with *M. leprae*.

We conducted a cross-sectional study of 397 paraffin-embedded blocks of palatine tonsils and adenoids extracted from 144 patients due to otorhinolaryngological indication during 2011–2016 at the Regional Hospital, Presidente Prudente, Brazil. The local Research Ethics Committee approved the study (protocol #1.920.994).

Microscopic analysis using hematoxylin-eosin staining did not reveal granulomas. We analyzed 50 fields in the 100× objective (1,000× magnification) per slide stained with Fite-Faraco; of the positive cases (9 [2.3%] slides from 8 [5.6%] patients, 6 men and 2 women [mean age 11 $\pm$ 5.5 years]), we observed only 1 acid-fast rod per slide. We studied all the blocks of these 8 patients, a total of 20 blocks (Table).

Immunohistochemistry with 1:20,000 anti–phenolic glycolipid-I (anti-PGL-I) antibody (Bei Resources, https://www.beiresources.org), specific for *M. leprae*, was conducted with the Mach 1 polymer-based biotin-free detection kit (Biocare Medical, https://biocare.net) (*5*). We used deparaffinized skin sections from multibacillary leprosy as positive control. For the negative control, we omitted the antibody. To confirm specificity, we used deparaffinized skin sections from paucibacillary leprosy and atopic dermatitis (excluding inflammatory cell recognition by the antibody), normal human scalp, and tuberculous lung section.

We extracted DNA from paraffin sections with isopropanol-ammonium acetate (*1*). The resulting DNA was used in conventional PCR with sense 5′-ATTTCTGCCGCTGGTATCGGT-3′ and antisense 5′-TGCGCTAGAAGGTTGCCGTAT-3′ primers (ThermoFisher Scientific, https://www.thermofisher.com) to amplify *M. leprae* microsatellite sequences, according to a previous report (*7*). We assessed amplicons of 148 bp on 2% agarose gel. The assays included negative (DNA omission) and positive (DNA from multibacillary leprosy skin sample) controls. We confirmed specificity with DNA extracted from *M. tuberculosis* culture. In addition, we conducted PCR with TB1/TB2 primers to detect *Mycobacterium* spp. and with T4/T5 primers to detect *M. tuberculosis* (*5*).

Immunohistochemistry was positive in 18/20 (90%) blocks. By PCR, 19 (95%) were positive with RLEP and 5 were simultaneously positive with TB1/TB2; all 19 positive by PCR were negative by T4/T5

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Table. Patient data and results of Fite-Faraco staining, immunohistochemistry with anti–PGL-I antibody, and PCR assays in study of *Mycobacterium leprae* on palatine tonsils and adenoids, Brazil, 2019*

Patient no.	Age, y/sex	Lymphoid organ	Fite-Faraco stain	IHC anti–PGL-I	PCR RLEP	*M. leprae* identification by DNA sequencing, %
1	20/F	AD	+	+	+	ND
2	19/M	RPT	+	+	+	ND
		LPT	–	+	+	ND
3	10/M	RPT	+	+	+	100
		LPT	–	+	+	ND
4	9/M	RPT	–	+	+	ND
		LPT	+	+	+	99
		AD	+	+	+	ND
5	7/M	RPT	–	+	+	ND
		LPT	+	+	+	ND
		AD	–	+	+	ND
6	4/M	RPT	–	+	+	ND
		LPT	+	+	+	99
		AD	–	–	+	ND
7	13/F	RPT	+	+	+	98
		LPT	–	+	+	ND
		AD	–	+	+	ND
8	6/M	RPT	–	–	–	ND
		LPT	+	+	+	ND
		AD	–	+	+	98
Total positive results			9	18	19	

*AD, adenoid; PGL-I, phenolic glycolipid I; IHC, immunohistochemistry; LPT, left palatine tonsil; ND, not determined; RLEP, *M. leprae* repetitive DNA sequence; RPT, right palatine tonsil; –, negative; +, positive.

primers. Samples that tested negative in IHC or PCR were also negative in Fite-Faraco staining (Table).

Little attention has been paid to the role that mucosa-associated lymphoid tissue (MALT) plays in the mechanisms used by mycobacteria during host invasion. In tuberculosis infection, bacilli cross mucous membranes and penetrate into palatine tonsils and adenoids, where they initiate an immune response. However, bacilli may develop immune-evasion strategies and disseminate into the organism or return to the mucosa surface and be eliminated to the environment (*8*). This process might also occur in leprosy, but to our knowledge there are no reports on this subject.

Our results corroborate the hypothesis that *M. leprae* bacilli infect palatine tonsils and adenoids. Prospective studies with a larger population group are necessary to clarify these findings. We could not infer from this retrospective study with paraffinized samples whether patients who had positive results for *M. leprae* identification had leprosy or were asymptomatic carriers. In both clinical scenarios, however, our findings indicate that palatine tonsils and adenoids may represent reservoirs for *M. leprae* bacilli in persons inhabiting a leprosy-endemic region.

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Fatal *Chlamydia avium* Infection in Captive Picazuro Pigeons, the Netherlands

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In 2016, an outbreak of *Chlamydia avium* infection occurred among Picazuro pigeons (*Patagioenas picazuro*) living in an aviary in the Netherlands. Molecular typing revealed a unique strain of *C. avium*. Our findings show that *C. avium* infection, which usually causes subclinical infection, can cause fatal disease in pigeons.

Until approximately 2014, *Chlamydia psittaci* was the only *Chlamydia* species detected in birds. Researchers have catalogued ≈465 bird species affected by this pathogen, which mainly causes subclinical infections but sometimes results in acute disease and death (*1*). In humans, *C. psittaci* is highly infectious and can cause severe pneumonia. *Chlamydia* bacteria, which are present in (dried) excreta or feather dust, are transmitted through direct contact or inhalation. In 2014, researchers proposed 2 new members of *Chlamydiaceae*: *C. avium* and *C. gallinacea* (*2*). *C. avium* affects pigeons and psittacine birds, whereas *C. gallinacea* affects poultry. Most *C. avium* and *C. gallinacea* infections in birds are subclinical, and the zoonotic potential of these species is unknown (*3*).

In 2016, an outbreak of *C. avium* infection occurred among 11 Picazuro pigeons (*Patagioenas picazuro*) housed in an aviary with other bird species in the Netherlands. The birds lost weight, had ruffled feathers, and were anorexic. Despite treatment with fluids, force-feeding, and in 1 bird, doxycycline treatment (50 mg/kg 1×/d), all 11 animals died or were euthanized. Necropsy revealed that 9 of these birds were in poor physical condition, lacking fat and pectoral muscle mass. The livers and spleens were enlarged; the livers extended an average of 0.5 cm beyond the rear edge of the sternum, whereas the mean diameter of the spleens was 1.0 cm, approximately twice as large as the normal size. We suspected *Chlamydia* infection because of intracellular inclusions in Stamp (modified Ziehl Neelsen)–stained cytology of liver and spleen. We found multifocal heterophilic and lymphoplasmacytic infiltrates with necrosis in the liver and lymphoid depletion with necrosis and heterophilic infiltrates in the spleen. We stained slides with polyclonal antibodies against *Chlamydia* (bioMérieux, https://www.biomerieux.com) after a standard Avidin Biotin Complex protocol (*4*); liver and kidney tissues from 7 birds tested positive for *Chlamydia*. We did not observe any histologic changes consistent with viral inclusions or bacterial infection.

Because psittacosis in birds is a notifiable disease in the Netherlands, we informed public health authorities of our results. We forwarded frozen tissue samples to the Wageningen Bioveterinary Research institute to confirm *C. psittaci* infection. We also collected and forwarded 2 Picazuro pigeon carcasses and 3 pooled fecal samples from contact birds (i.e., Roseate spoonbill [*Platalea ajaja*], Puna ibis [*Plegadis ridgwayi*], and Scarlet ibis [*Eudocimus ruber*]), from the aviary. Two liver samples, 2 conjunctival and cloacal swabs, and 3 pooled fecal samples initially tested negative for *C. psittaci*, *C. abortus*, *C. felis*, and *C. caviae* in a PCR selective for the *omp*A gene. Because the liver and kidney samples of 7 pigeons tested positive for antibodies against *Chlamydia*, we submitted samples from all 11 pigeons and the 3 pooled fecal samples for further testing with real-time PCR selective for the 23S gene of *Chlamydiaceae* (*5*) and a duplex real-time PCR selective for *C. gallinacea* and *C. avium* (*3*,*6*). All 11 pigeons tested positive for *C. avium* in ≥1 samples of conjunctiva, cloaca, liver or intestines. The pooled fecal samples of contact birds tested negative in a PCR for *Chlamydiaceae* (Appendix, https://wwwnc.cdc.gov/EID/article/26/10/20-0086-App1.pdf).