**Mycobacterium bovis** in Argentina: isolates from cats typified by spoligotyping

**M. J. ZUMÁRRAGA**1, M. MARTÍNEZ VIVOT2, D. MARTICORENA2, A. BERNARDELLI3, R. FASÁN4, R. IACHINI5, A. A. CATALDI1.

1Instituto de Biotecnología, CICVyA-INTA, Castelar; 2Facultad de Veterinaria, Universidad de Buenos Aires; 3Cátedra de Patología de la Universidad Nacional de Río IV, Córdoba; 4LACLIVET, Buenos Aires; 5Instituto Pasteur, Buenos Aires, Argentina.

*Correspondence. E-mail: mzumarraga@cia.inta.gov.ar

**ABSTRACT**

In the present work, 19 Mycobacterium bovis isolates from different cats were typified by spoligotyping. We detected nine spoligotypes. There was only one cluster, which grouped 11 of the isolates (57.9%), showing the main spoligotype from cattle from Argentina. The rest of the spoligotypes presented only one isolate each. Five of them were not found in cattle, and were unique and exclusive of cats. The isolates studied show that tuberculosis of bovine origin in cats constitutes a potential public health problem in Buenos Aires region. The identification of genotypes from non-natural hosts could contribute to understand the spread of bovine tuberculosis. This is the first report showing genetic profiles of *M. bovis* isolates in felines from Argentina.

**Key words**: tuberculosis, cats, spoligotyping

**RESUMEN**

*Mycobacterium bovis* en Argentina: aislamientos de gatos tipificados por spoligotyping. En el presente trabajo se tipificaron por spoligotyping 19 aislamientos de *M. bovis* de diferentes gatos. Se detectaron 9 espoligotipos y un único agrupamiento o cluster integrado por 11 aislamientos (57.9%) y relacionado con el principal espoligotipo de bovinos de Argentina. El resto de los espoligotipos detectados presentaron solamente un aislamiento cada uno; 5 de ellos no se encontraron en bovinos y fueron únicos y exclusivos de gatos. La presencia de estos aislamientos indica que la tuberculosis bovina en los gatos constituye un potencial problema de salud pública en la ciudad de Buenos Aires. La identificación de genotipos de aislamientos de *M. bovis* de hospedadores no convencionales podría contribuir a la mejor comprensión de la diseminación de la tuberculosis bovina. Este es el primer informe en el que se muestran los perfiles genotípicos de aislamientos de *M. bovis* obtenidos de felinos de Argentina.

**Palabras clave**: tuberculosis, gatos, spoligotyping

**INTRODUCTION**

Bovine tuberculosis is a chronic zoonosis that affects different wild and domestic animals, causing relevant economic losses in livestock. The causative agent is *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex, which also includes *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canetti*, *Mycobacterium caprae* and *Mycobacterium pinnipedii* (4). The main host of *M. bovis* is cattle, although several mammalian species can also be infected. In Argentina, the prevalence in cattle is around 1.2%, estimated by detection of lesions in the slaughterhouse (13). Among domestic animals, cats are the most susceptible hosts of bovine tuberculosis (14).

The main pathway of tuberculosis infection in felines is the digestive route. However, tuberculosis can also infect by the aerogenic pathway and/or injured scratches. Cats are more susceptible to *M. bovis* than dogs, probably because a common pet owner’s habit is to feed cats only with raw food such as raw lung, liver and other viscera (3, 6, 14). The milk of infected animals is a very important source of infection, especially in rural areas, where milk is commercialized without pasteurization. The evolution of the disease in cats is different from that in bovines, reaching earlier a generalization of the infection that leads to the death of the animal. The clinical signs of the disease are unspecific and affect several organs or fluids such as lymph nodes, intestines, kidneys, pleura, lungs, liver and/or brain. The confirmation of clinical suspicion must be carried out by culture from different organs or fluids (7). The National Plan of Control and Erradication of Bovine Tuberculosis does not recommend the use of the tuberculine test in dogs and cats because it can give negative false results (10). In Argentina, it is mandatory to report the cases of tuberculosis to sanitary agents. Nevertheless, the information about the incidence of bovine tuberculosis in cats in Argentina is incomplete and scarce (6, 11, 14). As an antecedent, 4% of the cats necropsies at the School of Veterinary Sciences of the
University of Buenos Aires (UBA) presented tuberculosis (14).

The transmission of bovine tuberculosis can be stopped by implementing control programs, feeding pets with balanced food, pasteurizing milk, and cooking meat products.

However, in the last years in Argentina, these barriers have not been enough to protect pets from tuberculosis. People who adopt and rear many cats, together with the increase of immunosuppressing diseases (feline immunodeficiency virus and feline leukemia virus), have led to an epidemiological change. Different molecular methods are used to type the M. tuberculosis complex. One of them, called spoligotyping, is a PCR reverse line blot hybridization method based on the polymorphism of the direct repeat (DR) region (8). This method is easy to perform and allows the differentiation of interspecies and intraspecies variability among the M. tuberculosis complex. However, although the method is not polymorphic enough to identify close relationships, it can be useful for the determination of more distant relationships between isolates (15).

Therefore, in order to gain a deeper insight into tuberculosis in cats, we have typed M. bovis isolates from cats from Buenos Aires city, Argentina, by spoligotyping.

MATERIALS AND METHODS

Stray cats from Buenos Aires city that had died by unknown causes between 1998 and 2006 were submitted to the School of Veterinary Sciences of the UBA to be necropsied. Nineteen isolates were obtained from the lymph nodes from all the animals except for one, which was obtained from bronchoalveolar lavage (3) from a pet belonging to a woman that lived with 20 other cats in her house. These cats were fed daily with "raw beef lung" (3). Macerated lymph nodes and bronchoalveolar exudate samples were cultured in Lowenstein–Jensen and Stonebrink media for mycobacteria after decontamination by the Petroff method (4% sodium hydroxide) (5).

A loopfull of bacteria was suspended in 200µl of distilled water into a 2-ml screw-cap tube and boiled for 30 min. After centrifugation at 12,000 rpm for 5 min, 5 µl of supernatant was used for PCR (polymerase chain reaction) to perform spoligotyping according to the protocol previously described by Kamerbeek et al. (8), and using the spoligotyping kit (Isogen Biosolutions B.V., Ocimun Biosolutions Company, Ijsselstein, The Netherlands). A cluster analysis of the spoligotype patterns was performed with the BioNumerics software (Windows NT, version 2.5; Applied Maths, Kortrijk, Belgium). The categorical coefficient was used to calculate the similarity of spoligotype patterns, and the UPGMA (Unweighted pair-group method with arithmetic averages) method was applied to calculate a dendrogram. Clusters of isolates were defined as two or more M. bovis strains with identical spoligotypes. Each of the different spoligotypes were allocated a number. M. tuberculosis H37Rv (ATCC 27294) and M. bovis Bacillus Calmette-Guerin (BCG) (ATCC 27289) were included as reference strains in each spoligotyping experiment.

RESULTS

Nine different spoligotypes were detected among the 19 M. bovis isolates from cats, eight of which were unique (Figure 1). All the strains lacked spacers 3, 9, 16 and 39 to 43, characteristic of M. bovis isolates. There was only one cluster that grouped 11 of the isolates (57.9%) and showed spoligotype Spo 34, identified as SB0140 in the international database of the University of Sussex, United Kingdom (UK) (http://www.mbovis.org/). This spoligotype is the most frequent among the 542 M. bovis isolates analyzed from Argentina (15, 16), representing 46.9% of the total collection. The remaining eight spoligotypes had only one isolate each. Five of them (Spo 48, 70, 71, 75 and 90) were exclusive of cats because neither bovine nor other host isolates from Argentina had been previously detected. The other three spoligotypes (Spo 3, 21 and 29) had been found in bovine. In addition Spo 21 was also found in human, pig and armadillo isolates and Spo 3 was also described in human isolates from Argentina. The spoligotypes not found in the University of Sussex database were included to perform further comparisons between laboratories.
DISCUSSION

Since cats are susceptible to the *M. tuberculosis*, *M. bovis* and *M. avium* complex, the isolates must be typed to adopt different health management programs. *M. bovis* is mainly transmitted to cats by the gastrointestinal route after the ingestion of contaminated food (6, 9, 14). It was not possible to know the infection source of the sampled cats, though they had probably been fed daily with "raw bovine lung". Feeding stray cats in this way is an old custom of people from our country. This practice could explain the probable infection source of these cats. Moreover, the availability of contaminated bovine lungs in the city, suggests a failure in slaughterhouse inspection.

The cluster that grouped 57.9% of the isolates from cats showed the main spoligotype (Spo 34/SB0140) detected in bovines from Argentina (15, 16). This spoligotype is also predominant in the United Kingdom and other countries that had imported cattle from the UK at the end of the nineteenth century (2). Spoligotypes Spo 3, 21 and 29, were identified in a previous epidemiological study, grouped at 3.3, 20.1 and 2.8% of all the isolates (15, 16). Spo 21 is the second spoligotype in frequency in Argentina and Spo 3 may be related to humans because 61% of the isolates with this spoligotype were from pulmonary isolates from non-related humans (15, 16). The presence of these spoligotypes in *M. bovis* isolates from cats, demonstrates the transmission from bovines to cats.

The distribution of the different patterns could be due to epidemiological factors and the virulence of the strain within the population. In low prevalence areas, a high genetic diversity is found in cattle populations, thus implying a variety of unrelated ancestor strains (12). Intriguingly, there were five spoligotypes that were unique and exclusive of cats, not detected among the cattle isolates from Argentina, differentiated by one or few spacers from those detected in bovines. These patterns could correspond to other genotypes from non-sampled bovines. On the other hand, the change of host could exert selective pressure on the mycobacterial genome, thus giving new related patterns. Other authors have also found spoligotypes from cats that were not found in isolates from other animals, thus spoligotyping represents a good tool to detect new types from different host species (1).

Further studies will be necessary to confirm the presence of *M. bovis* in bovine lungs from butcher's stores. It is our belief that it is very important to be aware of the risk of the very old custom of feeding cats with raw bovine lung. Thus, we suggest that the best way to decrease the incidence of bovine tuberculosis in cats is feeding them with balanced food pellets or well cooked food, reducing the risk of human infections.

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