# Case Report Rapport de cas

## Isolation of a *Mycobacterium microti*-like organism from a rock hyrax (*Procavia capensis*) in a Canadian zoo

Cyril Lutze-Wallace, Claude Turcotte, Gordon Glover, Debby Cousins, John Bell, Gloria Berlie-Surujballi, Yvon Barbeau, Geoff Randall

**Abstract —** A *Mycobacterium tuberculosis* complex organism was isolated from a zoo resident rock hyrax (*Procavia capensis*) imported into Canada from South Africa. The strain was identified biochemically as *Mycobacterium microti.* The spoligotype pattern obtained for this isolate was found to be rare. This represents the first report of isolation and spoligotyping of *M. microti* in North America.

**Résumé — Isolement d'un organisme semblable à *Mycobacterium microti* chez un daman des rochers (*Procavia capensis*) dans un jardin zoologique du Canada.** Un organisme du complexe Mycobacterium tuberculosis a été isolé chez un daman des rochers (*Procavia capensis*) pensionnaire d'un jardin zoologique, importé d'Afrique du sud au Canada. La souche identifiée par biochimie s'est révélée être *Mycobacterium microti.* Les caractéristiques des spoligotypes de cet isolat étaient peu fréquentes. Il s'agit du premier rapport d'isolement et de spolygotypie de *M. microti* en Amérique du Nord.

*(Traduit par Docteur André Blouin)*

*Can Vet J 2006;47:1011–1013*

A female rock hyrax (*Procavia capensis*) was captured in the wild and imported directly into Canada from South Africa in December 1997. The hyrax was held in quarantine for 60 d before being housed in an indoor exhibit, isolated from other zoo and indigenous wild animals. The animal (> 5 y) was euthanized following a progressive, chronic lameness that led to hind leg paralysis, approximately 30 mo after importation. Postmortem examination revealed granular pale lesions on the peritoneum, with similar nodular lesions in other organs. No lesions were found on necropsy related to the lameness, but there was some suggestion of thinning of a cranial thoracic intervertebral space on radiographs taken prior to euthanasia. The spine was not closely examined on necropsy. Large cauliflower-like pale nodules were found in the lungs. Companions were depopulated upon confirmation of the finding of tuberculosis. No similar lesions were observed on necropsy of the remaining 3 hyraxes in this colony. The exhibit in which these animals were housed was cleaned and disinfected and subsequently repopulated with birds and reptiles. There was no direct contact with other mammals, and zoo staff were monitored routinely for potential transmission. There were no reports of TB in any offspring.

Tissue from the pulmonary lesions was submitted to the Mycobacterial Diseases Centre of Expertise at the Canadian Food Inspection Agency, as a suspected tuberculosis case. Histopathological examination of the tissue revealed typical granulomatous lesions without mineralization and giant cells with large numbers of acid-fast bacilli resembling those of mycobacterial species. Cultures prepared from frozen pulmonary tissue yielded moderate growth on Lowenstein Jensen medium after 6 wk incubation, which subsequently stained acid-fast. Application of the polymerase chain reaction, using primers targeting the insertion element IS*6110* (1), confirmed the identification of the colonies as *Mycobacterium tuberculosis* complex (MTBC) organisms. The results of biochemical testing suggested that the organism was phenotypically consistent with *Mycobacterium microti.*

Strain typing by the spoligotyping method (2) showed a pattern distinctly different from that of *M. bovis* types commonly found in Canada (Figure 1). Reactions were evident within the group of oligonucleotides 39–43. This group is unreactive for isolates of *M. bovis.* Conversion of the spoligotype pattern to octal code was done in accordance with the method described by the National Tuberculosis Genotyping and Surveillance Network (3). The code obtained for this isolate was 772777400001671. Examination of the data base of spoligotypes (4) indicated that this type was rare, with only 2 isolates having been reported in Great Britain. However, the data base identified these isolates as MTBC organisms and not *M. microti*

Canadian Food Inspection Agency, Mycobacterial Diseases Centre of Expertise, Ottawa Laboratory Fallowfield, 3851 Fallowfield Road, Ottawa, Ontario K2H 8P9 (Lutze-Wallace, Turcotte, Bell, Berlie-Surujballi, Barbeau [retired], Randall [retired]); Assiniboine Park Zoo, 460 Assiniboine Park Dr., Winnipeg, Manitoba R3P 2N7 (Glover); Australian Reference Laboratory for Bovine Tuberculosis, Animal Health Laboratories, Department of Agriculture, Locked Bag 4 Bentley Delivery Centre 6983, Western Australia (Cousins).

Address all correspondence and reprint requests to Dr. C. Lutze-Wallace; e-mail: lwallacecy@inspection.gc.ca

Funding for this study was from the CFIA Technology Development Program.

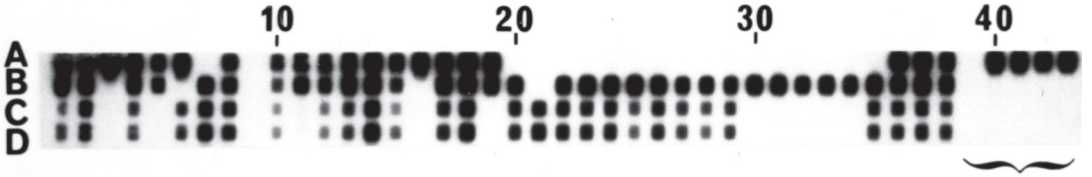


**Figure 1.** Spoligotype patterns for *M. microti* and recent Canadian isolates of *M. bovis.* Row A, rock hyrax isolate of *M. microti;* row B, *M. bovis* isolate from Ontario in 2002; rows C and D, *M. bovis* isolates from Manitoba in 2003. Numbers at the top indicate oligonucleotide position numbers. Note that the last 5 oligonucleotides do not react in rows B-D, characteristic for *M. bovis* (bracket).

specifically. This is the first report of *M. microti* isolation and identification of this spoligotype in North America.

Isolations of *M. microti* from mammals, such as the rock hyrax, or "dassie," from the Cape Province of South Africa, have been documented previously in 1958 (5) and more recently in 1994 in a zoo collection in Perth, Australia (6). The Australian case and this one confirmed in Canada have some common features. In both instances, the animals originated from South Africa and were captured from the wild. The animals imported to form the colony at the Perth zoo were held in quarantine in the UK for a period of months prior to arrival in Australia, while the hyraxes intended for Canada were imported directly from South Africa. Application of spoligotyping methodology to the 2 isolates obtained from the hyraxes in Australia generated the same spoligotype profile (octal code 772777400001671) as was obtained in this case. An epidemiological link is suggested by the finding of identical spoligotypes for these isolates. Further, earlier typing had indicated that the "Australian" hyrax isolates were related to the original "dassie" isolates of the 1950s (6). These findings strongly support the hypothesis that a reservoir for this clone exists in South African rock hyraxes.

Comparison of published spoligotype patterns for *M. microti* isolates (4,7,8) generates 3 separate strain types: a "vole" type, displaying reactions at positions 37 and 38 with variable reaction at oligonucleotide number 4; a "llama" type, with reactions at positions 4–7, 23, 24, 26 (variable), 37, and 38 (variable); and the "dassie/rock hyrax" type shown here (Figure 1) and previously (8). However, the latter isolate, designated 68/7171, did not show hybridization signals with oligonucleotides 15 and 16 (8), while that of the present case does. Geographically, *M. microti* isolations have been made in The Netherlands, Belgium, the United Kingdom, and France (vole and llama types). The dassie/rock hyrax type has been isolated in South Africa and in zoo animals in the United Kingdom, Australia, and now in Canada.

*Mycobacterium microti* is generally thought to be a disease agent of small mammals, but infrequently isolations have been reported from other species, such as pigs, cats, llamas, dogs, and humans (7–10). Given the potential for infection of species other than the conventional host, veterinarians and laboratory diagnosticians should not rule out the possibility of *M. microti* infection in zoo species or their close contacts.

Importation of zoo animals, such as rock hyraxes, presents problems for tuberculosis control programs, since there are no validated tests for unusual species. The conventional tuberculin skin tests are commonly applied is such circumstances as the default option; however, these tests have not been validated in rock hyrax. Additionally, such tests may not be practical in these animals and quarantine prior to import may not provide adequate protection against importation of the disease, as evidenced by the Perth zoo experience (6). Blood tests targeting a specific gamma interferon response to tuberculin or other antigens, as has been done for badgers (11); lymphocyte stimulation tests (12); or fluorescence polarization assays (13) could be evaluated for utility in this context. The problem of finding sufficient numbers of diseased and nondiseased animals to properly validate the sensitivity and specificity of such tests remains an issue. It may be that only by the continual global accumulation of test data will diagnosticians gain confidence in the use of such tests for the diagnosis of tuberculosis in unusual species.

## Acknowledgments

The authors thank staffs of the Mycobacterial Diseases Centre of Expertise (S. Chen, L. Rohonczy, D. Watchorn, and M. Sabourin) and the Canadian Food Inspection Agency (CFIA) Program and Operations (B. Thompson, Winnipeg and M. Koller-Jones, Ottawa) for their contributions to this study. CVJ

## References

1. Miller J, Jenny A, Rhyan J, Saari D, Suarez D. Detection of *Mycobacterium bovis* in formalin-fixed, paraffin-embedded tissues of cattle and elk by PCR amplification of an IS6110 sequence specific for *Mycobacterium tuberculosis* complex organisms. J Vet Diagn Invest 1997;9:244–249.
2. Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997;35:907–914.
3. Crawford JT, Braden CR, Schable BA, Onorato IM. National tuberculosis genotyping and surveillance network: Design and methods. Emerg Infect Dis 2002;8:1192–1196.
4. Filliol I, Driscoll JR, van Soolingen D, et al. Snapshot of moving and expanding clones of *Mycobacterium tuberculosis* and their global distribution assessed by spoligotyping in an international study. J Clin Microbiol 2003;41:1963–1970.
5. Wagner JC, Buchanan G, Bokkenheuser V, Leviseur S. An acid-fast bacillus isolated from the lungs of a cape hyrax, *Procavia capensis* (Pallus). Nature 1958;181:284–285.
6. Cousins DV, Peet RL, Gaynor WT, Williams SN, Gow BL. Tuberculosis in imported hyrax (*Procavia capensis*) caused by an unusual variant belonging to the *Mycobacterium tuberculosis* complex. Vet Microbiol 1994;42:135–145.
7. Kremer K, van Soolingen D, van Embden J, Hughes S, Inwald J, Hewinson G. *Mycobacterium microti:* more widespread than previously thought. J Clin Microbiol 1998;36:2793–2794.
8. van Soolingen D, van der Zanden AGM, de Haas PEW, et al. Diagnosis of *Mycobacterium microti* infections among humans by using novel genetic markers. J Clin Microbiol 1998;36:1840–1845.

9. Cavanagh R, Begon M, Bennett, et al. *Mycobacterium microti* infection (vole tuberculosis) in wild rodent populations. J Clin Microbiol 2002;40:3281–3285.
10. Deforges L, Boulouis HJ, Thibaud JL, et al. First isolation of *Mycobacterium microti* (Llama-type) for a dog. Vet Microbiol 2004; 103:249–253.
11. Dalley DJ, Hogarth PJ, Hughes S, Hewinson RG, Chambers MA. Cloning and sequencing of badger (*Meles meles*) interferon gamma and its detection in badger lymphocytes. Vet Immunol Immunopathol 2004;101:19–30.
12. Hutchings DL, Wilson SH. Evaluation of lymphocyte stimulation tests for diagnosis of bovine tuberculosis in elk (*Cervus elaphus*). Am J Vet Res 1995;56:27–33.
13. Surujballi O, Romanowska A, Sugden EA, Turcotte C, Jolley ME. A fluorescence polarization assay for the detection of antibodies to *Mycobacterium bovis* in cattle sera. Vet Microbiol 2002;87: 149–157.



# Book Reviews
## Comptes rendus de livres

### Reptile Clinician's Handbook: A Compact Clinical and Surgical Reference

Frye FL. Krieger, Malabar, Florida, USA, 1995, ISBN 0-89464-0948-5. US$21.78.

### Exotic Animal Formulary, 3rd ed.

Carpenter J. Elsevier, St Louis, Missouri, USA, 2005, ISBN 0-7216-0180-4, US$59.95.

Dr. Frederic Frye is well known as a prominent teacher, clinician, and pathologist, on reptile medicine and pathology. The Reptile Clinician's Handbook is extracted from his 2 ground-breaking large-format volumes on reptile medicine, "*Biomedical and Surgical Aspects of Captive Reptile Husbandry*," and a companion book on reptile nutrition "*A Practical Guide for Feeding Captive Reptiles*," both published in 1991. The size and cost of the former publications likely encouraged Dr. Frye to produce a compact synopsis for practitioners for whom reptiles were a small part of their practice. The book was published 12 years ago and is being resubmitted by the publishers.

The Handbook is indeed that — having 276 spiral-bound pages and measuring just 5 × 6 in. In order to keep the costs and page count down, there are no pictures or diagrams, no subheadings in the chapters, and the page margins are small. A short chapter on basic biology of reptiles is followed by an extensive and useful chapter with a series of tables on the practical aspects of nutrition and diets for all types of reptiles. Chapters titled "Clinical Laboratory Sample Collection" and "Processing and Clinical Methods" follow. Considerable space is devoted to sample handling, stains, and preservatives. Next, there is a section on "Surgical and Non-surgical Procedures," and a brief formulary. The last quarter of the book is an Appendix on poisonous plants, a large (34 p.) list of scientific and common names, and an index.

The book contents are of value and draw from Dr. Frye's many years as a practitioner and teacher. The main problem with the book is that it has now been 15 years since publication of the source texts, and the material is dated. For instance, most citations for the hematological data are pre-1960s. The tables of normal values are now less useful considering the amount of data accumulated over the past 35 years that have not been included. In the past few years, there has been a significant expansion in the reptile medicine and in the numbers of practitioners working with these animals. Two editions of another medicine textbook have been published; there is an organization (ARAV) with its own journal devoted to reptile medicine and, of course, we have the Internet. There are also now several extensive formularies and numerous other publications on the medical care of reptiles and other exotic animals.

Clinical techniques continue to evolve and many of those, as well as useful drugs, that have emerged in the past decade are not included. For instance, propofol and medetomidine are valuable reptile anesthetic agents, and this publication pre-dates their development and their use on reptiles. Newer sampling techniques and laboratory methods, such as molecular technology, and diagnostic methods including surgical and non-surgical endoscopy, that have become essential methodologies for very small animals, including reptiles, are also absent. Most surgical procedures are now commonly performed on reptiles, not just the few mentioned in the relevant chapter.

While the Handbook is not meant to be a reptile medicine or surgical text, there is some useful information contained inside especially considering the low price, but there is really not enough in it for even the casual reptile practitioner or veterinary student seeking up-to-date information on clinical reptile medicine or surgery.

A more recent publication is the ***Exotic Animal Formulary,*** the 3rd ed. of a book first published in 1996 with a 2nd version in 2001. Previously co-written with 2 other authors, Dr. Carpenter has co-opted twelve experts to update the sections in this edition. The need for a new version so soon after the last edition demonstrates the pace of development in non-traditional-animal medicine. The Formulary is principally a table of drug dosages with full references and comments, but also includes miscellaneous information on the species in question, such as biological and blood data, and dietary recommendations. The 3rd ed. includes the material from the earlier versions