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1 *Mycobacterium pinnipedii*: Transmission from South American Sea Lion
2 (*Otaria byronia*) to Bactrian Camel (*Camelus bactrianus bactrianus*) and
3 Malayan Tapirs (*Tapirus indicus*)

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10

11 **Abstract**

12 Tuberculosis infections caused by *Mycobacterium (M.) pinnipedii* in a South American sea
13 lion, Bactrian camel, and Malayan tapirs kept in two zoological gardens spanning a time pe-
14 riod of five years are reported. The zoos were linked by the transfer of one tapir. Conventional
15 bacteriological and molecular methods were applied to detect the pathogen. Spoligotyping
16 and MIRU/VNTR-typing performed to assess the genetic similarity revealed identical mo-
17 lecular characteristics of the isolates from all animals involved. Anti-tuberculosis antibodies
18 were detected using ELISA and a recently developed serological rapid test. The study shows
19 that (i), using molecular methods, the assessment of the genetic relationship of infectious
20 agents helps to confirm the routes of infection, and that (ii), immunological tests may help to
21 detect tuberculosis infections *ante mortem* more reliably and early. This would prevent the
22 transfer of tuberculosis by asymptomatic animals.

23

24 Key words: tuberculosis, *Mycobacterium pinnipedii*, molecular epidemiology, sea lion, camel,
25 tapir

26

27 **Introduction**

28 Members of the *Mycobacterium tuberculosis* complex (MTC) cause tuberculosis (TB) in
29 many warm-blooded animal species and man. The earliest reports on TB in marine mammals

1 were published in 1912 in the USA (2) and later on in 1956 in Germany (5) dealing rather
2 with the diagnosis of the disease at *post mortem* inspection than with the identification of the
3 mycobacterial agent and its route of transmission. Since then TB has been described in sev-
4 eral pinniped populations living in the southern hemisphere (1, 20). The disease was caused
5 by a unique member of the MTC formerly named *Mycobacterium* (*M.*) type seal which exhib-
6 ited some phenotypic and genotypic characteristics different from those of other members of
7 the MTC (4, 21). Therefore, it was proposed in 2003 to elevate *M.* type seal to species rank
8 and name it *M. pinnipedii* (4). Investigations on the molecular make up of the MTC presented
9 new insights into the evolution of MTC and suggested that close evolutionary ties exist be-
10 tween *M. microti* and *M. pinnipedii* (3, 8). In 1993, the transmission of a TB infection from
11 sea lion to human (seal trainer) was described, underlining the zoonotic and pathogenic poten-
12 tial of this mycobacterial species (18). Here we describe the transmission of *M. pinnipedii*
13 under conditions of animal keeping in zoological gardens from South American sea lion to a
14 camel and tapirs and show that molecular epidemiological methods can confirm relationships
15 between outbreaks in distant locations and completely unrelated species. Preliminary results
16 on serological *ante mortem* diagnosis of TB in these animals are presented.

17

18 **Materials and Methods**

19 **Animals.** One South American sea lion (*Otaria byronia*; I), four Bactrian camels (*Camelus*
20 *bactrianus bactrianus*; I - IV) and four Malayan tapirs (*Tapirus indicus*; I – IV) were included
21 in this study.

22 **Detection of mycobacteria.** Mycobacteria were isolated upon *post mortem* examination from
23 tissue samples (lymph nodes, lungs) of sea lion I, camel I and the tapirs I – IV. The samples
24 were decontaminated with NaLC-NaOH using standard procedures (10), inoculated on Stone-
25 brink agar containing pyruvate and PACT (polymyxin B, amphotericin B, carbenicillin,
26 trimethoprim), Loewenstein-Jensen agar as well as MGIT liquid medium (BD, Heidelberg,

1 Germany) and cultivated for six to eight weeks at 37°C. In parallel DNA was extracted from
2 tissue using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the
3 manufacturer's instructions except a prolongation of the proteinase K treatment over night
4 followed by heating at 95°C for 15 min. Mycobacterial DNA in tissue extracts was detected
5 using an in-house nested PCR.

6 **PCR.** DNA from culture was prepared by ultrasonication and boiling of heat-inactivated my-
7 cobacteria for 10 min each. PCR was performed according to Rodriguez et al. (15) using
8 primer sequences modified according to the GeneBank data base. The following sequences
9 were used: 5'- GAA CCC GCT GAT GCA AGT GC -3' as forward and 5'- ACG CCG CTG
10 ACC TCA AGA AG -3' as reversed primer. PCR was run using the following cycling condi-
11 tions: a denaturation step at 96°C for 60 sec, followed by 30 cycles of 96°C for 15 sec, 63°C
12 for 60 sec, 72°C for 60 sec, and a final extension at 72°C for 300 sec. An amplicon of 499 bp
13 was generated.

14 **Nested PCR.** The nested PCR based on the modified PCR of Rodriguez et al. (15) was per-
15 formed using 5'-GCA AGT GCC ACA ATG CTG-3' as forward and 5'-CGA ACG CTT
16 CGA CCA GCT CG-3' as reversed primer. A 435 bp DNA stretch was amplified.

17 The following cycling conditions were used: a denaturation step at 96°C for 60 sec followed
18 by 30 cycles of 96°C for 15 sec, 67°C for 60 sec, 72°C for 60 sec, and a final extension at
19 72°C 300 sec.

20 **Spoligotyping.** Spoligotyping (9; Isogen Bioscience, The Netherlands) was performed using
21 DNA extracted from cultures.

22 **MIRU typing.** MIRU typing was performed targeting 12 chromosomal loci as given by Ma-
23 zars et al. (12) using the primers described by Supply et al. (16). The MIRU copy numbers
24 were determined by gel electrophoresis.

25 **Enzyme-linked immunosorbent assay.** Serum ELISA was performed using an in-house test
26 based on crude extracts prepared from *M. bovis* and *M. avium* according to Hall and Thoen

1 (7) as well as recombinant MPB70 antigen. Briefly, bacterial suspensions of *M. bovis* AN5
2 and *M. avium* D4 were extracted using 3M KCl in phosphate buffer and subsequently centri-
3 fuge at 16,000 g for 30 min. After dialysis against phosphate-buffered saline the supernatant
4 containing the soluble antigen was stored at -20° C for further use. The recombinant MPB70
5 was expressed as a poly-histidine recombinant protein in *E. coli* as described earlier (19) us-
6 ing the expression vector pQE40 system and a nickel-nitrilotriacetic acid (Ni-NTA) resin for
7 purification according to the manufacturer's instructions (Qiagen). Microlon (655092,
8 Greiner, Frickenhausen, Germany) ELISA plates were coated with the respective antigens and
9 the coating concentration of the antigens was standardised using a positive control serum ob-
10 tained from a *M. bovis*-infected oryx antelope (*Oryx leucoryx*) (6). Bound antibodies were
11 detected using a recombinant protein G-conjugated peroxidase (Zymed Laboratories, San
12 Francisco, USA). ELISA results for the serum samples were expressed as titres relative to the
13 positive control serum using its titre (1:40) as cut-off value.

14 **Rapid antibody test.** A rapid test (RT) for diagnosis of animal TB was developed by Chem-
15 bio Diagnostic Systems, Inc. (Medford, USA). This antibody detection immunoassay is based
16 on the lateral-flow technology and employs a cocktail of selected recombinant proteins of *M.*
17 *tuberculosis*, including ESAT-6, CFP10, and MPT83 as described previously (11).

19 **Results**

20 **Course of the disease.** Between September 2001 and January 2003 one female sea lion (I)
21 and two camels (I, II) died or were euthanised in zoo A (Germany) due to severe respiratory
22 symptoms. The camels III and IV remained healthy. In May 2004 an asymptomatic male tapir
23 (I) was transferred from zoo A to zoo B (France). Early in 2005 tapir I had to be euthanised
24 after a period of progressive weight loss and respiratory symptoms (cough). Between August
25 2005 and June 2006 three other Malayan tapirs (female II, III and male IV) in zoo B were

1 euthanised due to suspicion of TB infection. The suspected course of transmission is shown in
2 figure 1.

3 **Pathological findings.** In sea lion I, camel I and the tapirs I, II and III severe granulomatous,
4 partially necrotising bronchopneumonia with mineralisation, multiple caseous foci, enlarged
5 lung lymph nodes and granulomatous foci in the lung lymph nodes were found upon *post*
6 *mortem* inspection. Tapir IV showed only moderate signs of TB.

7 In the camel II extreme diffuse condensation of the lung tissue, pleurisy, depletion of the
8 lympho-reticular tissue in the lymph nodes, purulent rhinitis, multiple whitish lentil spots in
9 the pancreatico-duodenal lymph nodes and yellow layers on the pharyngeal tonsils were
10 found. The lesions were not estimated to be characteristic for TB.

11 **Bacteriological and molecular biological findings.** DNA of the MTC bacteria was detected
12 in tissue extracts of all animals except camel II using nested PCR. MTC bacteria were culti-
13 vated from all animals except camel II. In camel II mycobacteria could not be detected. The
14 dates of the diagnostic events and the results are given in table 1.

15 **Spoligotyping patterns.** The spoligotyping patterns of all isolates were identical and charac-
16 teristic for *M. pinnipedii*. In all of them the spacers 4-7, 23-25, 29-38 of DNA yielded strong
17 signals. The spacers 26 and 28 gave weak signals, spacer 27 gave no signal. (Figure 2). The
18 spoligotyping pattern was deposited in the data base and designated as SB1162 by the data
19 base www.Mbovis.org.

20 **MIRU patterns.** The MIRU patterns for the 12 genomic loci determined from all isolates of
21 sea lion I, camel I and the tapirs I – IV were identical. The pattern is given in table 2.

22 **Rapid antibody test.** The sampling dates and the results of the tests are shown in table 1. The
23 sea lion and the camel sera were not tested because the RT was not available at that time. Ta-
24 pir I reacted strongly positive and was clinically ill at the time of testing. The serum samples
25 of the tapirs II and III also reacted strongly positive, while no clinical symptoms were ob-
26 served. Tapir IV was tested several times after its arrival in zoo B in May 2005 until the end

1 of May 2006. This animal did not show clinical symptoms over the whole time period but RT
2 started revealing sero-conversion about eight months after its arrival in zoo B.

3 **ELISA.** The sampling dates and the results of the tests are shown in table 1. Camel I was not
4 tested. Camel II showed a significantly elevated serum titre against *M. bovis* and *M. avium*
5 extracts, but no sero-conversion against recombinant MPB70. The camels III and IV remained
6 negative. Tapir I was not tested. The tapirs II and III reacted strongly in ELISA. Tapir IV
7 showed a weak sero-conversion against *M. bovis*.

8

9 **Discussion**

10 Tuberculosis in zoo animals concerns the survival of endangered species as well as public
11 health aspects. TB in sea lions has been reported by several authors and *M. pinnipedii* was
12 identified as pathogenic agent. The zoonotic potential of *M. pinnipedii* concerning terrestrial
13 mammals has been mentioned before but in the present report the duration of the incubation
14 period, the course of sero-conversion during incubation and the severity of the disease in ta-
15 pirs has been described in more detail for the first time. The identification of TB infection in
16 animals is most often only possible at *post mortem* examination, validated *ante mortem* diag-
17 nostic tests are not available or difficult to interpret. For understandable reasons standardized
18 experiments with artificial infections and negative controls cannot be performed in zoo animal
19 species. In the present study preliminary efforts were made to detect TB infection *ante mor-*
20 *tem* in two different exotic animal species. Due to the character of this study as a field study,
21 it is not possible to report results obtained under standardized conditions. Not all of the in-
22 volved animals were tested using all the test procedures mentioned. Not from all animals se-
23 rum samples were available. Nevertheless, the study includes nine animals, belonging to the
24 species South American sea lion, Bactrian camel and Malayan tapir and spanned altogether
25 five years. Most likely, the infection started in the sea lions' colony in zoo A and crossed the
26 species barrier to camels and tapirs. A possible way of transmission may have been the gen-

1 eration of infectious aerosols by the high-pressure steam cleaning procedure used routinely
2 for the sea lions' pool in zoo A. This mode of cleaning procedure has already been assumed
3 by other authors to support TB spreading in zoos (13). The tapirs and camels in zoo A had
4 been housed in close neighbourhood to the sea lions. In all three affected animal species the
5 manifestation of the disease primarily in the respiratory tract makes transmission by aerosol
6 the most likely route of transmission.

7 At the time of transfer of tapir I from zoo A to zoo B there was no indication that TB was pre-
8 sent in zoo B. The molecular relatedness of the isolates originating from zoo A and zoo B,
9 respectively, is very close and the likelihood that the group of sea lions in zoo A was the
10 source of the actual infection is very high. In zoo B the infection seemed to spread from the
11 male tapir I to its resident female partners (tapirs II and III) by direct contact. Tapir IV which
12 was introduced into zoo B when tapir I was already euthanized, had been tested negative by
13 RT before its arrival in zoo B (personal communication) and remained negative concerning
14 clinical appearance and serum antibody titre for approximately seven months before sero-
15 conversion emerged. Tapir IV did not have direct contact to the other infected tapirs but was
16 housed separately only sharing the outdoors' area with them but never at the same time.

17 In the camels' group except camel I and the sero-conversion in camel II no further TB cases
18 emerged. The sero-conversion of camel II could not be clarified.

19 The genotypic characterization of the *M. pinnipedii* isolates revealed that they were identical
20 at least in two aspects. When spoligotyping is performed, within the series of 43 spacers nor-
21 mally the spacers 4-7 and 23 – 38 are present in *M. pinnipedii*. In all the isolates presented
22 here the spacers 26 – 28 gave weak or no signal. In a previous report South American sea lion
23 isolates were described missing the spacers 23, 24, 28, 35 or 36 in numbers and combinations,
24 in addition to the general pattern (4). However, the available data base is too small to deduce
25 or reject an assumption of the geographical origin of the animal introducing the pathogen at
26 the beginning of the series of TB infections. MIRU analysis as second genotyping method

1 targets several genetic loci scattered all over the mycobacterial chromosome characterised by
2 a variable number of short repeating DNA sequences (12, 16). For *M. tuberculosis*, 15 loci
3 have recently been recommended to be analysed in order to achieve optimal discrimination of
4 isolates of global origin (17). In this study 12 loci were analysed which were previously used
5 to discriminate a broad collection of *M. caprae* isolates from animals and humans across
6 Europe (14). MIRU typing characteristics of *M. pinnipedii* have not been reported yet. There-
7 fore, no conclusions regarding the genetic diversity of this member of the MTC can be drawn.
8 The contribution of presently available *in vivo* tests to the diagnosis of TB infection is diffi-
9 cult to evaluate. RT was developed for serological diagnosis of TB in elephants (11) and was
10 used for the first time for TB diagnosis in tapirs.

11 Sea lion I and the camels I and II could not be tested by RT since the test was not available at
12 that time. The tapirs I, II and III showed significantly elevated antibody titres. The tapirs II
13 and III were euthanized because of known exposure to the TB-positive tapir I and the positive
14 serological results obtained. Both results were confirmed bacteriologically.

15 Tapir IV showed gradually rising RT serum titres starting eight months after potential contact
16 with infected environment. Five months later the titre was estimated to be “three plus” and a
17 comparative tuberculin skin test was performed with bovine and avian tuberculin (data not
18 shown). Tapir IV reacted strongly positive to bovine tuberculin. Therefore the animal was
19 euthanized and TB was bacteriologically confirmed. To our knowledge this is the first time
20 that the time course of antibody formation against TB in a tapir is recorded in detail.

21 Altogether, the RT yielded promising results to detect TB-infected tapirs. Nevertheless, fur-
22 ther validation studies are required to evaluate its diagnostic reliability.

23 The ELISA described is used as a routine test for the surveillance of tuberculosis in zoos for a
24 wide variety of mammals in the Netherlands by the National Reference Laboratory, CIDC-
25 Lelystad. In the report presented here the results are difficult to interpret. Camel II showed a
26 significantly elevated serum antibody titre against *M. bovis* and *M. avium* extracts, indicating

1 a mycobacterial infection, although mycobacteria could not be detected. The bacteriologically
2 confirmed TB-positive tapirs II and III yielded strong ELISA titres. Tapir IV showed low or
3 negative ELISA serum titres from the suspected onset of infection until the date of euthanaza-
4 tion and the bacteriological confirmation of the TB infection.

5 Up to now only one report exists on *M. pinnipedii* causing TB in humans (18). In the cases
6 presented here, no indication of infection of the zoo personnel was obtained by now.

7

8 **Conclusion**

9 The clinical courses of disease and pathological findings indicate that *M. pinnipedii* is patho-
10 genic not only for pinnipeds, but also for different terrestrial mammals, thus strongly confirm-
11 ing the wide host range and the zoonotic potential of this member of the MTC. The use of
12 molecular typing methods elucidated the potential routes of transmission. New specific and
13 sensitive serological tests for TB may enable us in future to avoid the spread of the disease by
14 movement of asymptomatic animals.

15

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19

20 **References**

1. Bernardelli, A., Bastida, R., Loueiro, J., Michelis, H., Romano, M. I., Cataldi, A., Costa, E., 1996. Tuberculosis in sea lions and fur seals from the south-western Atlantic coast. *Rev. Sci. tech. Off. Int. Epiz.* 15, 985-1005.
2. Blair, W. R., 1913. Report of the veterinarian, p 74. *In* 17th annual report of the New York Zoological Society.
3. Brosch, R., Gordon, S. V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K.,

- Garnier, T., Gutierrez, C., Hewison, G., Kremer, K., Parsons, L. M., Pym, A. S., Samper, S., van Soolingen, D., Cole, S. T., 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci. USA* 99, 3684-3689.
4. Cousins, D. V., Bastida, R., Cataldi, A., Quse, V., Redrobe, S., Dow, S., Duigignan, P., Murray, A., Dupont, C., Ahmed, N., Collins, D. M., Butler, W. R., Dawson, D., Rodriguez, D., Loureiro, J., Romano, M. I., Alito, A., Zumarraga, M., Bernardelli, A., 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. *Int. J. Syst. Evol. Microbiol.* 53, 1305-1314.
 5. Ehlers, K., 1965. Records of the hooded seal *Cystophora cristata* Erxleben, and other animals at Bremerhaven Zoo. *Int. Zool. Yearbook* 5, 148-149.
 6. Flamand, J.R., Greth, A., Haagsma, J, Griffin, F., 1994. An outbreak of tuberculosis in a captive herd of Arabian oryx (*Oryx leucoryx*), diagnosis and monitoring. *Vet. Rec.* 29, 115-8.
 7. Hall, M. R., Thoen, C. O., 1983. Preparation of biologically active components of *Mycobacterium bovis*, using Triton X-100 or potassium chloride. *Am. J. Vet. Res.* 44, 1602-1604.
 8. Huard, R. C., Fabre, M., de Haas, P., Lazzarini, L. C. O., van Soolingen, D., Ho, J. L., 2006. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. *J. Bact.* 188, 4271-4287.
 9. Kamerbeek, J., Schouls, L., Kolk, A., van Agterveld, M., van Soolingen, D., Kuijper, S., Bunschoten, A., Molhuizen, H., Shaw, R., Goyal, M., van Emden, J., 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis, and epidemiology. *J. Clin. Microbiol.* 35, 907-914.

10. Kubica, G. P., Dye, W. E., Cohn M. L., 1963. Sputum digestion and decontamination with N-acteyl-L-cysteine-sodium hydroxide for culture of mycobacteria. *Am. Rev. Respir. Dis.* 87, 775-779.
11. Lyashchenko, K. P., Greenwald, R., Esfandiari, J., Olsen, J. H., Ball, R., Dumonceaux, G., Dunker, F., Buckley, C., Richard, M., Murray, S., Payeur, J. B., Andersen, P., Pollock, J. M., Mikota, S., Miller, M., Sofranko, D., Waters, W. R., 2006. Tuberculosis in elephants: Antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. *Clin. and Vaccine Immunol.* 13, 722-732.
12. Mazars, E., Lesjean, S., Banuls, A. L., Gilbert, M., Vincent, V., Gicquel, B., Tibaurenc, M., Locht, C., Supply, P., 2001. High-resolution minisatellite-based typing as a portable approach to global analysis of *Mycobacterium tuberculosis* molecular epidemiology. *Proc. Natl. Acad. Sci. U.S.A* 98, 1901-1906.
13. Moisson, P., Lacroix, F., Manson, C., André-Fontaine, C., Bakker, D., Fiette, L., Thorel, M.-F. , Lernould, J.-M., 1998. Tuberculosis outbreak in some felid species and Southern sea lions in Mulhouse zoo from 1992 to 1996: Diagnostic perspectives. *European Association of Zoo and Wildlife Veterinarians*, p. 93-111. *Proceedings of the 2nd meeting. European Association of Zoo and Wildlife Veterinarians and the British Veterinary Zoological Society*, Chester, United Kingdom.
14. Prodinger, W. M., Brandstaetter, A., Naumann, L., Pacciarini, M., Kubica, T., Boschiroli, M. L., Aranaz, A., Nagy, G., Cvetnic, Z., Ocepek, M., Skrypnyk, A., Erler, W., Niemann, S., Pavlik, I., Moser, I., 2005. Characterization of *Mycobacterium caprae* isolates from Europe by mycobacterial interspersed repetitive unit genotyping. *J. Clin. Microbiol.* 43, 4984-4992.
15. Rodriguez, J., Fissanoti, G, J. C., del Portillo, P., Patarroyo, M. E., Romano, M. L.,

- Cataldi, A., 1999. Amplification of a 500-base-pair fragment from culture isolates of *Mycobacterium bovis*. *J. Clin. Microbiol.* 37, 2330-2332.
16. Supply, P., Lesjean, S., Savine, E., Kremer, K., van Soolingen, D., Locht C., 2001. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. *J. Clin. Microbiol.* 39, 3563-3571.
17. Supply, P., Allix, C., Lesjean, S., Cardoso-Oelemann, M., Ruesch-Gerdes, S., Willery, E., Savine, E., de Haas, P., van Deutekom, H., Roring, S., Bifani, P., Kurepina, N., Kreiswirth, B., Sola, C., Rastogi, N., Vatin, V., Gutierrez, M. C., Fauville, M., Niemann, S., Skuce, R., Kremer, K., Locht, C., van Soolingen, D., 2007. Proposal for standardization of optimized Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol.* 44, 4498-4510.
18. Thompson, P. J., Cousins, D. V., Gow, B. L., Collins, D. M., Williamson, B. H., Dagnia H. T., 1993. Seals, seal trainers and mycobacterial infection. *Am. Rev. Respir. Dis.* 147, 164-167.
19. Vordermeier, H. M., Cockle, P. C., Whelan, A., Rhodes, S., Palmer, N., Bakker, D., Hewinson, R. G., 1999. Development of diagnostic reagents to differentiate between *Mycobacterium bovis* BCG and *M. bovis* infection in cattle. *Clin. Diagn. Lab. Immunol.* 6, 675-682.
20. Woods, R., Cousins, D. V., Kirkwood, R., Obendorf D. L., 1995. Tuberculosis in wild Australian fur seal (*Arctocephalus pusillus doriferus*) from Tasmania. *J. Wildlife Dis.* 31, 83-86.
21. Zumarraga, J. M., Bernardelli, A., Bastida, R., Quse, A. V., Loureiro, J., Cataldi, A., Bigi, F., Alito, A., Ramos, M. C., Camper, S., Otal, I., Martin, L, C., Romano, M. I.,

1999. Molecular characterisation of mycobacteria isolated from seals. *Microbiology* 145, 2519-2526.

1

Figure 1 Suspected route of TB transmission in zoo A and zoo B

Figure 2 Spoligotyping patterns of MTC isolates from sea lion, camel, tapirs
Spacers 14 and 18: artefact (background on the membranes of Isogen Bioscience)

Table 1 Time table and results of serological and bacteriological examinations of sea lion, camels and tapirs

¹⁾ Antibody titre of 1:40 was used as cut-off value in ELISA; reciprocal titres are shown
results are shown as “-“, negative; “+”, weakly positive; “++”, positive; or “+++”, strongly positive.

³⁾ ND, not done.

Table 2 MIRU pattern of the MTC isolates from sea lion I, camel I, and tapirs I-III

2

Table 1

Animal	Location (zoo)	Sample date	Bacteriological culture	Molecular detection	Serological tests				
					ELISA (reciprocal serum dilution)				RT
					Sample date	<i>M. bovis</i>	<i>M. avium</i>	MPB70	
Sea lion I	A	09-2001	+	N. D.		N. D.	N. D.	N. D.	N. D.
Camel I	A	11-07-2002	+	N. D.		N. D.	N. D.	N. D.	N. D.
Camel II	A	07-02-2003	-	-	25-12-2002	320	320	<5	N. D.
Camel III	A		N. D.	N. D.	25-12-2002	<5	<5	<5	N. D.
Camel IV	A		N. D.	N. D.	25-12-2002	<5	<5	<5	N. D.
Tapir I	A/B	07-01-2005	+	+	03-01-2005	N.D	N.D	N.D	+++
Tapir II	B	25-08-2005	+	+	18-08-2005	>640	>640	320	+++
Tapir III	B	06-09-2005	+	+	25-08-2005	>640	>640	>640	+++
Tapir IV	B				19-05-2005	40 ¹⁾	<5	<5	-
Tapir IV					23-09-2005	80	<5	<5	-
Tapir IV					27-01-2006	80	<5	<5	+
Tapir IV					10-05-2006	80	<5	<5	++
Tapir IV					23-05-2006 *	40	<5	<5	+++
Tapir IV		08-06-2006	+	+					

* At this date a comparative tuberculin skin test was performed and read after 72h. Tapir IV reacted strongly positive towards bovine tuberculin (data not shown).

MIRU locus	2	4	10	16	20	23	24	26	27	31	39	40
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MIRU copy number	2	5	6	4	2	4	2	2	2	3	2	2
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Table 2

Figure 1

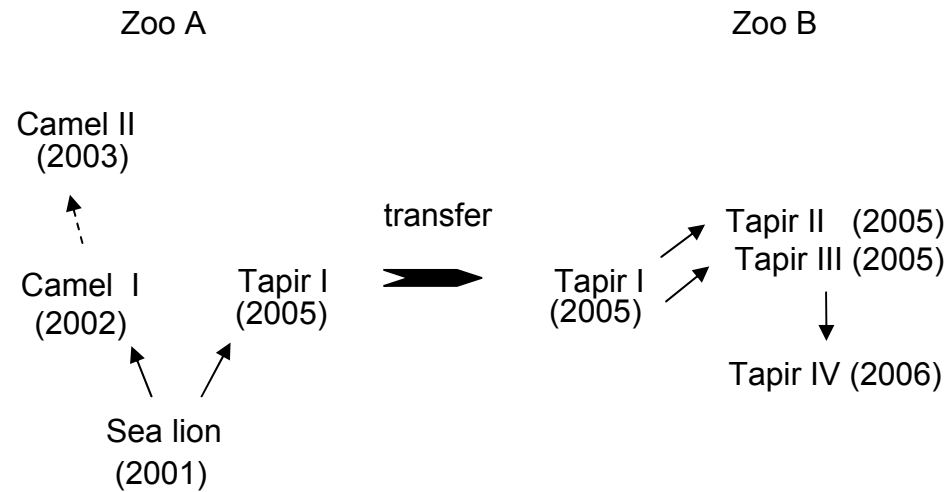


Figure 2

