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INFECTIOUS DISEASE

Outbreak of Tuberculosis in a Colony of Rhesus Monkeys (*Macaca mulatta*) after Possible Indirect Contact with a Human TB Patient

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Summary

Simian tuberculosis is one of the most important bacterial diseases of non-human primates. Outbreaks of tuberculosis have been reported in primate colonies almost as long as these animals have been used experimentally or kept in zoological gardens. Significant progress has been made in reducing the incidence of tuberculosis in captive non-human primates, but despite reasonable precautions, outbreaks continue to occur. The most relevant reason is the high incidence of tuberculosis (TB) amongst the human population, in which tuberculosis is regarded as an important re-emerging disease. Furthermore, many non-human primate species originate from countries with a high burden of human TB. Therefore, *Mycobacterium tuberculosis* remains a significant threat in animals imported from countries with high rates of human infection. We report an outbreak of tuberculosis among a group of rhesus monkeys (*Macaca mulatta*) living in a closed, long-term colony. The outbreak coincided with reactivation of a TB infection in a co-worker who never had direct access to the animal house or laboratories. Eleven of 26 rhesus monkeys developed classical chronic active tuberculosis with typical caseous granulomata of varying size within different organs. The main organ system involved was the lung, suggesting an aerosol route of infection. Such an outbreak has significant economic consequences due to animal loss, disruption of research and costs related to disease control. Precautionary measures must be improved in order to avoid TB in non-human primate colonies.

Keywords: chronic active tuberculosis; granulomatous pneumonia; *Mycobacterium tuberculosis*; rhesus macaque

Introduction

Simian tuberculosis (TB) is most often contracted from people and closely resembles the human illness (Brack, 1987; Simmons and Gibson, 2012). Non-human primates have been used widely for TB research including vaccine and drug efficacy studies and this has augmented our knowledge of the simian disease (Capuano *et al*., 2003; Lin and Flynn, 2012). Most simian cases are caused by *Mycobacterium tuberculosis*, but the incidence of *Mycobacterium bovis* infections is increasing (Zumpe *et al*., 1980; Stetter *et al*., 1995; Keet *et al*., 1996, 2000; Thorel *et al*., 1998; Garcia *et al*., 2004a; Biet *et al*., 2005). Infections with other members of the *M. tuberculosis* complex (MTBC), including *Mycobacterium africanum*, *Mycobacterium microti* or *Mycobacterium canettii*, are rare (Thorel, 1980). Mycobacteria other than tuberculosis (MOTT) belonging to Runyon groups I–III may lead to mycobacteriosis, a TB like disease, which is differential diagnosis. In most cases, mycobacteriosis is

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caused by the *Mycobacterium avium—intracellulare* complex, a group of phenotypically similar species comprising *Mycobacterium avium* with its four subspecies (*M. avium* ssp. *avium*, *Mycobacterium silvaticum*, *Mycobacterium paratuberculosis* and *Mycobacterium hominissuis*) as well as several species closely related to *M. intracellulare* (e.g. *Mycobacterium chimaera*). Agents of the *M. avium—intracellulare* complex are opportunistic pathogens that often occur in immunodeficient animals in association with simian immunodeficiency virus (SIV) infection (Mansfield and Lackner, 1997). This disease primarily involves the gastrointestinal tract and associated lymphatics or may be disseminated (Holmberg et al., 1982). Other more rarely involved mycobacteria include *Mycobacterium kansasii*, *Mycobacterium simiae* and *Mycobacterium chelonae* (Didier *et al*., 1999; Parsons *et al*., 2010).

The main route of transmission of simian TB is inhalation via the respiratory tract. The disease usually affects the respiratory tract, but TB can disseminate to almost any organ and is regarded as a systemic infection (Hines *et al*., 1995).

TB occurs in prosimians and New and Old World monkeys as well as in great apes. Although these species are variably susceptible to the disease, all non-human primates can develop TB. Old World monkeys are considerably more sensitive than great apes and New World monkeys (Brack, 1987). The most severe outbreaks have been observed in macaque species and African green monkeys (*Cercopithecus aethiops*) (Hines *et al*., 1995). The course of the disease may vary depending on the macaque species affected. Rhesus monkeys (*Macaca mulatta*) may develop an acute and progressive form of TB (Langermans *et al*., 2001). Cynomolgus monkeys (*Macaca fascicularis*) have a more variable course of infection. They appear to be more resistant and are able to carry the infection subclinically, mimicking latent infection in man (Walsh *et al*., 1996; Capuano *et al*., 2003). In this species, a chronic debilitating disease course is possible as well as a latent infection without overt disease depending on the infective dose (Capuano *et al*., 2003; Lin *et al*., 2009).

After primary infection, a spectrum of clinical syndromes can evolve. Often, primates are simply found dead with no previous clinical history. If clinical signs occur, they are usually non-specific and include weakness, paralysis, reduced appetite, weight loss, dull hair coat, coughing and general depression. Intermittent coughing is a characteristic clinical sign of pulmonary TB. Signs of extrapulmonary TB are determined by the organs involved. Cases of primary and secondary cutaneous TB are characterized by non-healing wounds, draining ulcers or fistulous tracts combined with enlargement of lymph nodes. Vertebral TB results in paraplegia or kyphosis and cerebral TB may cause epileptic seizures (Fox *et al*., 1974; Machotka *et al*., 1975). Intestinal TB presents with severe diarrhoea (Simmons and Gibson, 2012).

Pulmonary disease is the most common manifestation, so intermittent coughing and chronic weight loss should always be considered as an indicator of the disease (Garcia *et al*., 2004a). The diagnosis of TB can pose a challenge. Ante-mortem diagnosis is traditionally based on the intradermal tuberculin skin test (TST) using mammalian old tuberculin or tuberculin purified protein derivative injected into the eyelid. Unfortunately, there may be false-positive and false-negative reactions, so combination with other tests such as the interferon (IFN) gamma releasing assay (PRIMAGAM®; Prionics AG, Zurich, Switzerland) or (Quantiferon Gold Test®; Cellestis, QIAGEN, Venlo, Netherlands) is advised (Garcia *et al*., 2004b; Bushmitz *et al*., 2009; Parsons *et al*., 2009).

The aim of the present paper is to present the clinicopathological findings in a colony outbreak of TB amongst rhesus monkeys thought to have been initiated by indirect exposure to an infected person.

Materials and Methods

Details of the Colony and Disease Outbreak

The animals of the Max Planck Institute (MPI), Tübingen, Germany, are kept under the regulations for non-human primates and the guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC; Appendix A ETS 123). This includes health monitoring, housing conditions, primate husbandry, care and environmental enrichment. These conditions are consistent with the regulations of the Guide for Care and Use of Laboratory Animals of the National Research Council (USA). All experiments are performed in accordance with the German Animal Welfare Act. This includes supervising and advice by the institutional animal welfare officer and the institutional animal welfare body. The experiments were approved by the governmental veterinary authorities under numbers KY2/06, KY1/07, KY2/08, KY3/09, KY6/10 and KY1/11 given by the Regierungspräsidium (RP) Tübingen (Baden-Württemberg), Germany. Housing conditions and medical procedures performed on animals were approved by the local authorities (as above). The entire colony was closed for experimental work as soon as the diagnosis was confirmed, so ethical evaluation was not relevant for dealing with this medical problem.

The outbreak occurred among a group of 26 adult rhesus monkeys of different ages and sexes (13 male

and 13 female) living in one of the two facilities of the MPI. The animal facility is a separate building with electronic access control and double entrance doors. A limited number of staff had access. All staff members were monitored once a year for TB. The animals were co-housed in 12 group cages with two to five animals per cage (each 7 m^2 floor size and 2.3 m height) located in one large room with a common air ventilation system. Over the last 10 years new animals had been continuously integrated into the group. Only domestically reared animals with certificated origin and with a minimum of two negative TB tests prior to delivery were taken into the colony. All animals were given health checks including a TB test twice a year; all TB tests prior to the outbreak were negative. The individual composition of the animal groups changed constantly due to the needs of group housing and experimentation. The animals were allowed free access to food and water and had routine environmental enrichment. Animal rooms were maintained at 25°C on a 12/12 h light and dark cycle. The animals were fed primate pellets every day, supplemented by fruit, nuts, dried crop and vegetables of the standard for human consumption. Most of the animals were used in neurobiological studies covering both repeated electrophysiological as well as magnetic resonance imaging experiments under anaesthesia and during waking.

Clinical Investigation

Regular complete physical examinations were performed on each animal. The in-vivo diagnosis was based on the intradermal TST using tuberculin purified protein derivative (PPD RT 23) in combination with the PRIMAGAM® test. The palpebral area is used for tuberculin skin testing as it is easy to visualize the results of the test without anaesthetizing the animals. For the palpebral TST, 0.1 ml PPD RT 23 (2 TU/0.1 ml) (Statens Serum Institute, Copenhagen, Denmark) was injected intradermally as close as possible to the edge of the upper eyelid of each monkey using a sterile 27 gauge needle. Palpebral reactions were graded at 8, 24, 48 and 72 h after injection with the standard 0–5 scoring system (Bushmitz *et al*., 2009).

The PRIMAGAM® test is a whole blood IFN-γ stimulation assay licensed for use in cynomolgus and rhesus macaques. The assay may discriminate between MTBC and *M. avium* infection by including avian and bovine PPD separately in the test. The amount of IFN-γ produced in response to stimulation with different antigens can be used to differentiate a reaction due to *M. bovis*, *M. tuberculosis* or non-tuberculous mycobacteria. It is a quantifiable diagnostic test with good sensitivity and specificity compared with disease status determined by pathological examination (Vervenne *et al*., 2004; Garcia *et al*., 2004b).

As additional tests, dorsoventral and lateral chest radiographs and sonograms were used to detect thoracic and abdominal lesions. Furthermore, gastric lavage was performed to determine by culture or polymerase chain reaction (PCR) whether mycobacteria were present in the gastrointestinal system.

Histopathology

Samples for histopathology were fixed in 10% neutral buffered formalin. Tissues were processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE) and with the Ziehl–Neelsen and Fite Faraco Kynjon stains for acid-fast bacteria.

Microbiology

Attempts to culture mycobacteria from the infected monkeys were performed on gastric aspirates and organ samples taken during necropsy examination. Direct nucleic acid amplification test (NAAT) to detect *M. tuberculosis* complex DNA was performed using the ProbeTec ET DTB (Becton Dickinson and Company, Heidelberg, Germany). For identification of MTBC species, GenoType® MTBC (Hain Lifescience GmbH, Nehren, Germany) was used according to the manufacturer's instructions. As a gold standard, culture techniques using BACTEC™ MGIT™ 960 solid medium and liquid broth were applied. For direct susceptibility testing we used the BACTEC™ MGIT™ 960. The isolated strains were sent to the National Reference Centre for Mycobacteria (Borstel, Germany) for molecular genotyping. All isolates were analyzed by the spoligotyping technique (spacer oligonucleotide typing) according to standardized protocols described by Kamerbeek *et al*. (1997). In addition, 24-loci MIRU VNTR (mycobacterial interspersed repetitive units variable number of tandem repeats) was performed for all samples (Supply *et al*., 2006) and compared using a web-based database (http://www.miru-vntrplus.org).

Results

Timeline of the Outbreak

The first (index) animal (animal 1) developed coughing 5 months before the ultimate diagnosis of TB was made. In the following months the animal showed intermittent diarrhoea and weight loss. In light of the results of analyzed stool samples, this animal was treated with different antibiotics over a period

of several weeks with periods of recovery followed by relapse and deterioration. Over time, the cough gradually intensified. Since the animal recovered intermittently TB was not considered as a differential diagnosis. One month before diagnosis, the animal became anorectic and weight loss increased above 26% of its pre-disease weight. Finally, this animal was humanely destroyed.

Necropsy examination revealed caseous granulomas in the lung, spleen and liver. Microbiological studies confirmed that *M. tuberculosis* was the cause of disease. After confirmation of this case the remaining 25 animals were investigated and monitored. Over the following 2 weeks, 10 more animals were identified as likely to be infected with *M. tuberculosis* (Table 1). Two had shown signs such as cough and weight loss (animals 2 and 3); the other eight showed no clinical signs. All 10 animals had positive palpebral skin tests of grade 3–5 (Fig. 1). These animals showed swelling and drooping of the eyelid with varying degrees of erythema or marked swelling with necrosis and closed eyelids within 48–72 h. In five of these 10 cases, gastric lavage was positive for the presence of mycobacteria by culture and PCR (animals 2, 6, 7, 8 and 9). In eight animals radiographical lesions of different size, shape and density were detected in the lung (Table 1). Of the positively diagnosed animals, five were additionally tested for *Mycobacterium* infection using the PRIMAGAM® test and all were positive (animals 7–11). Based on these results, a TB outbreak in the group was confirmed. All 25 animals were immediately placed under quarantine with access only for care and medical staff and were handled under biosafety level 3 conditions.

Within 2 weeks, six animals were humanely destroyed. One of these animals had not shown positive results during testing, but had been exposed to a high infection risk. A therapeutic plan was designed for the remaining animals. Prophylactic treatment was also started for the 15 test-negative animals, which still had to be co-housed in order to prevent further spread of the infection. After a therapeutic trial with a combination of isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and pyrazinamid (PZA), daily for 6 weeks, as advised by the World Health Organisation for treatment of human TB, it became apparent that the housing and laboratory environment of the animals would not permit their re-use without a major risk of reactivation of the infection. Therefore therapy was stopped and the positive animals were humanely destroyed.

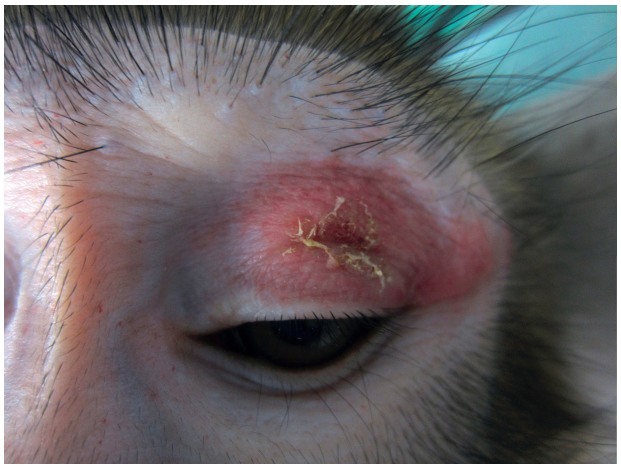

Fig. 1. Animal 4 showing a positive reaction after the palpebral TST. There is swelling and drooping of the eyelid with erythema and focal dermal necrosis 72 h after application.

The remaining 15 animals of the group did not show clinical signs of the disease. They were skin test and PRIMAGAM® test negative and had normal thoracic radiographs. They were used in a short experimental neurobiological study and were then humanely destroyed.

Table 1
Results of indirect TB tests and distribution of lesions

Animal	*Skin test*	*Gastric lavage*	*X-ray*	*PRIMAGAM® test*	*Lung*	*Lung lymph node*	*Spleen*	*Liver*	*Liver lymph node*	*Kidney*
1	ND	ND	ND	ND	Primary lesion	Primary complex	~20 granulomas	~50 granulomas	1 granuloma	5 granulomas
2	+	+	+	ND	Primary lesion	Primary complex	~10 granulomas	~30 granulomas	1 granuloma	4 granulomas
3	+	–	+	ND	Primary lesion	Primary complex	~40 granulomas	~20 granulomas	1 granuloma	4 granulomas
4	+	–	+	ND	Primary lesion	Primary complex	–	–	–	–
5	+	–	+	ND	Primary lesion	Primary complex	–	–	–	–
6	+	+	+	ND	Primary lesion	Primary complex	4 granulomas	~10 granulomas	–	–
7	+	+	+	+	Primary lesion	Primary complex	–	–	–	–
8	+	+	+	+	Primary lesion	Primary complex	~15 granulomas	~40 granulomas	–	–
9	+	+	+	+	Primary lesion	Primary complex	~20 granulomas	~8 granulomas	–	–
10	+	–	–	+	Primary lesion	Primary complex	–	–	–	–
11	+	–	–	+	Primary lesion	Primary complex	–	–	–	–

+, positive test; –, negative test; ND, not done.

After confirmation of the first case among the rhesus monkeys, all staff members of the facility and co-workers of the institute were monitored for signs of disease. One person working in a different department of the institute, who had never been in or near the animal facility, was identified with signs of acute pulmonary TB. This person had been suffering from a chronic form of the disease, which had been believed to be cured. All other staff tested negative and remained healthy.

To summarise: the index animal first developed signs of TB in September 2011 and was humanely destroyed at the end of January 2012. Ten positive animals were detected within the group and five of them were humanely destroyed shortly after the diagnosis was confirmed in February 2012. Five infected animals were treated, but were humanely destroyed in mid-March 2012 because of poor recovery. The short neurobiological study with the TB-negative contact animals was finished at the end of April 2012.

All 30 animals housed in the second facility of MPI remained healthy, despite the fact that the same staff worked in both facilities every day. Often, a single person was responsible for both facilities on weekends and holidays.

Gross Pathology

At necropsy examination, the index animal and all 10 TB-infected animals showed classical gross pathological findings. The main lesions were found within the respiratory system. In all animals firm nodules were noted within the lung, mainly in the right cranial and caudal lobes. The nodules were yellow—white or grey in colour and ranged in size from pinpoint to several millimetres in diameter (Figs. 2 and 3).

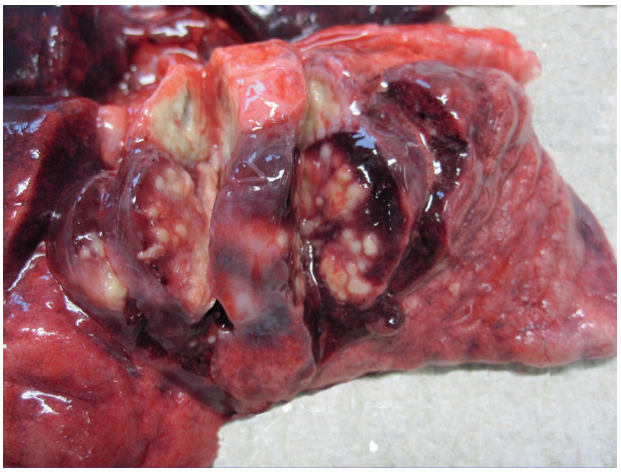

Fig. 3. Lung from animal 5 showing severe tuberculous pneumonia with large confluent granulomas within the cranial lobe.

Many of these nodules were coalescing, leading to classical tuberculous pneumonia with larger areas of necrosis (Fig. 3). In animal 2 there were large cavitating lesions connected with the bronchioles (Fig. 5). A classical 'Ghon complex' was demonstrated in all animals. The tracheobronchial and hilar lymph nodes were enlarged, having focal or multifocal granulomas or complete caseous effacement (Figs. 2 and 4). Comparable granulomatous nodules of similar or larger size were found in the liver and spleen of six animals (animals 1, 2, 3, 6, 8 and 9), indicating secondary spread of the disease. In general, granulomas in liver and spleen were larger than those in the lung and were often confluent and diffusely distributed within the parenchyma (Figs. 6 and 7). In three animals (animals 1, 2 and 3) granulomatous lesions were also found in the kidney

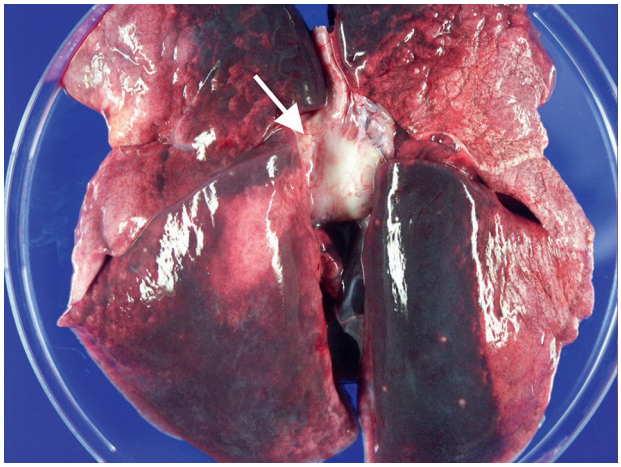

Fig. 2. Lung from animal 3 showing enlargement of the bronchopulmonary lymph node (arrow) and small pinpoint granulomas throughout the parenchyma of the right caudal lobe.

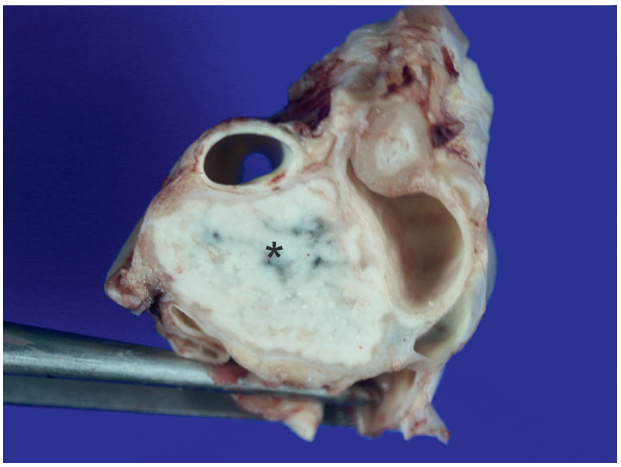

Fig. 4. Lung from animal 2 showing an enlarged bronchopulmonary lymph node with a caseous necrotic centre (asterix); fixed tissue.

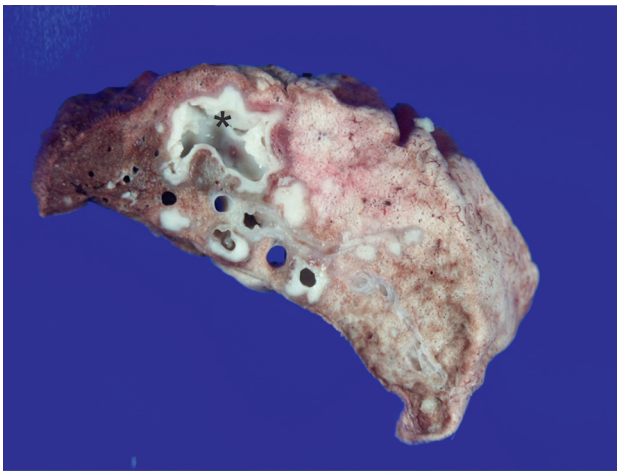

Fig. 5. Lung from animal 2 showing multiple granulomas of different sizes within the parenchyma and cavitating and invasive lesions (asterix); fixed tissue.

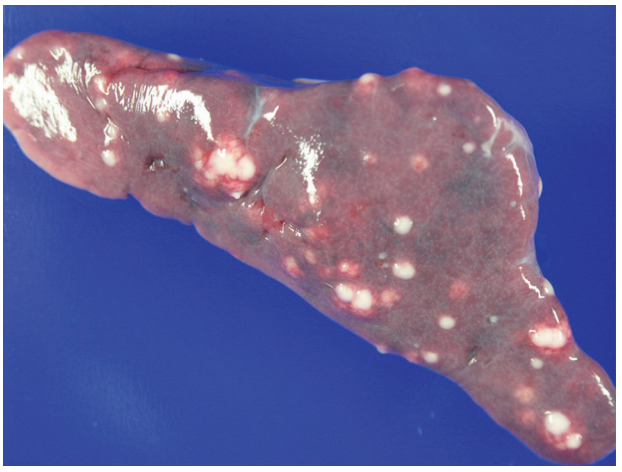

Fig. 7. Spleen from animal 3, showing multiple granulomas of different sizes distributed diffusely within the parenchyma.

and the hepatic lymph nodes. In animal 8 there were lesions in one testis.

Histopathology

Microscopically, the pulmonary lesions ranged from scattered, discrete small organized caseous granulomas to coalescing areas of caseous necrosis. These lesions were accompanied by pulmonary oedema or haemorrhage. Caseous granulomas had a central area with amorphous eosinophilic debris surrounded by a border zone of epithelioid macrophages and small numbers of giant cells with peripheral nuclei (Langhans giant cells). Mineralization of the necrotic cores was not observed. Granulomas were surrounded by an outer margin with small amounts of fibrous connective tissue accompanied by lymphocytes and plasma cells. In the periphery of the larger granulomas there were small non-necrotizing or solid granulomas composed of epithelioid macrophages and Langhans giant cells. In some locations tuberculous pneumonia was evident, characterized by areas with multiple coalescing large granulomas extending into large areas of necrosis (Fig. 8). Some of the larger granulomas had extended into the major airways and formed cavities. In these cases the bronchial epithelium was ulcerated and heavily infiltrated with neutrophils. Expanded bronchial airways contained necrotic debris admixed with inflammatory cells (Fig. 9). Animal 5 showed necrosis and ulceration of the trachea with extension to a severely affected hilar lymph node.

The microarchitecture of the lymph nodes was effaced by large areas of caseous necrosis consisting of amorphous eosinophilic material surrounded by

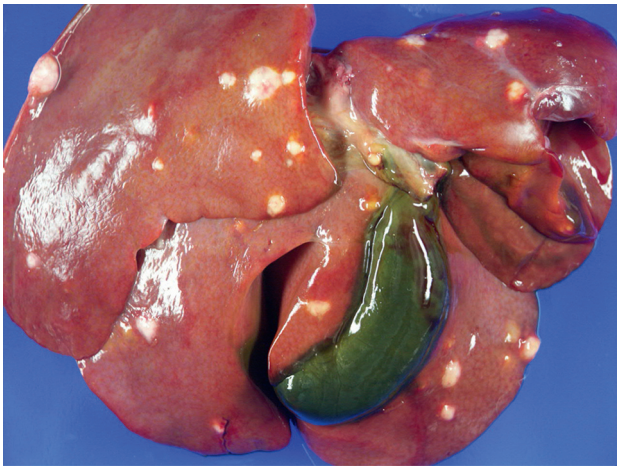

Fig. 6. Liver from animal 1 showing multiple granulomas of different sizes distributed diffusely within the parenchyma.

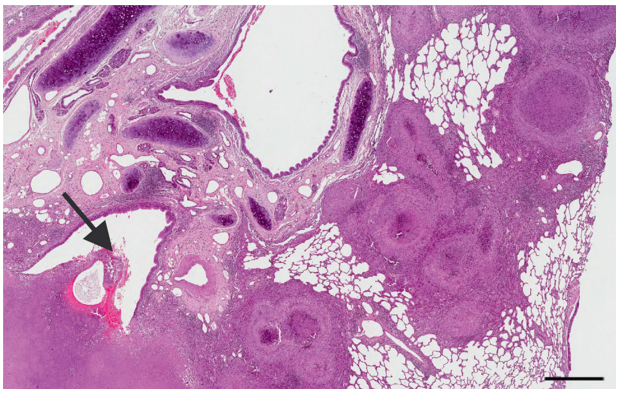

Fig. 8. Lung from animal 8 showing tuberculous pneumonia with multiple large coalescing granulomas extending into and involving the lumen of the major airways (arrow). Airways contain necrotic debris. HE. Bar, 1 mm.

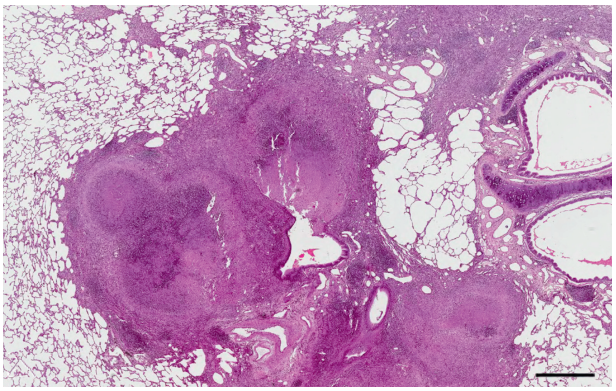

Fig. 9. Lung from animal 8 showing tuberculous pneumonia with multiple large coalescing granulomas extending into and involving the lumen of the major airways (arrow). Airways contain necrotic debris. HE. Bar, 1 mm.

dense infiltrates of epithelioid macrophages, fewer multinucleated giant cells and few lymphocytes and plasma cells within the periphery. In other cases, variably sized granulomas were found within a background of activated lymphoid tissue.

Typical granulomas with a caseous centre consisting of acellular necrotic debris were also found within the liver and spleen. The central cores were surrounded by a zone of epithelioid cells interspersed with few Langhans-type giant cells (Figs. 10—12). Peripheral to the larger granulomas were small non-necrotizing granulomas composed of epithelioid macrophages and Langhans giant cells. These solid granulomas had a small outer rim of lymphocytes (Fig. 13). Calcification of the larger granulomas was minimal or absent. It was not possible to demonstrate acid-fact bacteria within the granulomas, but the characteristic gross and microscopical findings enabled a diagnosis of TB to be made. The diagnosis was confirmed by microbiological investigation.

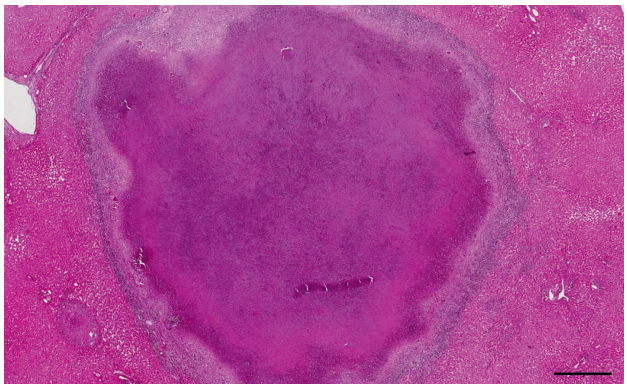

Fig. 10. Liver from animal 2. There is a large caseous granuloma with central necrosis surrounded by a narrow mantle zone. HE. Bar, 100 μm.

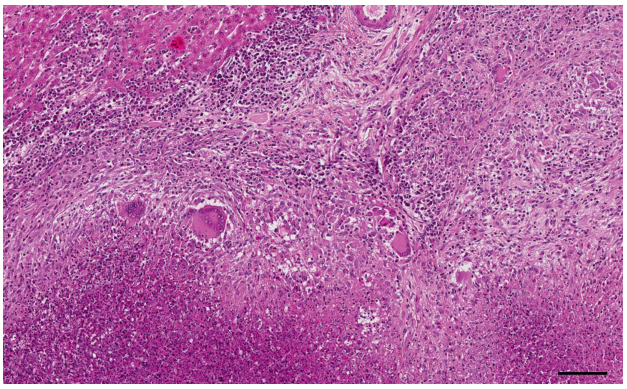

Fig. 11. Liver from animal 2. The mantle zone consists of epithelioid cells plasma cells and lymphocytes and a small amount of fibrosis. HE. Bar, 100 μm.

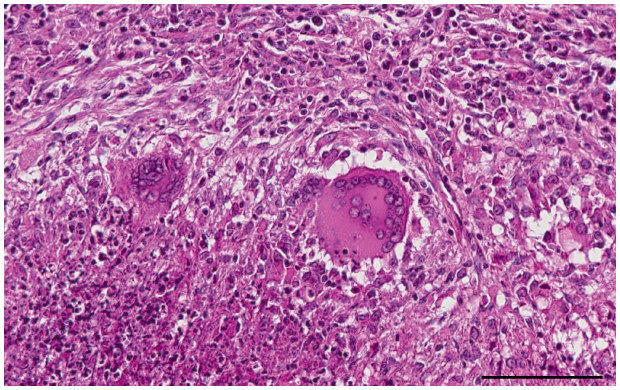

Fig. 12. Liver from animal 2. Single multinucleated giant cells of Langhans-type are present in the transition zone to the inner necrotic area. HE. Bar, 100 μm.

Necropsy examination of the 14 negative monkeys revealed no evidence of TB, even after detailed examination of all lung lobes and draining lymph nodes (Lin and Flynn, 2012).

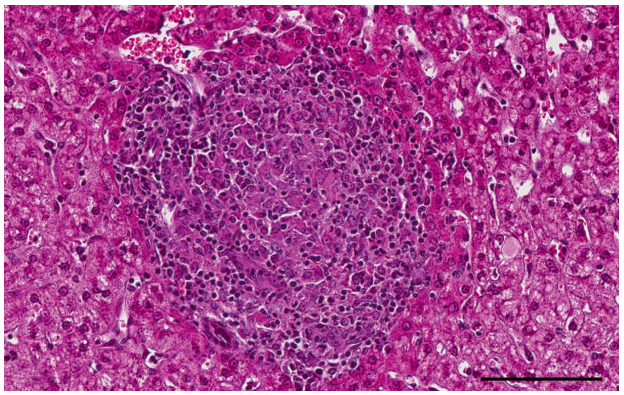

Fig. 13. Liver from animal 2. Small solid granulomas are present in the periphery. HE. Bar, 100 μm.

Microbiology

M. tuberculosis was identified microscopically and by culture of lung and liver samples from the index animal. Gastric lavage samples from five more animals were positive by culture and PCR (animals 2, 6, 7, 8 and 9). In five cases (animals 1, 2, 6, 7 and 8) spoligotyping and MIRU-VNTR analysis showed that the isolated *M. tuberculosis* strains were completely identical (*M. tuberculosis*, lineage: Dehli/CAS [Central Asian], MLVA MtbC15-9: 317−35; Table 2).

Mycobacteria isolated from the human patient were identical to the strain isolated from the animals as confirmed by spoligotyping.

Discussion

TB is rare in natural populations of non-human primates living in areas remote from man. In contrast, TB is the most frequently reported infectious disease of captive non-human primates living in close contact to man (King, 1993). The adoption of strict quarantine procedures and routine tuberculin testing of non-human primates and animal care personnel has reduced the prevalence of this classical anthropozoonosis in zoos and biomedical research facilities over the years. Nevertheless, the occurrence of this outbreak, together with other reports, shows that TB remains a challenge for primate housing facilities (Panarella and Birnes, 2010; Rocha *et al.*, 2010).

As shown in the present outbreak, simian TB is most often acquired after transmission from people followed by monkey-to-monkey transmission (Michel and Huchzermeyer, 1998; Payne *et al.*, 2011). Retransmission from monkey to man offers a potential health risk for other staff members. Simian TB is mainly a disease of captive primates. Little evidence exists of *M. tuberculosis* infections in wild populations (Coscolla *et al.*, 2013). It is predicted that with the growing human population and the disappearance of undeveloped wildlife areas, in combination with an increase of rescue and rehabilitation projects for primates, TB will pose an increasing risk to native primate populations. The detection of TB among free living baboons in peri-urban environments in African countries confirms this suspicion (Keet *et al.*, 2000; Parsons *et al.*, 2009).

The incidence of human TB is still high. In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease (WHO, 2013). The absolute number of cases is still growing, because slow decreases in incidence rates are outweighed by the increase in the world's population. The WHO has identified 22 high-burden TB countries that contribute more than 80% of the global burden of TB. These

Table 2

Genotyping results for six clinical isolates in comparison with the H37Rv reference strain (ATCC 27294).

	24- loci - MIRU VNTR																						Spoligotyping pattern																							
	580	2996	802	1955	960	2163b	3192	1644	424	577	2165	4052	154	2531	4348	2401	2059	2687	3007	4156	2347	2461	3171	3690	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
H37Rv reference strain	3s	3	1	2	3	5	3	2	4	3	5	2	2	6	2	2	2	1	3	2	4	3	3	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Animal 1	2	6	3	4	5	2	5	4	5	2	4	8	2	5	3	2	2	1	3	4	4	2	3	3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Animal 2	2	6	3	4	5	2	5	4	5	2	4	8	2	5	3	2	2	1	3	4	4	2	3	3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Animal 6	2	6	3	4	5	2	5	4	5	2	4	8	2	5	3	2	2	1	3	4	4	2	3	3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Animal 7	2	6	3	4	5	2	5	4	5	2	4	8	2	5	3	2	2	1	3	4	4	2	3	3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Human case	2	6	3	4	5	2	5	4	5	2	4	8	2	5	3	2	2	1	3	4	4	2	3	3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Animal 8	2	6	3	4	5	2	5	4	5	2	4	8	2	5	3	2	2	1	3	4	4	2	3	3	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Numbers of repeats for each of 24 loci are shown according to the MIRU-VNTR nomenclature (http://www.miru-vntrplus.org/MIRU/nomenclature.faces). Spoligotyping results are visualized in a binary form for 43 digits (1, presence of spacer; 0, absence of spacer). All strains investigated were completely identical and belong to the Dehli/CAS lineage.

include India and China, both countries with large breeding colonies for non-human primates intended for export. International travel and trade provide manifold sources for infection. Moreover, the impact of latent infection among the human population, which could be reactivated by numerous cofactors, is underestimated. Human immunodeficiency virus infection is the most common risk factor for reactivation of TB in man.

The present outbreak was linked to a case of human TB. The person in question was a staff member who had worked for a long time in a different department of the institution, but without contact with the animal facilities. This was proven by the electronic locking system, which provides entrance to the animal rooms. The person had suffered from TB in the past and was believed to have been cured. This individual then developed a rheumatic disease and was given prolonged glucocorticotherapy. At the time that the outbreak occurred among the monkeys this person had developed a respiratory disease with typical signs of respiratory TB. Microbiological examination confirmed TB and spoligotyping showed that this was the same organism found in the monkeys. The strain was of lineage Dehli/CAS, which is widely distributed in Asian countries, but also found in Europe. It was suspected that an old TB process in this person was reactivated by the prolonged cortisol treatment. Infected people may harbour the organism for life and have a risk of reactivation of the disease. This could not clearly have been proven, because no data were available from the earlier medical history of this patient. The possibility could not be completely excluded that the person obtained the infection from the animals. The patient was immunosuppressed and therefore highly susceptible to TB. However, since the person had no direct contact with the primates, possible routes of transmission could not be identified. It was assumed that the mycobacteria were carried with clothes, food, other vectors, dust or aerosols. Both the human patient and the primates showed lung involvement and so aerosol transmission would seem to be the most likely route of infection. Low numbers of mycobacteria may infect highly susceptible individuals like the monkeys or an immunosuppressed human patient and a single accidental contact may have been sufficient to establish infection.

Once brought into a non-human primate colony, the disease spreads rapidly, as seen in the present case. Infection occurs by inhalation or occasionally by digestion. The lung involvement in all cases of the present study makes aerosol transmission most likely. Furthermore, all 11 infected animals lived close together in groups of two to three individuals, sharing cages and frequent physical contact (e.g. grooming). Close contact would appear to be the most likely factor involved in transmission of the agent. The 15 healthy animals lived in the same unit and shared the same air ventilation system and attending staff, four of them were in close contact with the infected animals. The disease progresses slowly and clinical signs may be absent until advanced disease is present. TB in macaques commonly results in debilitating pulmonary disease (Garcia *et al*., 2004a). The severity of clinical disease is directly associated with the extent of the gross pathology. In the present case, all 11 infected monkeys developed a chronic active disease state, which is often associated with involvement of multiple lung lobes and extrapulmonary tissues (Lin *et al*., 2009; Lin and Flynn, 2012).

The primary lesion of simian tuberculosis is a typical tubercle. The pathology is similar to that observed in human TB. Lesions can vary from almost non-detectable to widely disseminated, firm yellow–white nodules of different sizes (King, 1993). Granulomas can be seen on the pleural surface or within the lung parenchyma. A variable distribution, ranging from focal, to multifocal or coalescing granulomas within one or more lung lobes, as seen in the present outbreak, is also described in experimental studies (Lin *et al*., 2009). In severe cases, tuberculous pneumonia occurs consisting of coalescing granulomas, completely effacing entire lung lobes. Firm nodules may be seen in all major organs, but the lung is the most commonly affected organ. Gross lesions also include large cavitary and coalescing lesions resulting from ectatic bronchi, and tubercles may extend into the trachea (Simmons and Gibson, 2012). Tracheobronchial lymph nodes may be enlarged and contain focal or multifocal granulomas with various degrees of loss of nodal architecture (Simmons and Gibson, 2012). A classic 'Ghon complex' (i.e. pulmonary granulomata and lymph node involvement) is frequently found and was present in all cases of the present study (Kumar *et al*., 2007). Advanced stages of the disease, as in the present study, are characterized by secondary spread to the spleen, kidney, liver and various lymph nodes (King, 1993; Garcia *et al*., 2004a). Dissemination to the spleen, liver and other organs is variable among monkeys and does not necessarily correlate with the severity of lung involvement.

Microscopical findings depend on the duration and extent of the disease. The histopathological hallmark is the tuberculous granuloma. In experimental studies, a spectrum of different granuloma types can be seen depending on the stage of disease (Lin *et al*., 2009; Lin and Flynn, 2012). Early stages of the

disease are characterized by small granulomas consisting of circumscribed accumulations of epithelioid cells and few Langhans-type giant cells confined to the lung or the intestinal tract. Advanced stages are characterized by classical tubercle formation. Tubercles are granulomas of varying size with a caseous centre consisting of acellular necrotic debris or proteinacious material. The central cores are surrounded by a mantle zone of epithelioid cells and a band of plasma cells and lymphocytes interspersed with few Langhans-type giant cells. All animals of the present investigation developed caseous granulomas, indicating an active stage of disease. In contrast to other animal species, encapsulating fibroplasia is usually not found in non-human primates. The granulomas were unencapsulated and not demarcated from the lung parenchyma (Simmons and Gibson, 2012). Non-necrotizing granulomas are composed of epithelioid macrophages without necrosis and are surrounded by lymphocytes and exclusively occur in active TB (Lin and Flynn, 2012). In the present study this granuloma type was observed at the periphery of larger confluent caseous granulomas and was regarded as an early lesion. Fibrosed and calcified granulomas were not observed. These lesions are generally observed in monkeys with latent infection (Flynn *et al.*, 2003; Lin *et al.*, 2009). Experimental studies show that only long-term non-progressive lesions tend to calcify (Capuano *et al.*, 2003). The number of acid-fast bacilli within granulomatous lesions can vary considerably. Hence, it can be difficult to demonstrate the bacteria within tissue sections, as in the present cases. Therefore, microscopical examination alone is not sufficient for the diagnosis of simian TB.

Rhesus macaques develop a broad range of disease manifestations similar to that observed in man (Walsh *et al.*, 1996). A spectrum from latent to rapidly progressive infection with high mortality is described. The course of disease depends on the dose, the inoculation route and the *Mycobacterium* species. In rhesus macaques, TB tends to be a rapidly progressive disease characterized by early and widespread dissemination, as seen in the present outbreak.

M. tuberculosis infection in man does not usually lead to active progressive disease. The majority of infections are clinically latent. In the present outbreak, none of the animals developed signs of a latent infection that would suggest high infective pressure in indoor group-housed animals.

As a consequence of this outbreak, preventive measures were improved to protect non-human primates and the personnel according to guidelines for both animals and personnel (Bushmitz *et al.*, 2009).

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