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Case Report

Natural outbreak of *Mycobacterium caprae* infection in imported laboratory cynomolgus macaques (*Macaca fascicularis*): diagnostic pitfalls and management of safety precautions

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Abstract: Tuberculosis (TB) is a major health threat for humans and for non-human primates used for toxicology or research purposes. Emerging mycobacterial species represent a major challenge for diagnosis and surveillance programs. Here, we report a natural outbreak of *Mycobacterium caprae* in imported cynomolgus macaques (*Macaca fascicularis*) that occurred at AnaPath Research S.A.U. (APR). The macaques underwent repeated negative intradermal tuberculin tests (IDT) before importation and at the European quarantine station. Exhaustive TB screening was started at APR after confirmation of one positive case at another facility. The animal in question belonged to the same colony received at APR. Diagnostic approaches included clinical examination, PCR, culture, spoligotyping, IDT testing, interferon-γ release assay (IGRA), and thoracoabdominal ultrasound (US). Three regulatory toxicity studies and stock animals were affected. The macaques lacked clinical signs, except for one showing a fistulizing nodule in the right inguinal area, which tested positive for the Mycobacterium tuberculosis complex by PCR. All animals were necropsied and 10 macaques (n=114) showed gross and histologic findings compatible with TB confirmed by PCR and culture. *M. caprae* was identified as the etiological agent by Direct Variable Repeat spacer oligonucleotide typing (DVR spoligotyping). The infection was traced to Asia via the SB1622 spoligotype involved, confirming that the animals were infected prior to their import into Europe. Tuberculin skin test (TST), IGRA, and US were only sensitive in detecting advanced cases of *M. caprae* infection. One staff member showed a positive TST reaction, which was handled in accordance with the Spanish government's health regulations. All the sanitary measures implemented were effective in eradicating the disease. (DOI: 10.1293/tox.2024-0048; J Toxicol Pathol 2024; 37: 197–206)

Key words: tuberculosis, cynomolgus monkey, diagnostics, spoligotyping, prophylactic measures

Introduction: Tuberculosis (TB) was declared a global public health emergency in 1993 by the World Health Organization[1], becoming the 13th most significant cause of death and the second leading infectious killer worldwide. In 2021, it was estimated that 10.6 million people contracted the disease, and one in three infected humans suffer from drug-resistant TB, thus increasing mortality rates, especially in people co-infected with immunodeficiency virus (HIV)[2]. Mycobacterial infections have re-emerged over the past decade in industrialized countries linked to immigration and in Africa associated with the human HIV epidemic[3]. In 2018, the United Nations reported that $13 billion (USD) is needed annually for prevention, diagnosis, treatment, and care[2]. The estimated direct cost of a four- or six-month regimen for drug-susceptible TB treatment in the United States was calculated as $23,000 (USD) per person[4].

TB is a significant threat when nonhuman primates (NHP) are imported from countries with high infection rates. Numerous TB outbreaks have been reported in primate colonies used in experimental studies and in zoological gardens[5–13]. Transmission is not limited to NHP to humans, as infected staff members are equally important sources of animal contagion[8]. Despite the strict measures implemented

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in the early 1990s regulating the importation, quarantine, and animal holding, outbreaks in laboratory NHP continue to occur[14].

To date, two mycobacterial species, *M. tuberculosis* and *M. bovis* (both members of the Mycobacterium tuberculosis complex; MTBC), have been implicated in outbreaks in laboratory cynomolgus macaques (*Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*)[7, 8, 10, 15]. TB infection in both NHP species results in granulomatous lesions, principally in the tracheobronchial lymph nodes and lungs, with occasional dissemination to other organs such as the spleen, liver, and kidney[7, 8, 10, 15–17]. Cynomolgus macaques can better tolerate the disease, showing a higher incidence of subclinical forms[18]. Because cynomolgus macaques are now the most imported NHP for research purposes, these animals represent a major challenge when screening for TB and elevate the risk of introducing emerging mycobacterial species.

Here, we describe a recent TB outbreak in cynomolgus macaques imported from South Vietnam that took place at AnaPath Research S.A.U. (APR), a monkey test facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The objectives of this case report were as follows: i) to describe the outbreak of TB infection in cynomolgus macaques caused by *Mycobacterium caprae*, an emerging member of the MBTC; ii) to present the diagnostic tools implemented and additional investigations performed during the outbreak; and iii) to describe how the outbreak was managed with a focus on safety precautions.

Chronological description of the outbreak: On April 27, 2023, AnaPath Services GmbH (APS) and AnaPath Research S.A.U. (APR) were notified of gross lesions compatible with TB in a cynomolgus macaque necropsied on April 17, 2023, at a test facility in France. On May 4, 2023, APR was formally notified by the quarantine station, as ordered by the National Ministry of Agriculture, confirming the case as MTBC PCR-positive. The infected macaque belonged to the same colony as those animals acquired by APR in March 2023. All cynomolgus macaques were imported from a breeding farm in South Vietnam. According to the certificates, animals tested seronegative for cercopithecine herpesvirus 1 (B-virus), simian immunodeficiency virus (SIV), simian T-lymphotropic virus (STLV), and simian retrovirus type D (SRV). Additionally, all cynomolgus macaques transported to Europe were IDT test-negative (Mantoux) at the breeding farm and in repeated tests performed at the European quarantine station, where the animals were housed prior to relocation to several European test facilities.

APR received 114 adult cynomolgus macaques that were housed in separate animal rooms. Eighty-four animals (42 per sex) were allocated to three regulatory toxicity studies (Studies 1, 2, and 3), and 15 animals per sex were kept as a stock colony. Procedures for all three regulatory toxicity studies were approved by the local authorities (*Generalitat de Catalunya, Departament d'Acció Climàtica, Alimentació i Agenda Rural, Direcció General de Polítiques Ambientals i Medi Natural*) and were in compliance with animal welfare act regulations (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010; *Real Decreto* (Royal Decree) 118/2021 of 23 February and *Real Decreto* 1386/2018 of 19 November both amending *Real Decreto* 53/2013 of 1 February) and the Guide for the Care and Use of Laboratory Animals.

In light of the communicated positive case, exhaustive veterinary clinical inspections and diagnostic surveillance were conducted in all macaques at APR. The animals were clinically healthy, with no signs of respiratory disease, inappetence, and/or weight loss. One male macaque (no. 22921M) had an irregular round nodule with central fistulae in the right inguinal area (Fig. 1A). The caseous contents of the nodule and urine from aleatorily selected macaques were sampled and sent for PCR analysis to an accredited private diagnostic laboratory (Laboratorio Echevarne, Barcelona, Spain). Urine samples are recommended as matrix for PCR testing[19–21]. All urine PCR results were negative on May 12, 2023, whereas the inguinal exudate was positive for MTBC. The animal health authorities (*Generalitat de Catalunya, Departament d'Acció Climàtica, Alimentació i Agenda Rural, Secció de Ramaderia i Sanitat Animal de Barcelona; AHA*) were informed immediately. On May 17, 2023, APR received an inspection by the local authorities, after which precautionary measures were imposed by the *Generalitat de Catalunya* (Government of Catalonia) and were immediately implemented at the facility.

The MTBC-infected macaque (male, no. 22921M) belonged to Study 1. Necropsy was decided and performed on May 17, 2023, in the presence of the local authorities. On postmortem examination, the fistulizing nodule in the right inguinal area corresponded to a superficial inguinal lymph node effaced by multifocal caseonecrotic granulomas contiguous to the dermis. Multiple different-sized granulomas were also observed within the lungs, tracheobronchial, mediastinal, and hepatic lymph nodes, and spleen and were regarded as disseminated TB (Fig. 1B, 1C, and 1D). Another five male macaques were sacrificed on the same day, in accordance with the planned study schedule. One animal (no. 22647) had a minimally enlarged superficial left inguinal lymph node with hemorrhage. Animal no. 21173 exhibited ulcerative dermatitis on one finger, an enlarged left inguinal lymph node with a suppurative exudate, and coalescing abscesses in the left kidney. Both animals were suspected cases because these changes are not typically compatible with TB. The remaining male macaques were unremarkable grossly, as were the six animals sacrificed on May 18, 2023. Twelve females from Study 1 were euthanized on May 25, 2023, and three (nos. 23250, 23280, and 23242) displayed granulomatous lesions compatible with TB. Animal nos. 23250 and 23280 had disseminated disease, whereas granulomas in animal no. 23242 were confined to the tracheobronchial lymph nodes and lungs. One animal (no. 22996) had a focal cystic change in the lungs and was considered a suspected case.

Animals from Study 2 were necropsied between May

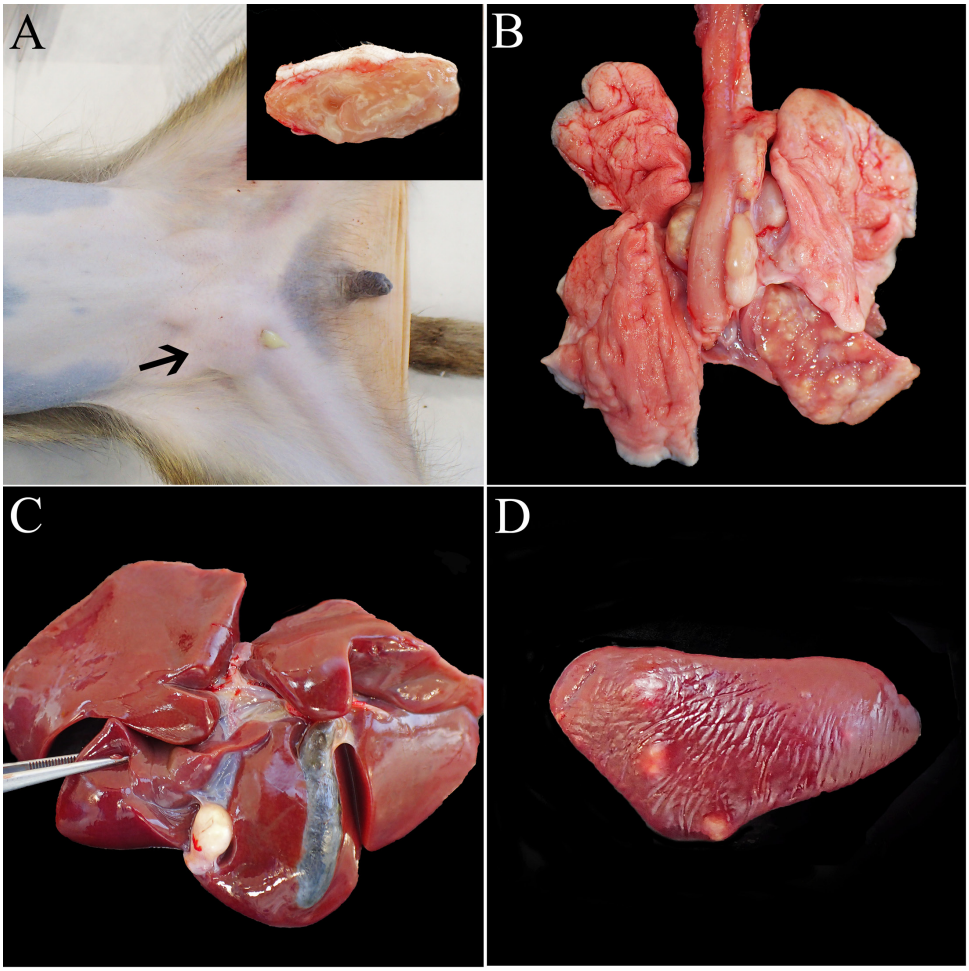

Fig. 1. Macroscopic findings in *M. caprae* infection. Male, no. 22921M. A. Right inguinal area with a nodular change, fistulizing caseo-suppurative exudate (arrow). Inset: affected right inguinal lymph node with multiple-coalescing granulomas. B. Tracheobronchial lymph nodes and pulmonary lobes with multiple-coalescing granulomas. C. Hepatic lymph node effaced by a large granuloma. D. Spleen showing multiple granulomas.

23 and 24, 2023, of which one female macaque (no. 23274) had disseminated TB, and another female (no. 24008) had two suspicious white nodules of approximately 0.3 cm confined to the liver.

Complementary diagnostic approach: After discovering that macaques from these two studies had gross lesions compatible with TB, APR performed intrapalpebral IDTs, and ultrasound examinations (US) on all surviving animals. The aim was to investigate whether positive cases could be detected by combining both approaches to prioritize necropsies of affected animals, including those from Study 3 and recovery animals from Studies 1 and 2. IDT was conducted on May 30, 2023 (13 animals) and June 6, 2023 (64 animals). Readings were performed at 24, 48, and 72 hours post-inoculation, following the classification from the OIE Terrestrial Manual (2022), where reactions of Grade 0 to 2 are considered negative, Grade 3 is inconclusive, and Grades 4 and 5 are positive (methods described in Supplementary Materials 1a). Animal health authorities recommended sacrificing all animals with Grade 4 but did not make it mandatory for animals with Grade 3. An external veterinarian (and ultrasound specialist) carried out thoracoabdominal examinations on May 29, 2023 (13 animals), June 5, 2023 (24 animals). June 8, 2023 (33 animals), and June 12, 2023 (10 animals) (methods described in Supplementary Materials 1b). Particular attention was paid to the presence of B lines[22] and possible irregularities in the spleen. The IDT and US results compared with the gross findings at necropsy are summarized in Supplementary Table 1.

Three macaques scored Grade 4 in the IDT tests: two (nos. 21099 and 22096) belonged to Study 3, and one (no. 22911) to the stock colony. US examination revealed B lines in both hemithoraxes and homogeneous splenomegaly in animal no. 21099. Postmortem examination confirmed granulomas within the tracheobronchial lymph nodes and lungs but no gross changes in the spleen. Animal no. 22096 showed hyperechogenic stippling in the spleen and unaltered lungs by US. However, necropsy showed granulomas within the tracheobronchial lymph nodes and lungs and confirmed the presence of granulomas in the spleen and liver. Animal

no. 22911 showed heterogeneous and hyperechogenic spleen and prominent lung irregularities by US. Grossly, this animal showed granulomas within the mediastinal and tracheobronchial lymph nodes, lungs, pericardium, myocardium, paracostal pleurae, spleen, liver, and kidneys.

Twelve animals from Study 3 and the stock colony showed Grade 3 reactions at 24, 48, or 72 hours. Although not mandatory, a necropsy was decided for two stock animals (nos. 22825 and 23339) with Grade 3 reactions to investigate whether macroscopic findings were present with inconclusive IDT results and/or US findings. Animal no. 22825 showed pulmonary irregularities by US and an unremarkable spleen. Necropsy confirmed granulomas within the tracheobronchial lymph nodes and lungs and revealed granulomas within the spleen and liver. Animal no. 23339 did not undergo US examination, and necropsy was unremarkable for this animal. The remaining animals with Grade 3 reactions and animals with no findings or the presence of B lines within the hemithorax that tested negative for IDT were kept alive under safety precautions. These animals were sacrificed according to the corresponding study plan, and none showed gross lesions compatible with TB.

Altogether, four animals were regarded as suspected cases, and 10 animals had macroscopic findings compatible with TB (14 animals out of 114). The lungs and tracheobronchial lymph nodes were affected in all macaques with TB, and there was evidence of ruptured granulomas releasing caseous material into the bronchial lumen (endobronchial disease). Disseminated disease was observed in eight macaques, whereas in two animals, the lesions were confined to the tracheobronchial lymph nodes and lungs.

All 14 animals with gross lesions were sampled during necropsy and sent to the official laboratory (IRTA-CReSA, Bellaterra, Barcelona, Spain) for MTBC-specific quantitative PCR, mycobacterial culture, and Ziehl-Nielsen (ZN) staining, as previously described[23]. In addition, whole-blood IGRAs were performed using the Primagam™ kit (all methods are described in Supplementary Materials 1c). The gross findings per organ and the laboratory results are summarized in Table 1. Samples of affected organs for histopathology were processed at APS (methods described in Supplementary Materials 1d). All ten animals with TB-compatible gross lesions were PCR-positive for MTBC, and mycobacteria were isolated in culture, whereas animals regarded as suspected cases were PCR- and culture-negative. DVR spoligotyping was performed at VISAVET (Universidad Complutense de Madrid, Madrid, Spain) on DNA samples extracted from all ten isolates and revealed *M. caprae* as the implicated species with the spoligotype profile SB1622. Histologically, granulomas elicited by *M. caprae* were distinctively characterized by large numbers of foamy macrophages, epithelioid cells, scattered multinucleated giant cells (MNGC), low numbers of lymphocytes and plasma cells, the presence of spindle cells arranged perpendicularly from the necrotic centers, and the absence of a fibrous capsule. In larger granulomas, spindle cells formed glomeruloid-like structures wrapping macrophages and epithelioid cells. Based on these microscopic findings, granulomas could be classified using five broad developmental stages as described by Kegler *et al.* (manuscript in preparation)[†]. Briefly, small clusters of foamy macrophages and epithelioid cells were considered Stage I. Stage II granulomas had foamy macrophages and epithelioid cells forming a well-discernible nodule with the presence or absence of MNGC and small numbers of infiltrating/peripheral lymphocytes (Fig. 2A). Stage III granulomas had a central necrotic core with or without mineralization and presence of spindle cells impairing a radiating appearance. In the Stage IV granulomas, evident glomeruloid-like structures were present at the periphery (Fig. 2B and 2C). Stage V granulomas were coalesced with areas of necrosis/mineralization with disorganized cellular arrangements. The granuloma size increased at higher stages, illustrating the progressive development of the lesions. Stage V was predominant in the lungs and tracheobronchial lymph nodes, and evidence of endobronchial invasion was observed in all animals (Fig. 2D). In the remaining affected organs, a combination of several different stages was found. Additionally, in four macaques from Studies 1, 2, and 3, in which gross lesions were not observed at necropsy, and hence no further laboratory diagnostics were performed, a Stage I granuloma was detected histologically in the lung (animal no. 23278, Study 1), Stage II granulomas were present in the tracheobronchial lymph node and thymus (animal no. 22471, Study 1), and Stage III granulomas were observed in the liver (animal nos. 23073 and 23000, Study 3). The macroscopic and histologic correlates in animals with suspected TB that were PCR and culture negative are briefly described in Supplementary Table 2.

Implementation of prophylactic measures: Prophylactic measures were reviewed, reinforced, and immediately implemented by the time of a confirmed positive case at APR; three regulatory toxicity studies (Studies 1, 2, and 3) and stock colony animals were affected. For safety reasons, the main treatment period in Study 3 was reduced from 13 to 10 weeks, and the recovery period was eliminated. Similarly, the recovery period from Study 2 was reduced from eight to four weeks.

The areas designated for animal procedures, animal boxes, and rooms were cleaned daily and disinfected by spraying with 10% diluted bleach (DAC, 4% sodium hypochlorite; Lleixius DAC, S.L.). Shavings and possible dietary remains were swept out of the boxes and disposed of in bags as biohazardous waste. The floor, walls, and bars of the boxes were rinsed weekly with low-pressure water and a de-

[†] Kegler K; Mayoral F, Vallejo C, Sanchez R, Kant R, Ortega P, Vallejo R, Sironen T, De Miguel R, Weber K. Emerging *Mycobacterium caprae* in laboratory cynomolgus macaques (*Macaca fascicularis*): distinctive granulomatous inflammation with high transmission risk. Manuscript in preparation.

tergent solution (Daronit 1000). The central water channel of the room was disinfected with 10% diluted bleach (DAC, 4% sodium hypochlorite; Lleixius DAC, S.L.). Brushes, brooms, and dustpans were disinfected with 10% diluted bleach (DAC, 4% sodium hypochlorite, Lleixius DAC, S.L.) in the corresponding antechamber. During cleaning procedures, animals were temporarily housed in an empty box. The materials and equipment in the necropsy room were disinfected with Virkon™ daily, and the waste was eliminated. Carcasses were disposed of by a specialized company in accordance with AHA protocols.

The sanitary break disinfection protocol for empty rooms and their respective ventilation systems included nebulization with 8% hydrogen peroxide (F-66 SR, Reg. 01610P) using manual Aeroturbex® equipment and a two-step rinse with Sanit P20 (Proder Pharma, AEMPS reg. no. 564-DES), DX50 223 (Dessol 111, R.D.G.S.P. register no. 13-20/40-04630), and Germosan-Nor BP1 (D.G.S.P. register no. 18-20/40/90-09463 and HA). Before introducing new animals into the disinfected boxes, environmental samples were collected from the rooms by AHA staff with sponges specifically designed to detect mycobacteria. If the pathogen was detected, the disinfection process was repeated until negative results were obtained. Figure 3 shows an example of the disinfection process in an animal room.

To ensure the safety of APR workers, personal protective equipment for working with NHP was reinforced: double gloving, FFP3 face mask, coverall, cap, eye shield or protection, and disposable shoe covers. All equipment was disposed of before leaving the room. Maintenance personnel were requested to use FFP3 masks when cleaning the ventilation system duct filters.

Furthermore, technicians who had been in direct or indirect contact with infected animals underwent tuberculin PPD (purified protein derivative) RT23 SSI tests. One worker tested positive and was referred for unremarkable

Table 1. Gross Findings at Necropsy and Results from Laboratory Tests Performed at The Reference Laboratory (IRTA-CReSA, Bellaterra, Barcelona, Spain)

Study	Animal ID	Inguinal LN: enlarged with hemorrhage	Inguinal LN: fistulizing granuloma	Inguinal LN: abscess	Mediastinal LN: granuloma	TBLN: granuloma	TBLN: enlarged and pale	Lung: granuloma	Lung: focal cystic cavernous change	Pericardium: granuloma	Myocardium: granuloma	Paracostal pleura: granuloma
1	22921M		x		x	x		x				
	21173M	x		x								
	22647M	x										
	23250F				x	x		x				
	23242F					x		x				
	23280F					x		x				
	22996F						x		x			
2	23274F				x	x		x				x
	24008F											
3	21099M					x		x				
	22066F					x		x				
	22096F					x		x				
Stock	22911M				x	x		x		x	x	x
	22825M					x		x				

Study	Animal ID	Paravertebral pleura: granuloma	Liver: granuloma	Spleen: granuloma	Kidney: granuloma	Kidney: abscess	Diaphragm: granuloma	Finger: ulcerative dermatitis	Ziehl-Neelsen	Primagam™	PCR (MTC)	Spoligotype: *M. caprae* SB1622
1	22921M		x*	x					pos	pos	pos	x
	21173M					x		x	neg	neg	neg	np
	22647M								neg	neg	neg	np
	23250F		x	x					pos	pos	pos	x
	23242F								pos	pos	pos	x
	23280F		x						pos	np	pos	x
	22996F								neg	np	neg	np
2	23274F	x	x	x	x				pos	np	pos	x
	24008F		x						neg	np	neg	np
3	21099M						x		pos	np	pos	x
	22066F		x	x	x				pos	np	pos	x
	22096F		x	x	x				pos	np	pos	x
Stock	22911M		x	x					pos	np	pos	x
	22825M		x						pos	np	pos	x

*Unremarkable hepatic parenchyma but granuloma within hepatic LN. LN: Lymph node; TBLN: Tracheobronchial lymph node; pos: positive; neg: negative; np: not performed.

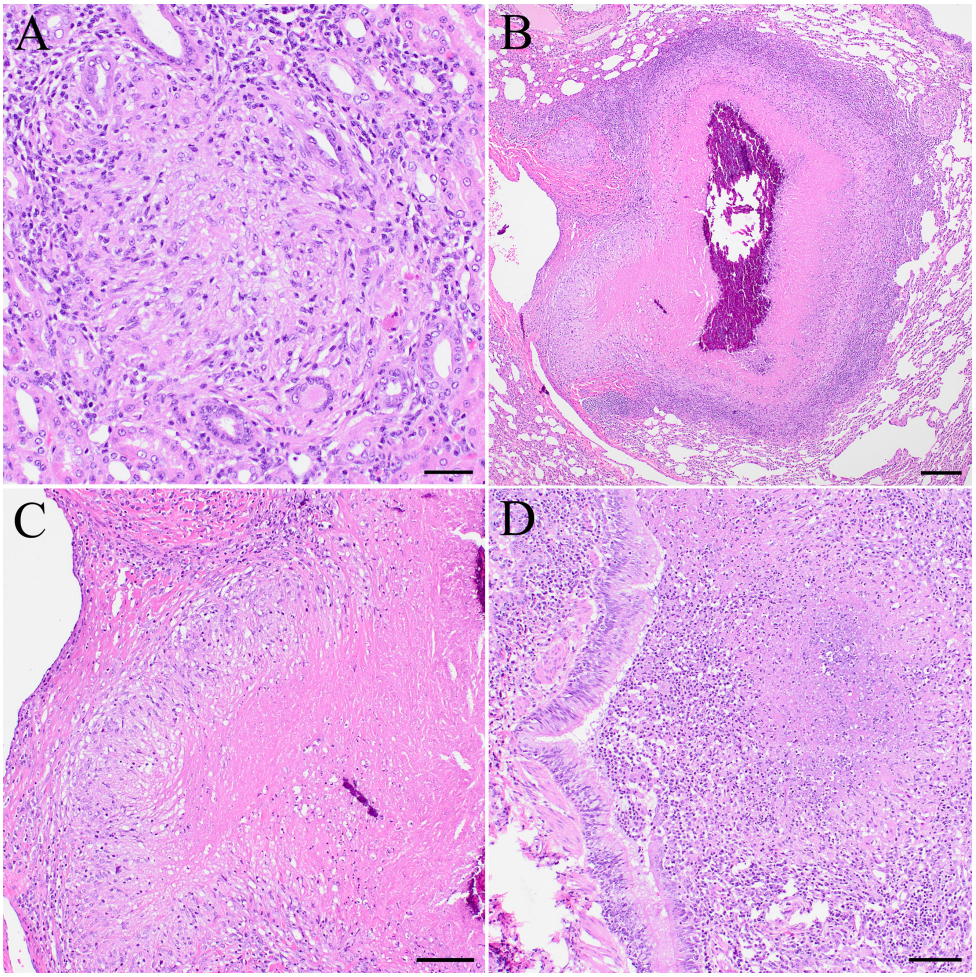

Fig. 2. Histopathology of *M. caprae* infection in formalin-fixed paraffin-embedded sections stained with hematoxylin and eosin (HE). A. Stage II granuloma within the kidney. Bar 50 µm. B. Stage IV granuloma within the lung with central mineralization and necrosis, peripheral presence of glomeruloid-like structures, and lack of fibrous capsule. Bar 200 µm. C. High magnification of B depicting spindle cells entrapping macrophages and epithelioid cells forming packages giving the appearance of glomeruloid-like structures. Bar 100 µm D. Lung of an infected animal showing a ruptured granuloma with release of exsudate within a bronchus. Bar 100 µm.

chest radiography findings. Two other workers had doubtful results, and IGRA tests were recommended; both tested negative. The remaining staff members tested negative for tuberculin. New PPD tests for personnel were repeated after three months, and negative results were confirmed.

Epidemiology, diagnostic, and prophylactic challenges in *M. caprae* natural outbreak: This report describes a TB outbreak caused by *M. caprae* (spoligotype SB1622) in a colony of imported cynomolgus macaques at the APR test facility. The occurrence of this outbreak, together with published evidence, clearly demonstrates that TB in NHP remains an important problem despite strict regulatory measures. Diagnosing and managing TB are becoming increasingly challenging as new mycobacterial species emerge.

M. caprae was first detected in samples from goats in Spain and was established in 2003 as a unique species in the MTBC[24]. Since then, it has been isolated from domestic and wild animal, and human samples[25–27]. Thought to be restricted primarily to Europe, *M. caprae* infections were recently described in farming buffalos from Thailand, captive elephants in Japan, three NHPs from China, and a 70-year-old patient who grew up in Bangladesh and immigrated to the United States[28–30]. Spoligotyping has proven successful in clustering mycobacterial species restricted to specific demographic areas[26, 31]. Particularly, the spoligotype SB1622 was identified in the Chinese NHP and the human patient from Bangladesh and is phylogenetically clustered in a lineage exclusive from Asia[29]. Although the incidence of *M. caprae* infection is high in European countries, to our knowledge, *M. caprae* SB1622 has never been identified previously in animal or human samples from Europe[26, 27, 31, 32]. Therefore, published data tracing this specific spoligotype to Asian countries, together with the advanced disease observed in macaques from APR, clearly indicate that infection with *M. caprae* occurred before the animals arrived in Europe.

Alarmingly, repeated IDT tests performed at the breed-

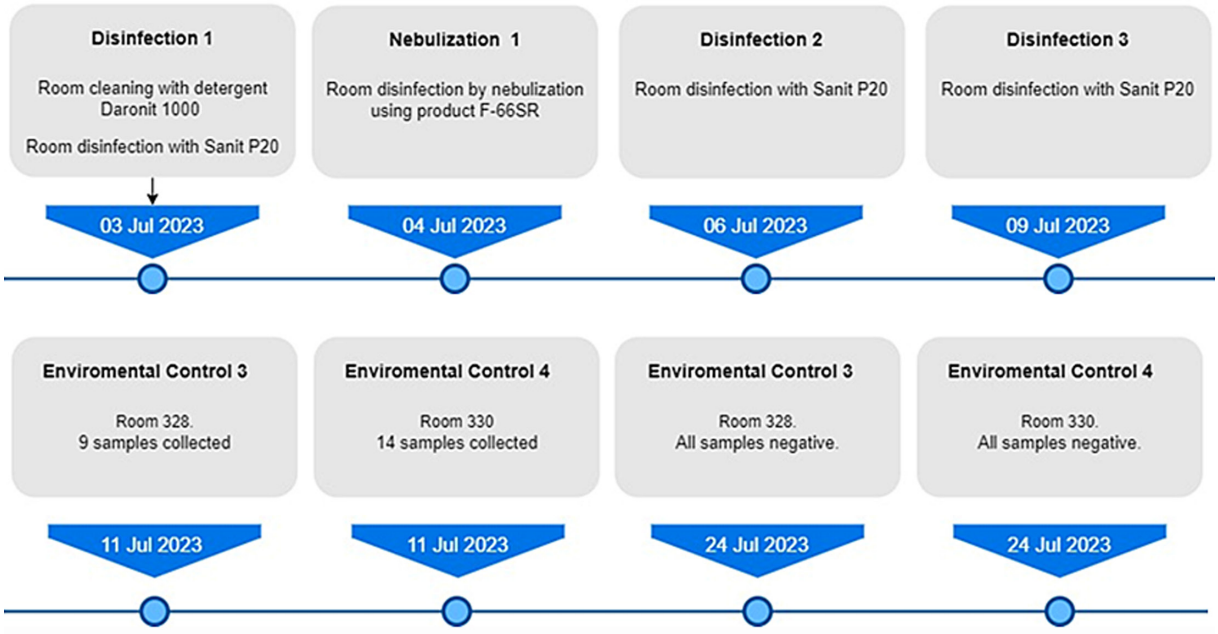

Fig. 3. Example of disinfection (above) and sample collection for swab analysis for one animal room. On the first day of the disinfection procedure, the room is cleaned using low pressure water and detergent solution (Daronit 1000) and then rinsed in two steps with Sanit P20 (Proder Pharma AEMPS reg. no. 564-DES), DX50 223 (Dessol 111, R.D.G.S.P. reg. no. 13-20/40-04630), and Germosan-Nor BP1 (D.G.S.P. reg. no. 18 20/40/90-09463 and HA). On the second day, the room is disinfected using 8% hydrogen peroxide (F-66 SR, reg. 01610P). Swab samples are taken in the animal room and laboratories for environmental monitoring. When swab samples are positive, the disinfection process is repeated.

ing farm in South Vietnam and at the quarantine station in Europe completely failed to detect positive cases of *M. caprae* infection. The IDT test remains the gold standard for screening TB in NHP and humans[31, 33, 34]. While a positive IDT reaction is detected in cynomolgus macaques four to six weeks after being experimentally infected with *M. tuberculosis*[35], the first positive results for a natural outbreak of *M. bovis* in this species were reported 12 weeks after the last negative quarantine test[7]. Similarly, we observed positive reactivity approximately nine weeks after the animals left the quarantine station. Three cases with advanced disease scored Grade 4, whereas only one out of 13 animals scoring Grade 3 had TB based on gross findings, PCR, and culture. Our results provide novel insights in that a positive IDT test is confirmatory of *M. caprae* infection in cynomolgus macaques, whereas inconclusive results do not correlate well with the disease. Furthermore, we provide evidence that IDT testing leads to 100% false-negative results in early *M. caprae* infections during natural outbreaks.

Several authors, as well as the European Primate Veterinary Association Working Group on TB, recommend that alternative screening methods, including whole blood interferon gamma (IFN-γ) release assays (IGRAs) such as Primagam™ and ESAT-6 ELISA should be incorporated into NHP screening programs to aid in early identification of TB[7, 33]. Five animals from APR were tested with Primagam™, three with advanced disease and positive PCR/culture results, and two with suspicious gross findings but negative PCR/culture results. Primagam™ was positive for all three animals with confirmed TB but negative in both negative cases, highlighting the sensitivity of this test. Unfortunately, we were unable to determine whether Primagam™ is helpful in the early detection of *M. caprae* infection because it was first employed after advanced TB cases appeared at our test facility.

Cynomolgus monkeys at APR were further screened by thoracoabdominal US to improve the internal monitoring of possible cases and help prioritize the order of necropsies for surviving animals. We were also eager to investigate whether IDT tests combined with US might increase the chances of overcoming TST false negatives during early infection. Diagnostic imaging methods such as US, X-ray, CT scan, and MRI, are widely employed complementary tools to detect TB lesions in humans[36]. MRI has also proven useful in measuring the disease burden in macaques experimentally infected with *M. tuberculosis*[37]. We chose US because it is a rapid and noninvasive approach. TB-compatible changes were detected sonographically only in animals with advanced granulomatous lesions at necropsy. However, there was no clear correspondence between US findings and necropsy status, as this method failed to reveal inconspicuous granulomas in some animals. Correspondingly, US offered nothing in detecting false-negative TST results.

The lack of clinical signs, even in animals with advanced granulomatous changes and disseminated disease, further complicates the diagnosis of TB in cynomolgus ma-

caques. Most cases during natural TB outbreaks are detected at the test facilities after quarantine periods have ended and when animals are necropsied for other purposes[15]. A nodular fistulizing change in the right inguinal area of one cynomolgus macaque was the only finding during clinical examination of animals at APR; the contents tested PCR-positive for MTBC. In the context of TB, scrofuloderma is clinically defined in humans by the presence of subcutaneous, painless, slowly growing nodules that evolve to ulcers or draining fistulous tracts overlying an infected lymph node, bone or joint[38–42]. Necropsy of the affected cynomolgus macaque confirmed the nodule as a superficial inguinal lymph node fistulizing through the dermis. As such, this is the first report of a *M. caprae* infection describing a nodular change sharing similarities with human scrofuloderma. Apart from this uncommon finding, all other affected cynomolgus macaques had typical granulomatous gross lesions involving multiple pulmonary lobes and tracheobronchial lymph nodes or manifested as disseminated disease. Histologically, however, *M. caprae* infection appears to elicit a distinctive phenotype of granuloma when compared to *M. tuberculosis* and *M. bovis* infections in NHP (Kegler *et al.*, 2024, manuscript in preparation). Importantly, all animals infected with *M. caprae* showed gross and histologic evidence of ruptured granulomas into the main airways (endobronchial disease), which increased the risk of transmission among monkeys and facility personnel.

Sanitary measures implemented at APR were effective in preventing spreading and later in eradicating the disease. New NHPs were introduced to the facility only after environmental swabs tested PCR negative for MTBC at least three times. Workers with positive or doubtful IDT test results were handled according to the Spanish government's health regulations and have shown no signs of disease to date. The reinforcement of protective equipment used by all personnel in direct or indirect contact with NHP seems effective in maintaining a TB-free status for both newly introduced monkeys and workers.

To the best of our knowledge, this is the first report describing a natural outbreak caused by *M. caprae* infection in imported cynomolgus macaques and its management. These results clearly demonstrate that the detection of early TB cases remains a huge challenge and is a major handicap for NHP used for toxicologic or research purposes. Therefore, regulatory measures for importation and quarantine should be reviewed, and new screening strategies should be investigated to significantly reduce the risk of importing emerging mycobacterial species to Europe.

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Authors' Contribution: KW: planification and general guidance, histopathology/peer reviews, client contacts, drafting of publication; FM: necropsy; CV: ultrasound imaging, authority contact, drafting of publication; RS: necropsy, drafting of publication; RH: organization of materials and information, drafting of publication; PM: authority and external lab contact, organization of necropsies, PCR, IGRA, TST; BPV: PCR, culture, IGRA analyses; JS: organization of animal facility and disinfection procedures; YO: histopathology; PO: histotechnique and histopathology; LR: immunophenotyping; AS: hematology, immunophenotyping, plasma PCR analysis; RV: histopathology; RM: histopathology, drafting of manuscript; KK: planification and guidance of necropsy, necropsy, histopathology, drafting of publication.

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