A new *Mycobacterium bovis* spoligotype in a domestic cat – case study

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Abstract

A 2-year-old mixed-breed domestic cat was presented with blindness, persistent skin lesions, and diffuse interstitial lung disease. A diagnosis of *Mycobacterium bovis* infection was established based on histopathological examination revealing multifocal granulomatous inflammation with caseous necrosis and acid-fast bacilli, positive Ziehl-Neelsen staining, bacteriological culture confirmation using the Mycobacteria Growth Indicator Tube system, and spoligotyping. Treatment with a combination of ciprofloxacin (10 mg/kg per os (p.o.) q12 h), rifampicin (11 mg/kg p.o. q24 h), and azithromycin (9 mg/kg p.o. q24 h) resulted in clinical improvement within the first month of therapy. Since the cat's history prior to adoption was unknown, the route of infection could not be determined. Spoligotyping revealed a previously unreported *M. bovis* pattern, which was submitted to the international database and assigned the SB number SB2784. This case report describes the first molecularly characterized *M. bovis* infection with a novel spoligotype diagnosed *ante mortem* in a cat in Türkiye and highlights the importance of molecular typing in the epidemiological evaluation of feline tuberculosis.

Feline tuberculosis, spoligotyping, zoonosis, lymphadenitis, skin

Bovine tuberculosis is a zoonotic mycobacterial disease that can infect many animal species as well as humans (Alves et al. 2019). *Mycobacterium bovis, M. microti*, and rarely *M. tuberculosis*, are members of the *Mycobacterium tuberculosis* complex, and are primarily responsible for tuberculosis in cats (Gunn-Moore 2014). *Mycobacterium bovis* is transmitted directly between infected animals or by consuming contagious animal products. In particular, cats are at risk of contamination if fed unpasteurized milk, raw meat, or offal. The prevalence of bovine tuberculosis has decreased over recent years due to the increased consumption of ready-to-eat foods for pets and preventive medicine practices (Gunn-Moore et al. 2011; Gunn-Moore 2014). However, it is possible that the increasing popularity of feeding raw diets might increase the incidence of tuberculosis in cats (O'Halloran et al. 2019). The possibility of cat-to-cat transmission cannot be ruled out (Roberts et al. 2014; Černá et al. 2019), and *M. bovis* infection also poses a zoonotic risk (Ramdas et al. 2015; O'Connor et al. 2019; Didkowska et al. 2024).

In Türkiye, bovine tuberculosis has been investigated primarily through epidemiological and molecular studies focusing on cattle and humans. Molecular typing approaches, including spoligotyping and Mycobacterial Interspersed Repetitive Units - Variable Number Tandem Repeats (MIRU-VNTR) analyses, have demonstrated substantial genetic heterogeneity among *Mycobacterium bovis* strains isolated from different regions of the

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country, and have shown that certain spoligotypes are shared between animal and human isolates (Çavuşoğlu and Yılmaz 2017; Tuzcu and Köksal 2020; Güven Gökmen et al. 2022; Şahin et al. 2022). In addition, the isolation of *M. bovis* from raw milk samples has highlighted the potential role of foodborne transmission in the epidemiology of bovine tuberculosis in Türkiye (Aydın et al. 2012). Although *M. bovis* infection in cats is rare in Türkiye, sporadic feline cases have been reported (Haligur et al. 2007; Gokalp et al. 2011; Eroksuz et al. 2019). However, available reports provide limited information regarding molecular characterization of feline isolates by spoligotyping or their comparison with international databases. Consequently, the molecular epidemiology of feline *M. bovis* infections in Türkiye remains insufficiently defined.

In this case report, a *M. bovis* strain isolated from a cat in Türkiye, diagnosed *ante mortem* and molecularly characterized as a novel SB spoligotype, is described. The clinical, pathological, and molecular findings are presented and discussed in the context of the existing literature.

Case description

A 2-year-old, female, indoor crossbreed domestic cat was referred from a private clinic for further investigation after the detection of an abdominal mass. The cat had lived with the owner from the age of 4 months and had been fed only commercial foods since then. On physical examination, the cat was bright, alert and responsive with a good body condition score (BCS; body weight: 3.6 kg, BCS 5/9), normal vital indicators, and a normal rectal temperature. Abdominal palpation identified a firm and non-painful mass in the mid-abdominal region. A complete blood count revealed no clinically significant abnormalities, and the only abnormal finding on routine serum biochemistry was an elevated alanine aminotransferase (ALT) level (Table 1). Three-view thoracic radiography performed without sedation revealed a diffuse, mixed, bronchial lung pattern, especially in the caudal lobes. Three-view abdominal radiography revealed a well-defined abdominal mass lesion, raising the suspicion of lymphadenopathy (Plate VII, Fig. 1). The abdominal mass lesion was identified as an enlarged mesenteric lymph node, which was surgically removed and submitted for histopathology. Following an uneventful anaesthetic recovery and admission for ongoing monitoring, the cat was discharged the following day with appropriate analgesia and prophylactic antibiotics as determined by the surgical team.

The mass was fixed in 10% formalin solution, routinely paraffin embedded, sectioned, and stained with haematoxylin and eosin (H&E). In addition, Ziehl-Neelsen staining (Bio-Optica, Milan, Italy) was performed to demonstrate acid-fast bacilli in the affected areas. Gross examination revealed a 1 cm diameter, firm, soft-tissue mass characterized by multi-lobular caseated necrotic foci on the cross-section. Histopathological examination revealed multi-focal coalescing granulomatous inflammation characterized by large areas of caseous necrosis with central calcification surrounded by epithelioid cells, lymphocytes, and neutrophil leukocytes. In caseous necrotic areas, randomly distributed large amounts of acid-fast bacilli were observed by Ziehl-Neelsen staining. The histopathologic diagnosis was caseous lymphadenitis with mycobacterial infection (Plate VII, Fig. 2).

The patient did not attend follow-up examinations for two months after the surgery. Then, the patient presented to the hospital with complaints of vision loss and non-healing wounds on the skin. Blood analysis revealed no abnormalities, while signs of improvement were observed on lateral thoracic radiographs (Plate VIII, Fig. 3). In the ophthalmological examination, retinal degeneration and bilateral toxic cataract (Plate VIII, Fig. 4) were identified, and it was determined that the patient's vision loss was attributable to these pathologies. During physical examination, submandibular and popliteal lymphadenopathy and cutaneous ulcerative and nodular lesions on the face and tail were found. A punch

biopsy was taken from the skin lesion on the tail tip and was submitted for bacteriological culture. Since the Mycobacteria Growth Indicator Tube (Becton Dickinson, Maryland, USA) gave positive results, the isolate obtained was subjected to identification by spoligotyping method (Kamerbeek et al. 1997).

A spoligopattern of *M. bovis* 674073637777600 was obtained and compared with the international database, Mbovis.org (www.mbovis.org). Since no match could be found with the existing patterns within the database, the new pattern was submitted to the database. This finding suggests that the identified *M. bovis* strain represents a novel genetic variant that has not been previously documented in the database. The absence of a match in the database indicates that this strain may be unique to the region, previously undetected in surveillance studies, or possibly an emerging strain with distinct molecular characteristics. It has been inserted in the Mbovis.org database and SB number SB2784 allocated (Fig. 5). Following the isolation of the causative agent, the cat's owners were also subjected to *M. bovis* Purified Protein Derivative (PPD) testing, and both tested negative.

Fig. 5. The unique spoligopattern of *Mycobacterium bovis* SB2784 recovered from the cat's tail, depicted in binary and octal formats.

Treatment was initiated with ciprofloxacin (10 mg/kg q12h), *per os* (p.o.) rifampicin (11 mg/kg q24h), and azithromycin (9 mg/kg p.o. q24h). Skin lesions showed improvement after one month of treatment. Treatment was then continued for additional three months with rifampicin (11 mg/kg p.o. q24h) and azithromycin (9 mg/kg p.o. q24h). Throughout the treatment period, complete blood counts and blood biochemistry were monitored at least once per month (Table 1). Following the resolution of all clinical signs, the owner declined to continue the treatment, and antimicrobial therapy was therefore discontinued prematurely. After cessation of treatment, the patient was monitored at regular intervals for a period of one year through clinical examinations and routine laboratory assessments (Table 2). During the follow-up period, no recurrence of dermatological lesions or systemic abnormalities was observed, and the patient remained clinically stable.

Discussion

Bovine tuberculosis in cats is a rare zoonotic disease (Pesciaroli et al. 2014). *Mycobacterium bovis*, the main cause of tuberculosis in cats, is transmitted by direct contact with an infected animal or by consuming infectious animal products. In particular, cats fed unpasteurized milk, raw meat, or offal are at risk for infection (Gunn-Moore et al. 2011). The current epidemiology of feline tuberculosis is complex. There are occasional cases of ingestion due to drinking tuberculous cow's milk. However, skin lesions are thought to probably result from infected bite wounds, as lesions typically involve the face, extremities, base of the tail or perineum. (Murray et al. 2015).

In this case, the cat's unknown pre-adoption history limits definitive conclusions regarding the route of infection, although environmental exposure or contact with infected animals cannot be excluded. Feline tuberculosis can manifest as localized skin lesions or respiratory disease (Gunn-Moore 2014). Primary respiratory tuberculosis in cats is rare and can be asymptomatic until late in the disease (Černá et al. 2019). However, the secondary spread of tuberculosis agents from cutaneous lesions to the lungs is common

Table 1. Complete blood count and serum biochemical analysis results of the case.

	11/2/2022	21/2/2022	23/3/2022	1/4/2022	29/4/2022	20/5/2022	29/6/2022	26/8/2022	Reference range
WBC (10^9/l)	16.1	17.5	25.5	20.5	10.0	12.7	12.5	9.5	5.5-19.5
RBC (10^12/l)	10.7	9.3	11.2	10.0	9.9	9.8	10.6	10.1	4.6-12.0
HGB (g/dl)	13,.6	11.6	14.5	13.0	12.4	12.6	13.2	13.5	9.0-15.3
HCT (%)	40.6	34.4	44.0	39.8	38.4	38.5	40.9	40.6	26.0-49.0
MCV (fl)	37.8	37.2	40.4	39.9	39.0	39.4	38.6	40.1	39.0-53.0
MCH (pg)	12.7	12.5	12.9	13.0	12.5	12.8	12.4	13.3	13.0-20.0
MCHC (g/dl)	33.5	33.7	32.2	32.6	32.2	32.7	32.2	33.2	29.0-37.0
PLT (10^9/l)	453.0	426.0	355.0	378.0	306.0	327.0	297.0	295.0	100.0-518.0
ALB (g/dl)	2.9	2.7	2.6	2.5	25.0	2.7	2.5	2.7	2.3-3.5
ALP (U/l)	29.0	18.0	32.0	29.0	35.0	22.0	28.0	27.0	9.0-53.0
ALT (U/l)	242.0	94.0	48.0	53.0	49.0	63.0	68.0	173.0	22.0-84.0
BUN (g/dl)	17.0	18.0	21.2	24.3	19.7	27.9	27.5	24.9	17.6-32.8
CREA (mg/dl)	2.0	1.5	1.3	1.4	1.2	1.2	1.0	1.1	0.8-1.8
GLOB (g/dl)	4.4	4.2	4.3	3.8	3.4	3.6	3.7	3.0	2.8-5.1
GLU (mg/dl)	101.0	101.0	129.0	116.0	124.0	113.0	142.0	117.0	71.0-148.0
TP (g/dl)	7.3	6.8	6.9	6.3	5.9	6.3	6.2	5.7	5.7-7.8

WBC, white blood cell count; RBC, red blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet count; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CREA, creatinine; GLOB, globulin; GLU, glucose; TP, total protein

Table 2. Follow-up blood analysis during the post-treatment period.

	18/11/2022	23/12/2022	23/02/2023	07/04/2023	02/06/2023	27/07/2023	17/11/2023	Reference range
WBC (10^9/l)	13.8	9.1	12.2	11.6	8.7	13.2	10.8	5.5-19.5
RBC (10^12/l)	10.7	10.0	10.4	9.8	10.1	10.0	11.0	4.6-12.0
HGB (g/dl)	13.8	13.4	12.7	13.2	13.8	14.1	14.9	9.0-15.3
HCT (%)	43.0	40.7	39.4	41.3	40.2	41.6	45.5	26.0-49.0
MCV (fl)	40.3	40.6	38.0	42.3	40.0	41.5	41.5	39.0-53.0
MCH (pg)	12.9	13.3	12.2	13.4	13.7	14.0	13.5	13.0-20.0
MCHC (g/dl)	32.0	32.9	32.2	31.9	34.3	33.8	32.7	29.0-37.0
PLT (10^9/l)	304.0	320.0	261.0	392.0	224.0	335.0	300.0	100.0-518.0
ALB (g/dl)	2.8	2.9	2.7	2.9	2.7	3.0	2.9	2.3-3.5
ALP (U/l)	17.0	15.0	15.0	18.0	21.0	17.0	17.0	9.0-53.0
ALT (U/l)	137.0	84.0	110.0	57.0	141.0	115.0	122.0	22.0-84.0
BUN (mg/dl)	22.4	25.4	21.4	28.4	25.4	24.1	21.6	17.6-32.8
CREA (mg/dl)	1.1	1.1	0.9	1.1	1.3	1.5	0.9	0.8-1.8
GLOB (g/dl)	3.7	NT	3.4	3.5	NT	3.6	3.8	2.8-5.1
GLU (mg/dl)	95.0	91.0	95.0	106.0	104.0	119.0	104.0	71.0-148.0
TP (g/dl)	6.5	6.5	6.1	6.4	6.0	6.6	6.7	5.7-7.8

WBC, white blood cell count; RBC, red blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet count; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CREA, creatinine; GLOB, globulin; GLU, glucose; TP, total protein; NT, not tested

and produces diffuse interstitial lung disease. This disease is characterized by bronchial infiltration, dyspnoea, and cough (Mitchell and Gunn-Moore 2019). Consistent with previous reports, this case demonstrated both cutaneous lesions and pulmonary involvement indicative of secondary dissemination (Plate VII, Fig. 1).

In most cases of tuberculosis in cats, cutaneous lesions often involve the face, extremities, tail base, or perineum, frequently accompanied by local or generalized lymphadenopathy (Gunn-Moore 2014); in this case, localized skin lesions were found on the anterior palpebrae, tail region, and lumbar vertebrae. This disease can also lead to various clinical manifestations such as weight loss, pleural and pericardial effusions, and hepatosplenomegaly (O'Halloran et al. 2019). However, these findings were not observed in the presented patient. Ocular manifestations, such as cataracts and retinal degeneration, had also been previously documented (Mitchell et al. 2022), consistent with the findings in the present case.

Diagnosing feline tuberculosis is challenging due to the unreliability of conventional tuberculin skin testing. Molecular techniques such as PCR and DNA hybridization, bacteriological culture and interferon-gamma release test are used to diagnose cats (O'Halloran et al. 2019). However, species confirmation is crucial for treatment and prognosis, so culture isolation, which is still the gold standard for diagnosing feline tuberculosis, is crucial (Pesciaroli et al. 2014). In this case, bacteriological culture and subsequent spoligotyping identified a novel SB2784 strain. The detection of a novel spoligotype requires careful epidemiological evaluation. Although the absence of the SB2784 spoligotype from international databases suggests that this genotype has not been previously reported, it does not allow definitive conclusions regarding its geographical origin or source of transmission. Previous molecular epidemiological studies conducted in Türkiye have already demonstrated considerable genetic diversity among *M. bovis* strains isolated from cattle and humans, indicating that not all circulating genotypes may yet be fully characterized, even when spoligotyping and MIRU-VNTR analyses are applied (Çavuşoğlu and Yılmaz 2017; Tuzcu and Köksal 2020; Güven Gökmen et al. 2022; Şahin et al. 2022). In addition, the isolation of *M. bovis* from raw milk samples in Türkiye has highlighted the relevance of foodborne transmission in the epidemiology of this infection (Aydın et al. 2012). Therefore, the SB2784 spoligotype identified in the present case was evaluated, based on the available data, as a previously unreported molecular variant.

Tuberculosis in cats can primarily develop in the digestive tract, lungs, or skin (Malik et al. 2000). The pathogenesis of gastrointestinal tuberculosis in cats develops through contaminated food consumption, so granulomatous lesions are seen in the intestine and mesenteric lymph nodes where they may disseminate through the infected phagocytes to other organs (Zachary 2017). In this case report, macroscopic and histopathological findings were observed only in the mesenteric lymph node of the patient, and the pathological alterations were consistent with the typical features of tuberculosis described previously (Maxie 2015). Ideally, a combination of three drugs (rifampicin, pradofloxacin, and clarithromycin/azithromycin) for two months is preferred for treating *M. bovis* in cats. This is followed by a continuation phase in which two drugs (rifampicin and pradofloxacin or clarithromycin/azithromycin) are used for four more months, depending on the extent of the disease and for at least three months after the resolution of the lesions (Gunn-Moore et al. 2011). In this case, treatment was initiated based on established protocols in the literature, resulting in improvement of tuberculosis-related symptoms. The prognosis depends on the mycobacterial species involved, the severity of the infection, and whether the treatment includes effective drugs given over an appropriate period. Cutaneous tuberculosis generally responds favourably, even with secondary pulmonary involvement (Gunn-Moore 2014), as observed in this case.

Mycobacterium bovis infection carries a zoonotic risk as it can be transmitted from cats to humans (Ramdas et al. 2015; O'Connor et al. 2019). The zoonotic potential of *M. bovis* increases the importance of testing for cat owners, breeders, and veterinarians who come into contact with cats suspected to be infected with this pathogen (Didkowska et al. 2024). In this case, PPD testing was performed on the owners and the results were negative. The presented case represents the first report of a *M. bovis* infection in Türkiye diagnosed *ante mortem* in a cat that recovered following appropriate treatment.

Conclusion

In conclusion, this case report describes a *M. bovis* infection in a domestic cat in Türkiye in which a previously unreported spoligotype (SB2784) was identified through molecular characterization. The findings highlight the value of combining bacteriological culture with spoligotyping for accurate diagnosis and epidemiological evaluation of feline tuberculosis. Although the source of infection could not be determined, the identification of a novel spoligotype underscores the genetic diversity of *M. bovis* and the need for continued molecular surveillance. This case contributes to the limited data on molecularly characterized feline *M. bovis* infections in Türkiye and emphasizes the importance of considering feline tuberculosis in the differential diagnosis of cats presenting with cutaneous and respiratory manifestations.

Conflict of interest

No conflicts of interest have been declared.

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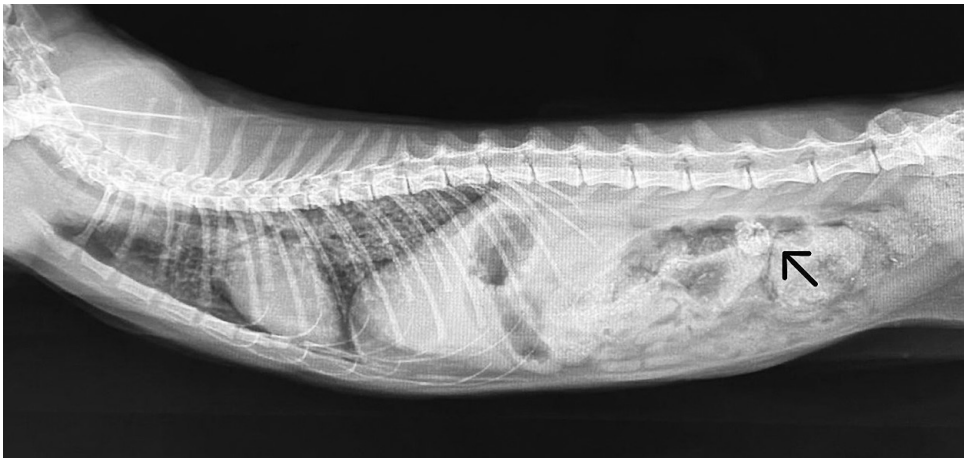

Fig. 1. Right lateral thoracic radiograph showing a diffuse mixed bronchial pattern in the lungs and a mass in the abdomen. Black arrow: mass found on the physical examination

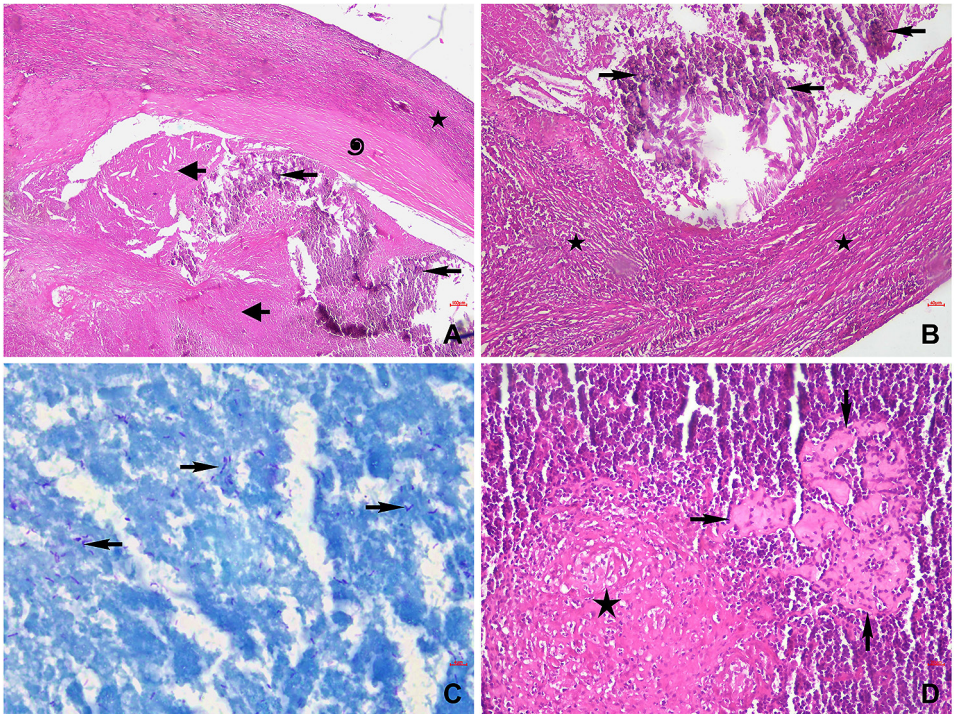

Fig. 2. A: Areas of caseation necrosis in the mesenterium (thick arrow), calcified areas (thin arrow), hyalinised capsular area (spiral), and fibrous capsule (line of demarcation) (star), scale bar 100 µm. B: fibrous capsule (line of demarcation) (star) surrounding the caseated and calcified areas (arrows) in the mesenterium, scale bar 40 µm. C: Ziehl-Neelsen stained section showing acid-fast bacilli within caseous necrotic areas (arrows), scale bar 4 µm. D: Area of caseation necrosis (star) and hyalinised amyloid-like structure (arrows) in the mesenteric lymph node, scale bar 20 µm. H&E staining

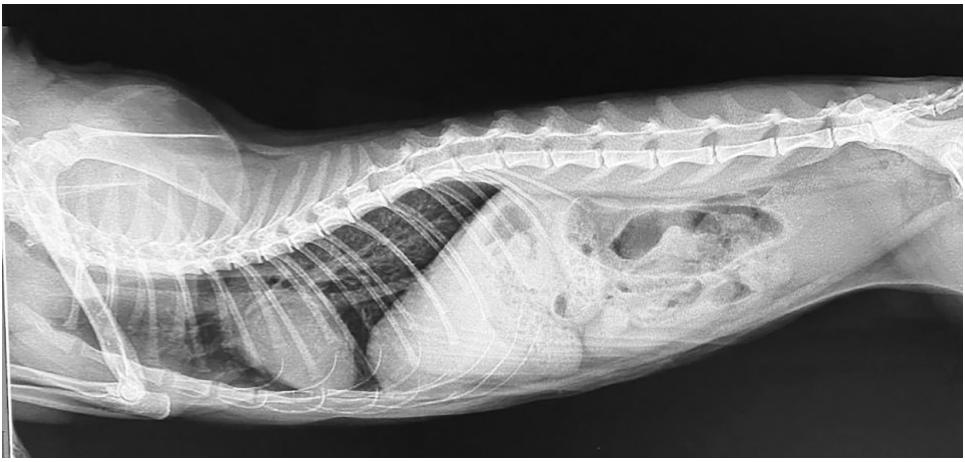

Fig. 3. A right lateral thoracic radiograph taken two months after the surgery reveals moderate improvement in the previously identified diffuse bronchial lung pattern.

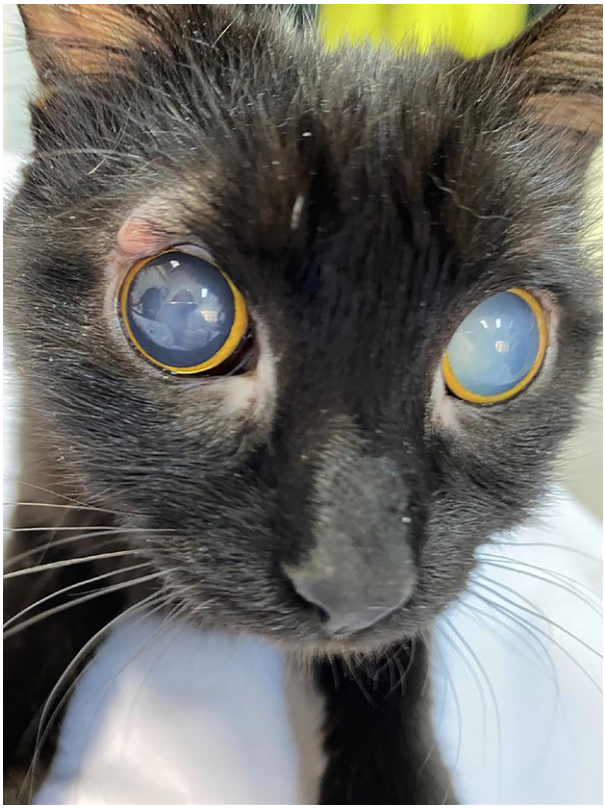

Fig. 4. Bilateral toxic cataracts and retinal degeneration were detected in the cat, and a nodular skin lesion associated with tuberculosis was present on the palpebra superior.