






Tuberculosis due to *Mycobacterium bovis* in pet cats associated with feeding a commercial raw food diet

Journal of Feline Medicine and Surgery
1–15

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DOI: 10.1177/1098612X19848455
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Abstract

Objectives *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex, can infect cats and has proven zoonotic risks for owners. Infected cats typically present with a history of outdoor lifestyle and hunting behaviour, and cutaneous granulomas are most commonly observed. The aim of this study is to describe an outbreak of tuberculous disease commencing with six young cats, living exclusively indoors in five different households across England, being presented to separate veterinarians across the UK with a variety of clinical signs.

Methods Investigations into the pyogranulomatous lesions, lymphadenopathy and/or pulmonary disease of these cases consistently identified infection with *M bovis*. Infection was confirmed by PCR, where possible, or was indicated with a positive interferon-gamma release assay (IGRA), where material for PCR was unavailable. In-contact, cohabiting cats were screened by IGRA and follow-up testing was undertaken/advised where results were positive. A lifestyle investigation was undertaken to identify the source of infection.

Results Six clinically sick cats and seven in-contact cats were identified with evidence of *M bovis* infection. Five clinical cases were either too sick to treat or deteriorated despite therapy, giving a mortality rate of 83%. Lifestyle investigations revealed the common factors between clusters to be that affected cats had mycobacterial infections speciated to *M bovis*, were exclusively indoor cats and were fed a commercially available raw food product produced by a single manufacturer. The Food Standards Agency, Animal & Plant Health Agency, Public Health England and the food manufacturer concerned have been notified/informed. Other possible sources of exposure for these cats to *M bovis* were explored and were excluded, including wildlife contact, access to raw milk, the presence of rodent populations inside the buildings in which the cats lived and exposure to known infectious humans.

Conclusions and relevance Upon investigations, our results provide compelling, if circumstantial, evidence of an association between the commercial raw diet of these cats and their *M bovis* infections.

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Keywords: Tuberculosis; infectious disease; outbreak; raw food diet

Accepted: 11 April 2019

Introduction

The increasing importance of mycobacterial infections in companion animals in the UK has become apparent in recent years.^{1,2} It has been demonstrated that approximately 1% of all feline biopsies submitted for histopathological analysis show changes consistent with mycobacteriosis (pyo/granulomatous inflammation dominated by epithelioid macrophages) and at least one-third of these have demonstrable Ziehl-Neelsen (ZN)-positive organisms when stained, with a thin rod-like appearance, indicative of mycobacteria; these are also referred to as acid- or alcohol-fast bacilli (AFB).³⁻⁵

Clinically, about a third of feline mycobacteriosis cases in the UK are caused by *Mycobacterium tuberculosis* complex (MTBC) pathogens, with *Mycobacterium microti* being cultured from 19% of all cases of feline mycobacteriosis, and a further 15% caused by *Mycobacterium bovis*.^{6,7}

There is a strong geographical predisposition to feline infections with members of the MTBC.⁶ *M bovis* infections are strongly coincident with where there are high levels of endemic infection in local bovine and wildlife populations, such as in the south-west of England.⁶⁻⁸ Feline MTBC infections, particularly those caused by *M bovis*, pose a potential zoonotic risk to owners;⁹ they may also act as a potential source of environmental contamination, which is critically important, as *M bovis* is a pathogen of major animal health significance in the UK and other countries as it is the causative agent of bovine tuberculosis (TB).¹⁰

In contrast to human TB, the majority of feline TB cases present with localised nodular cutaneous disease, frequently with a degree of ulceration and occasionally with draining sinus tracts.^{1,2,6,7,11,12} The lesions are typically distributed around the face, extremities and tail base: the so-called 'fight and bite' sites.^{1,2,6,7,11,12} Skin lesions may be accompanied by a localised or occasionally generalised lymphadenopathy, or lymphadenopathy (usually of the submandibular, prescapular or popliteal lymph nodes) may be the only presenting sign, termed an incomplete primary complex.^{1,2,6,7,11,12}

Primary pulmonary lesions do occur in cats, but rarely.^{1,6} They can result from bacteria being inhaled and causing tubercle formation in the lungs and hilar lymph nodes.^{1,2,6,7,11,12} However, much more commonly, pulmonary disease is secondary to the putative haematogenous spread of bacteria from the site of inoculation in the skin.^{1,2,6,7,11,12} This generates a diffuse interstitial pattern of disease that eventually becomes bronchial, and is clinically observable as progressive dyspnoea followed eventually by a soft productive cough.^{1,2,6,7,11,12}

Disseminated disease can cause a range of clinical signs, including hepatosplenomegaly, pleural and pericardial effusions, generalised lymphadenopathy and weight loss.^{1,2,6,7,11,12} Historically, TB in cats commonly presented as alimentary disease, caused by cats drinking tuberculous cow's milk, but following the introduction of milk pasteurisation this form of TB is now seen extremely rarely.^{1,2,6,7,11,12}

Owing to the putative transmission of *M bovis* and *M microti* to cats by hunting infected prey, TB is most frequently diagnosed in adult cats, with a history of hunting reported in almost every instance.⁶ Male cats are over-represented, possibly secondary to contamination of fight-inflicted wounds.⁶ The median age of infection is 3 years for *M bovis* and 8 years for *M microti*.^{1,2,6,7,11,12} There appears to be no link between MTBC infection and classical immunosuppression (ie, feline immunodeficiency virus [FIV] and feline leukaemia virus [FeLV] infection) in cats.⁶

The diagnosis of mycobacterial disease in cats is challenging; the traditional tuberculin skin-testing technique has been shown to be ineffective in domestic cats.¹³ Molecular techniques such as PCR and DNA hybridisation^{1,14,15} are available through human reference laboratories in the UK, but remain expensive, in some circumstances prohibitively so. These techniques also require the organism to be present within the sample being tested, and are most sensitive when performed on a cultured isolate. Where this is not possible for a cat, then an interferon-gamma (IFN- γ) release assay (IGRA) is available.^{16,17} This test uses specific mycobacterial proteins to stimulate peripheral immune cells in order to determine if a patient has been infected with an organism that either contains or secretes those peptides.¹⁶⁻¹⁸ The production of IFN- γ in response to protein purified derivative (PPD) from *M bovis* (PPDB), at a greater concentration than that produced in response to PPD from *Mycobacterium avium* (PPDA), confirms infection with an MTBC organism.^{16,17} A further peptide cocktail of 6 kDa early secreted antigenic target (ESAT-6) and 10 kDa culture filtrate protein (CFP-10) can be used to determine if a patient has been infected with an MTBC organism that encodes the genetic region RD-1; *M tuberculosis* and *M bovis* do encode this region, while *M microti* does not.^{19,20} Experimental challenge studies have shown that *M tuberculosis* does not cause clinical disease in cats, so a positive response to ESAT-6/CFP-10 and PPDB in a feline patient indicates infection with *M bovis*.²¹ This is not the case for canine patients, which can present with disease due to *M tuberculosis*.²²

Contrary to the common presentation of feline TB as cutaneous lesions on non-pedigree adult cats that hunt, we recently published our findings regarding three highly unusual systemic/abdominal cases of TB caused by *M bovis* that had occurred in young pedigree cats with no outdoor access in two households in England.^{23,24} Of particular note, both of these households are located well within areas of the country deemed to be low risk for *M bovis* in cattle and other animal species. However, we noted that all three cats had been fed a commercially available complete raw food diet and that the epidemiological significance of this was unclear at the time. Following the publication of letters in the *Veterinary Record* and *Veterinary Times*,^{23,24} we have now identified and investigated a total of five households infected with *M bovis* involving 13 cats. Here we report on all of the households and show that the raw food diet is epidemiologically implicated as a possible source of these infections. Further to these cases, we have investigated a continually growing number of cases, which has allowed us to begin a more extensive epidemiological investigation into this outbreak that will be published separately upon its completion.

Materials and methods

Each cluster is designated by its regional geographical location, which is represented on the map (Figure 1); the locations given are not exact (county level) in order to comply with client data protection (Figure 1). Furthermore, a timetable of the key dates for the outbreak is given in Table 1.

Cluster 1: Durham

A 2-year-old male neutered Siamese cat was referred with lethargy, hyporexia and pyrexia (40.5°C) of 2 weeks' duration. The cat had been acquired from a breeder at 12 weeks of age, had not received any vaccinations since the kitten course and was indoor-only with one other cat (see below). The cat had been fed only a single commercial frozen raw food diet since acquisition (Natural Instinct; <https://www.naturalinstinct.com>). Routine haematology and serum biochemistry (Table 2) documented a mild non-regenerative anaemia. The cat tested negative for FeLV antigen and FIV antibodies. An abdominal mass attributed to an enlarged mesenteric lymph node was palpated and surgically biopsied, with pyogranulomatous, necrotising lymphadenitis identified histopathologically.

On presentation at the referral centre, a thin body condition was noted (body condition score [BCS] 3/9; weight 4.3 kg). The respiratory rate was elevated at 42 breaths/min, with wheezing detected on inspiration and expiration. An 8 cm firm mass was palpable in the mid-abdomen, without evidence of pain.

Repeated routine haematology and serum biochemistry (Table 2) identified marginal mature neutrophilia, mild hyperglycaemia and mildly elevated urea. A CT scan of the thorax showed a diffuse ground-glass appearance to the lung parenchyma, which also contained disseminated micronodular lesions (Figure 2). The sternal lymph node was mildly enlarged. A CT scan of the abdomen showed the enlarged and lobulated appearance of the mesenteric lymph nodes (Figure 3).

Lymph node biopsies showed pyogranulomatous lymphadenitis, with no infectious organisms seen on ZN staining. To begin to investigate the differential diagnoses for pyogranulomatous lymphadenitis in the cat, feline coronavirus (FCoV) antibodies, serum alpha-1-acid glycoprotein and serum albumin:globulin ratio were tested; they were not suggestive of feline infectious peritonitis (FIP). *Toxoplasma gondii* serology was negative and *Bartonella* species serology was weakly positive (6; reference interval [RI] <5.5). On suspicion of mycobacterial infection, an IGRA was conducted at Biobest Laboratories and indicated infection with *M bovis*.

The cat began treatment with the recommended antimycobacterial triple antibiotic therapy,^{1,2} comprising pradofloxacin (Veraflox; Bayer, 3 mg/kg PO q24h), azithromycin (Zithromax; Pfizer, 9 mg/kg PO q24h) and rifampicin (generic, 11 mg/kg PO q24h). Two months after starting medication the abdominal mass was no longer palpable and the cat's weight had increased to 5.12 kg. Treatment is ongoing at the time of writing.

A 2-year-old female neutered Oriental cat that resided in the same household as the case described above was referred to the same centre with a chronic intermittent cough, and a 3 week history of increased respiratory rate and effort, lethargy and hyporexia. The cat was acquired from a different breeder at the same time as the above cat, had not received any vaccinations since the kitten course and was indoor-housed only. The cat was fed exclusively on the same commercial complete raw diet as the above cat (Natural Instinct). Prior to referral, pyrexia (39.8°C) had been recorded and routine serum biochemistry had identified a significant hyperbilirubinaemia (64 µmol/l; RI 0–12 µmol/l), while haematology was unremarkable.

On presentation at the referral centre, the cat's respiratory rate was 100 breaths/min, with bilateral moist rales on thoracic auscultation and a tracheal wheeze on auscultation of the cervical neck. A thoracic CT scan showed a marked diffuse nodular lung pattern with areas of alveolar infiltrate and an IGRA test confirmed infection with *M bovis*.

This cat was not amenable to medication for behavioural reasons, and the evident respiratory signs increased the risk of transmission to the owners, so this cat was euthanased. No post-mortem examination was conducted.

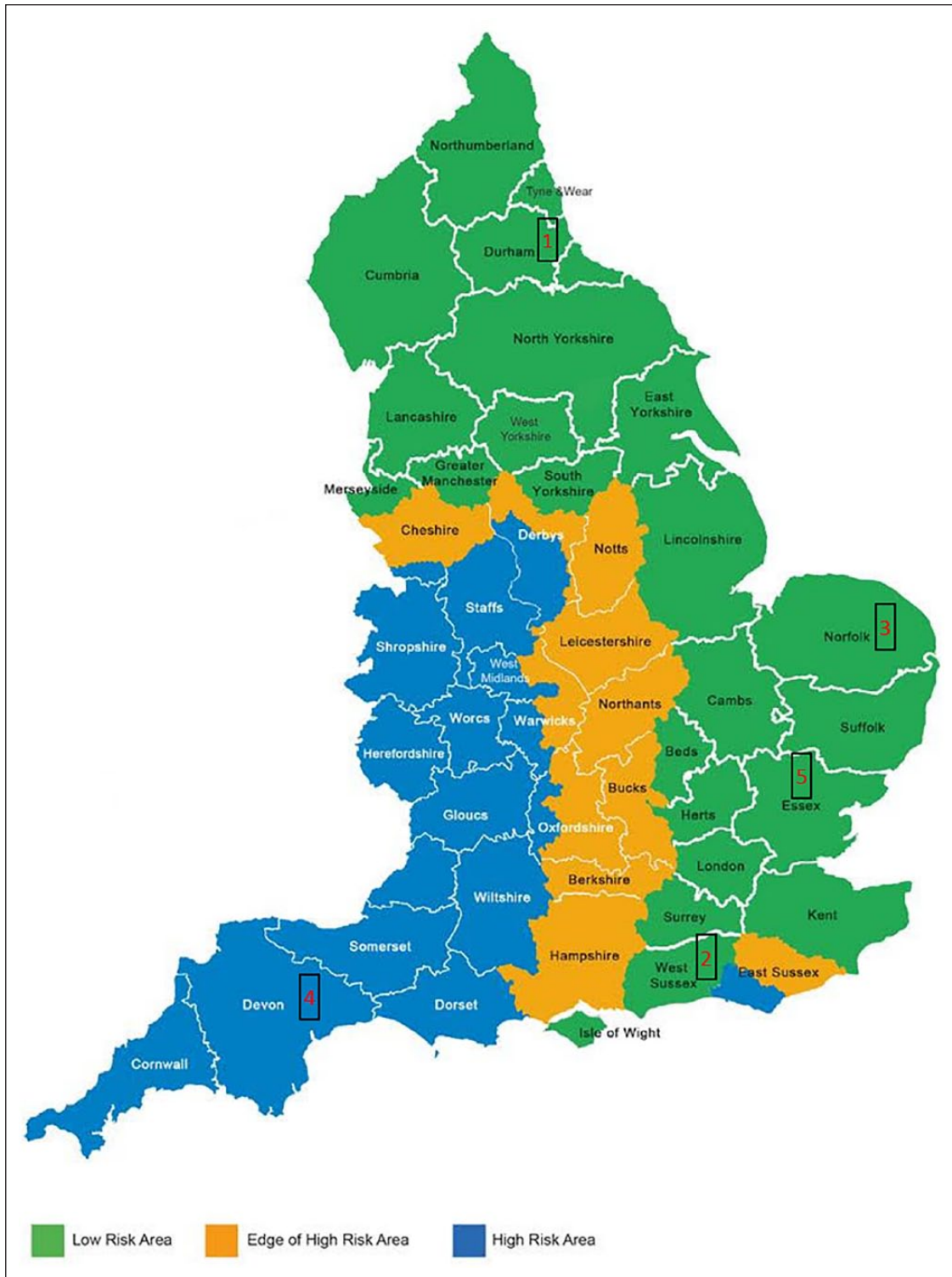


Figure 1 Map showing the approximate location of each of the clusters throughout England. Exact locations are withheld for client data protection purposes. The map is adapted from TBhub (<https://www.tbhub.co.uk/>, accessed 3 April 2019), a joint industry initiative supported by the Agriculture and Horticulture Development Board (AHDB), the Animal & Plant Health Agency (APHA), the British Cattle Veterinary Association (BCVA), the Department for Environment, Food & Rural Affairs (Defra), Landex and the National Farmers' Union (NFU)

Table 1 Timetable of the dates on which the first cat in each cluster was diagnosed with tuberculosis and when the relevant government authorities were alerted to our concerns

Date (2018)	Event
15 August	First cat in cluster 1 diagnosed by NR, Wear Referrals
21 September	First cat in cluster 2 diagnosed by ED, Top Cat Veterinary Centre
24 September	University of Edinburgh (DGM/COH) notify Animal & Plant Health Agency (APHA) and begin a collaborative epidemiological investigation
29 September	University of Edinburgh (DGM/COH) open investigations with Public Health England
10 October	University of Edinburgh (DGM/COH) contact food manufacturer with concerns
29 October	First cat in cluster 3 diagnosed by RH, Wymondham Veterinary Clinic
3 November	First cat in cluster 4 diagnosed by JG, City Vets
14 November	First cat in cluster 5 diagnosed by SVP, Millennium Veterinary Practice
11 December	Food manufacturer recalls suspect product

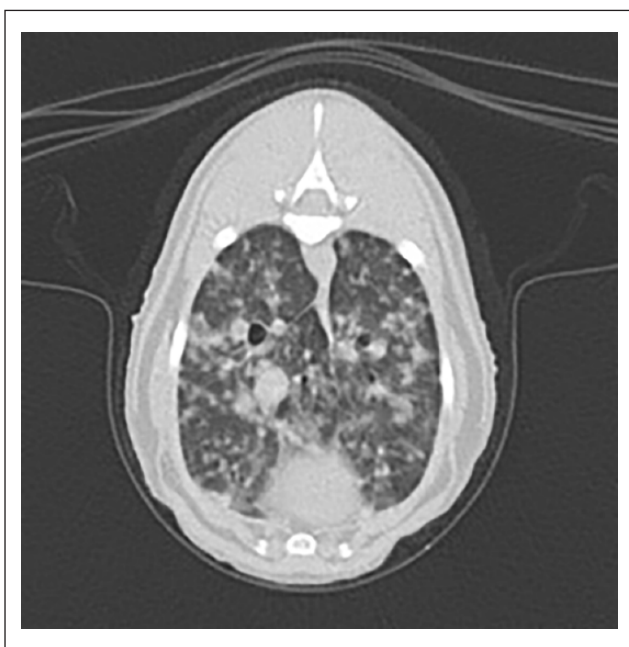


Figure 2 Transverse CT scan of the thorax of the first clinical case from cluster 1, showing an extensive multifocal nodular and alveolar lung pattern, consistent with mycobacteriosis

Cluster 2: Sussex

A 12-month-old female neutered indoor-only domestic shorthair cat was presented with a history of lethargy, hyporexia and significant weight loss (reduced by 12.5% over 4 months, BCS 2/5). This cat, and three other co-habiting cats, had been fed on the same commercial raw food (Natural Instinct) as those in cluster 1 since acquisition as kittens. On presentation, this cat was pyrexia (41.1°C) and an abdominal mass was palpable cranial to the bladder. The cat was referred for further investigation, which revealed mild generalised peripheral lymphadenopathy and diffuse bilateral wheezes and crackles on lung auscultation. Routine haematology and serum biochemistry (Table 2) showed non-regenerative

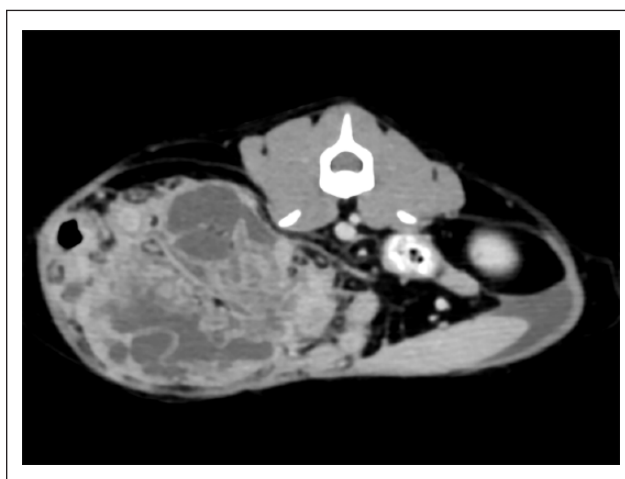


Figure 3 Transverse CT scan of the abdomen of the first clinical case in cluster 1. The scan shows a large multinodular mass occupying approximately two-thirds of the abdominal cavity

anaemia, a mature neutrophilia and monocytosis. Serum biochemistry revealed elevated globulin levels. As for cluster 1, the differential diagnoses included FIP, a lymphoproliferative disease, mycobacterial disease or other infectious disease. The cat tested negative for FeLV antigen and FIV antibodies. A chest and abdominal CT scan revealed multifocal pulmonary nodular-like lesions, alveolar foci and partially consolidated left and right cranial lung lobes, generalised severe lymphadenopathy, a multilobulated multicavitated right cranial abdominal mass around the ileum with perilesional peritonitis, and moderate splenomegaly.

A fine-needle aspirate from the enlarged nodes revealed extensive pyogranulomatous inflammation, containing numerous AFB with typical mycobacterial morphology (Figure 4). Unstained sections were submitted to Leeds Teaching Hospital for mycobacterial PCR, which identified MTBC DNA; subsequent speciation

Table 2 Abnormalities identified on routine haematology and serum biochemistry analysis of the presenting cat in each cluster

	Analyte	Measured value	Reference interval
Cluster 1	Haematocrit (%)	25.8	27.0–45.0
	Red blood cell count ($\times 10^{12}/l$)	4.7	5.0–10.0
	Neutrophil count ($\times 10^9/l$)	12.85	2.5–12.8
	Glucose (mmol/l)	8.1	4.5–8.0
	Urea (mmol/l)	15.8	2.8–11.0
Cluster 2	Haematocrit (%)	21.8	27.0–45.0
	Neutrophil count ($\times 10^9/l$)	25.00	1.48–10.29
	Monocyte count ($\times 10^9/l$)	2.00	0.07–0.85
Cluster 3	Packed cell volume (%)	14	25–45
	Neutrophil count ($\times 10^9/l$)	14.34	1.48–10.29
	SDMA ($\mu g/dl$)	16	<14
Cluster 4	Haematocrit (%)	25.2	30.3–52.3
	Total white blood cell count ($\times 10^9/l$)	18.16	2.87–17.02
	Neutrophil count ($\times 10^9/l$)	13.49	1.48–10.29
Cluster 5	Haematocrit (%)	21.0	27.0–45.0
	Neutrophil count ($\times 10^9/l$)	24.32	1.48–10.29

SDMA = symmetric dimethylarginine

using GenoType MTBC kit (Hain LifeScience) confirmed infection with *M bovis*.

Given the severity of disease the cat was euthanased on welfare grounds. On IGRA testing, the three cohabiting cats had results indicative of infection with *M bovis*. They remain clinically well, although owing to financial restrictions further investigation (eg, diagnostic imaging) has not been undertaken at the time of writing.

Cluster 3: Norfolk

A 15-month-old male neutered domestic longhair cat that had been exclusively indoor-housed since acquisition as a kitten along with a female sibling was presented. Both cats had been fed exclusively the same brand of raw food (Natural Instinct) as the cats in the other clusters from the time that they were kittens.

The cat was presented with diarrhoea and on examination had pale mucous membranes, abdominal distension, poor muscle condition and marked generalised lymphadenomegaly involving the submandibular, pre-scapular and popliteal lymph nodes, in addition to an abdominal mass assumed to be an enlarged mesenteric lymph node(s). The cat was borderline pyrexia ($39.5^{\circ}C$). Routine haematology and serum biochemistry (Table 2) revealed anaemia, a mature neutrophilia and a mild increase in symmetric dimethylarginine. Fine-needle aspirates of the abdominal mass/lymph node showed large numbers of macrophages (Figure 5) and intracytoplasmic AFB; confirmatory mycobacterial PCR and speciation at Leeds on the remaining slide confirmed that MTBC DNA was present, but as the slide had been previously stained the DNA was of insufficient quality to differentiate between MTBC organisms (ie, *M bovis* or

M microti). An IGRA test showed a weakly positive result suggestive of *M bovis* infection.

Treatment was attempted with pradofloxacin (Veraflox; Bayer, 3 mg/kg PO q24h), azithromycin (Zithromax; Pfizer, 9 mg/kg PO q24h) and doxycycline (generic; Summit, 10 mg/kg PO q24h). Unfortunately, the cat continued to deteriorate and was euthanased on welfare grounds. The sibling remains clinically well at the time of writing and screening by IGRA test gave a positive result for infection with a non-MTBC mycobacterial species.

Cluster 4: Devon

A 6-year-old female neutered Bengal-cross cat was presented to its primary veterinary surgeon with lethargy and hyporexia. The cat was an indoor-only cat and had been in the owner's possession since it was 7 weeks old. This cat lived with another exclusively indoor-housed cat, a 4-year-old male neutered Abyssinian cat that had asthma; both had been fed the same commercial raw food diet (Natural Instinct) as the cats in the other clusters since they were acquired, although they were also given occasional meals of commercial cooked processed fish-based foods (Sainsbury's Delicious Recipes 1+ Adult Cat Food Tuna in Jelly; Whiskas 1+ Years Complete Dry Cat Food with Tuna 340 g).

On presentation, the cat was pyrexia ($39.9^{\circ}C$) with an enlarged inguinal lymph node and localised overlying cellulitis. Routine haematology (Table 2) revealed non-regenerative anaemia, leukocytosis and mature neutrophilia. The cat tested negative for FeLV antigen and FIV antibodies. Histopathology of the lesion biopsy revealed marked necrotising panniculitis containing AFB with mycobacterial morphology. Unstained sections were submitted to

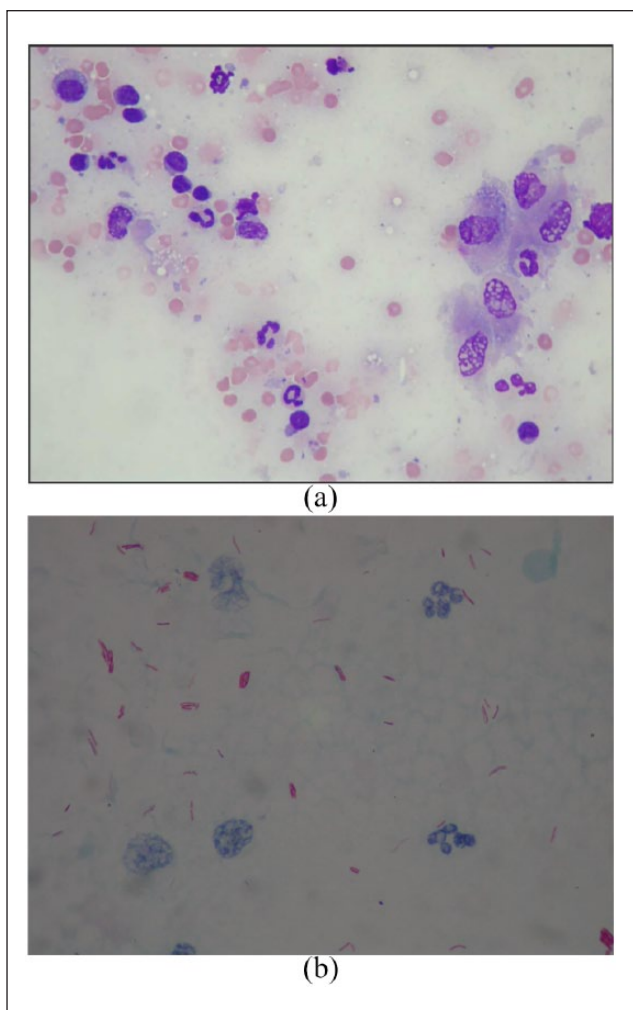


Figure 4 (a) Fine-needle aspirate from the inguinal lymph node of the clinically affected cat in cluster 2. The slide is stained with Wright's stain ($\times 50$ magnification). It shows reactive, vacuolated 'foamy' macrophages and mature neutrophils. (b) Ziehl-Neelsen stain of a second slide made from a fine-needle aspirate (as shown in Figure 4a) viewed under oil immersion ($\times 100$ magnification). Similarly, the slide shows reactive, vacuolated 'foamy' macrophages. Throughout the slide there are positively stained (fuchsia-coloured) extracellular rod-shaped bacilli with typical mycobacterial morphology

Leeds for mycobacterial PCR and speciation, as described above, and confirmed *M bovis*.

Pending these results, the necrotic area of the lesion was surgically debrided and the cat was given potentiated-amoxicillin (Clavaseptin; Vetoquinol, 18 mg/kg PO q12h) and marbofloxacin (Marbocare; Animalcare, 2 mg/kg PO q24h). Unfortunately, the wound broke down irreparably; the cat was euthanased on welfare grounds. A post-mortem examination was conducted within the Containment Level 3 facility of the Roslin Institute, University of Edinburgh; there was a surgical defect in the right inguinal area where the affected skin, fat and lymph

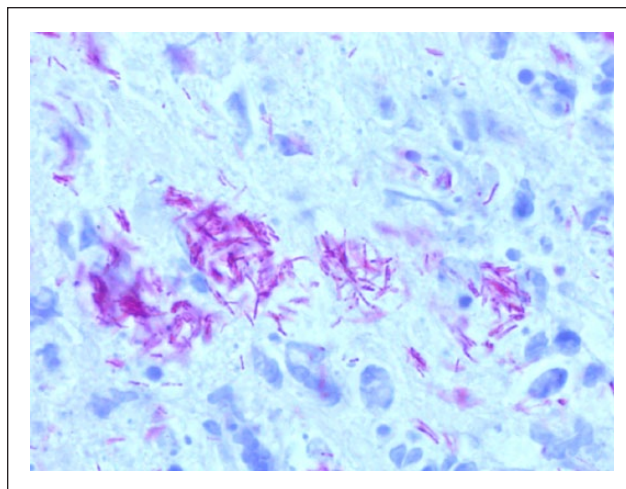


Figure 5 Ziehl-Neelsen stain of a slide made from a fine-needle aspirate of the abdominal mass from the case in cluster 3, viewed under oil immersion ($\times 100$ magnification) and containing high numbers of acid-fast bacilli

node had been removed, as well as pathological tissue breakdown (Figure 6). On examination of the abdomen, many small white, well-demarcated hard lesions were present throughout the spleen on both the visceral and cut surfaces. Within the thorax, similar lesions were visible in the caudal lung lobes and pericardium; there was a moderate degree of pericardial effusion.

Grossly pathological tissues were taken from the lung and spleen, homogenised in 1% collagenase solution containing 10% penicillin-G, decontaminated with 4% NaOH for 30 mins and sown onto OADC-supplemented 7H11 (Middlebrook) slopes, Löwenstein-Jensen medium with pyruvate and Stonebrink slopes in order to isolate and genotype the causative strain. After 9 weeks, small colonies were visible on the plates with the typical morphological appearance of *M bovis*. Initial in-house PCR testing at the University of Edinburgh (protocol unpublished, manuscript in preparation) followed by PCR performed at Leeds (as described above) confirmed the colonies to be *M bovis*; the Animal & Plant Health Agency (APHA) was notified and sent a second sample for independent analysis, which is pending.

The in-contact cat remains clinically well at the time of writing, but the results of a screening IGRA test indicate *M bovis* infection. Thoracic radiographs and abdominal ultrasound have identified no pathology. However, this cat has experienced significant recent weight loss (~12% body weight), so the owner has elected to treat it with standard antimycobacterial therapy (drug dosages as given above in cluster 1).

Cluster 5: Essex

A 15-month-old indoor-only male neutered Maine Coon cat was presented with lethargy and constipation of approximately 48 h duration. The cat had been fed

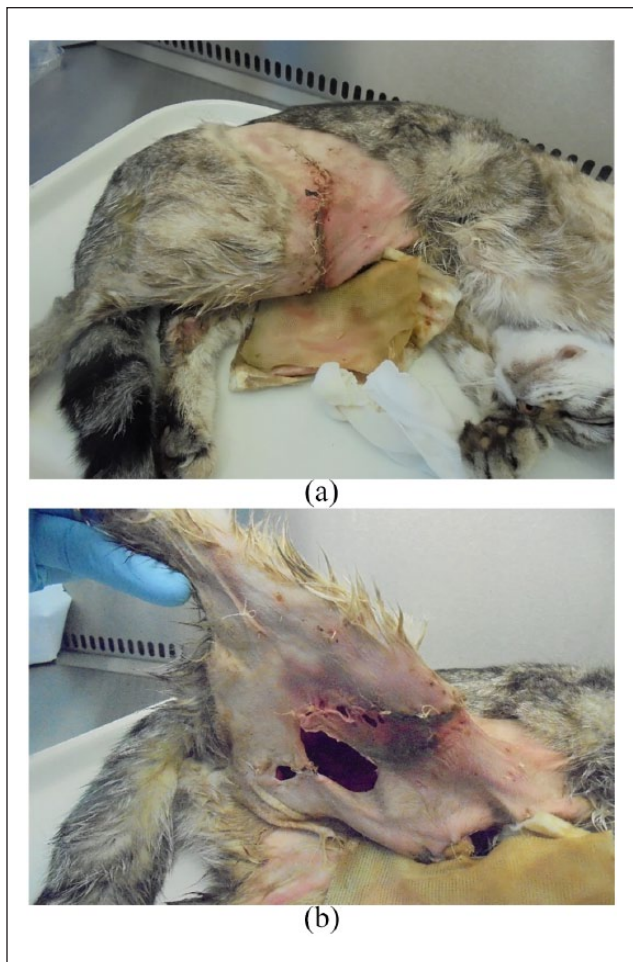


Figure 6 (a) Post-mortem examination of a 6-year-old female neutered Bengal-cross cat (cluster 4) shows evidence of the extensive nature of surgical resection required to remove the granulomatous panniculitis present. (b) Evidence of the breakdown of the surgical wound and surrounding tissue damage, which ultimately led to the euthanasia of this patient on welfare grounds

exclusively on the same diet (Natural Instinct) as the cats in the other clusters since it was acquired at 12 weeks of age. Physical examination revealed pyrexia (40.0°C) and a palpable, mobile, non-painful abdominal mass in the region of the mesentery. Ultrasound examination confirmed the presence of an 8 × 13 cm mass adjacent to an enlarged mesenteric lymph node and a small amount of free fluid. Fine-needle aspirates of the mass revealed large, foamy macrophages, mesenchymal cells and histiocytic infiltration. Routine haematology and serum biochemistry (Table 2) revealed non-regenerative anaemia and marked mature neutrophilia.

An exploratory laparotomy was performed and the fluid, mass and mesenteric lymph node were resected (Figure 7) and submitted for histological examination. Large numbers of macrophages were observed and a presumptive diagnosis of FIP was made. However,

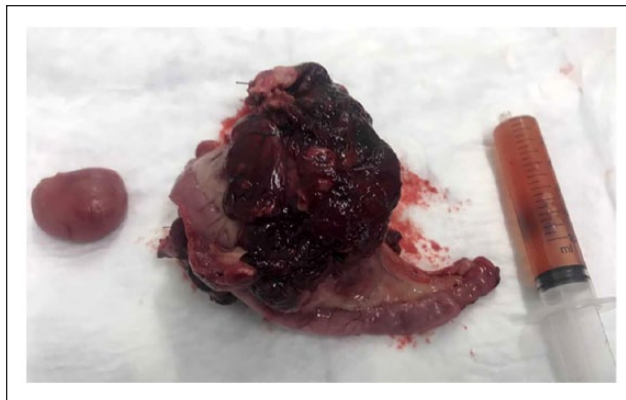


Figure 7 The Maine Coon in cluster 5 had a large abdominal mass (centre) measuring 8 × 13 cm removed during an exploratory laparotomy. The local draining mesenteric lymph node (left) was also removed during surgery, as was the ~12 ml ascitic fluid (right). The fluid was tested for mutated feline coronavirus by reverse transcription quantitative PCR but was negative. Retrospective histopathological examination of the mass (centre) showed scattered mycobacteria

further investigation to confirm FIP, including immunohistochemistry on the biopsy material, quantitative PCR for mutated FCoV on the abdominal fluid, and serum FCoV titre quantification and alpha-1-acid glycoprotein, was not compatible with FIP. During this investigation, retrospective staining of the original biopsy revealed individual AFB with mycobacterial morphology scattered in large areas of necrosis.

The cat improved clinically postoperatively but 6 weeks later developed a cough; thoracic radiography revealed a mass dorsal to the carina (Figure 8), while a repeat abdominal ultrasound revealed a 2 cm mid-ventral mass caudal to the spleen. Cytology of the abdominal mass again revealed macrophage infiltration with intracellular non-staining rods present. Cytology with ZN staining revealed AFB, initial PCR testing at the University of Edinburgh (as described above) indicated infection with *M bovis* and the remaining slides were submitted for mycobacterial PCR. Analysis at Leeds was unable to isolate sufficient mycobacterial DNA for definitive speciation. IGRA screening tests on the two cohabiting domestic shorthair cats, which ate mainly commercial dry food, separately from the Maine Coon cat, showed positive responses indicative of *M bovis* infection, although they were clinically well.

The Maine Coon was treated with the recommended antimycobacterial triple-antibiotic therapy,^{1,2} comprising marbofloxacin (Marbocyl; Vetquinol, 2 mg/kg PO q24h), azithromycin (Zithromax; Pfizer, 9 mg/kg PO q24h) and rifampicin (generic, 11 mg/kg PO q24h). Two months after starting medication the abdominal mass was no longer palpable and the cat's weight had increased. However, following owner-led

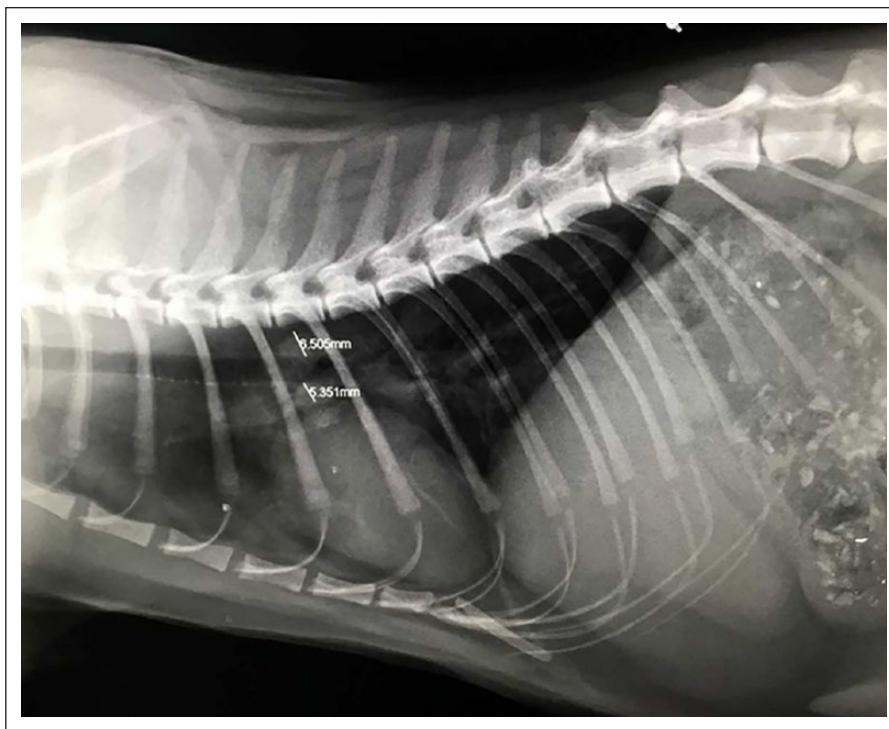


Figure 8 Left laterolateral radiograph of the thorax of the Maine Coon cat from cluster 5. There is a mass (total diameter 1.2 cm) at the level of the carina. A cough had become clinically evident at the time this radiograph was taken. It resolved within 3 days of antimycobacterial triple-antibiotic therapy

cessation of therapy at this stage, the cat subsequently relapsed and was euthanased on welfare grounds and has been submitted to APHA officials for post-mortem examination, the results of which are pending at the time of writing. Owing to the owner being immunocompromised, the two in-contact IGRA-positive cats were also euthanased.

Follow-up investigations

The current legislation applicable to England (The Tuberculosis [England] Order 2014) Article 6²⁵ states that suspicion of TB in the carcase of any pet mammal is notifiable, and as such the Secretary of State has been duly notified of all of the cases presented here via the APHA. However, there is no statutory obligation for investigation in non-bovine animals and so current investigations have been led by the University of Edinburgh and largely financed, where conducted, by the university and/or owners.

Public Health England (PHE) has similarly been informed of the cases and all owners have been offered the opportunity to discuss the zoonotic aspects that these cases present should they wish to do so with PHE. PHE is currently adopting a precautionary public health approach similar to that which has been described previously.²⁶ The owners of two of the affected cats were found to be IGRA and Mantoux (tuberculin skin test)

positive, respectively; one has required the instigation of antituberculosis medical therapy.

Potential sources of exposure to *M bovis* were explored with the owners; all except the raw commercial food were excluded. Excluded sources included wildlife contact (all cats were solely indoor cats), the presence of rodent populations inside the buildings in which the cats lived, access to raw milk and exposure to any known infectious humans.

As the only common factors between all clusters are that they were diagnosed with the same infection (*M bovis*), were exclusively indoor cats and were fed a commercially available raw food product produced by a single manufacturer (Natural Instinct), the Food Standards Agency (FSA) has similarly been informed and is investigating appropriately.

The authors at the University of Edinburgh have been sent detailed batch information and photographs and/or actual samples of the diet from 3/5 clusters, as was being fed to the cats at the time the diagnoses were made (Figure 9). Notably, all three of these samples were the venison product produced by the same company. On questioning the owners, the venison product from the single manufacturer comprised ~80% of the ration of one cat (cluster 4), the sole intake of another (cluster 5) and the 'vast majority' of the diet of a third (cluster 2). The remaining two clusters (clusters 1 and 3)

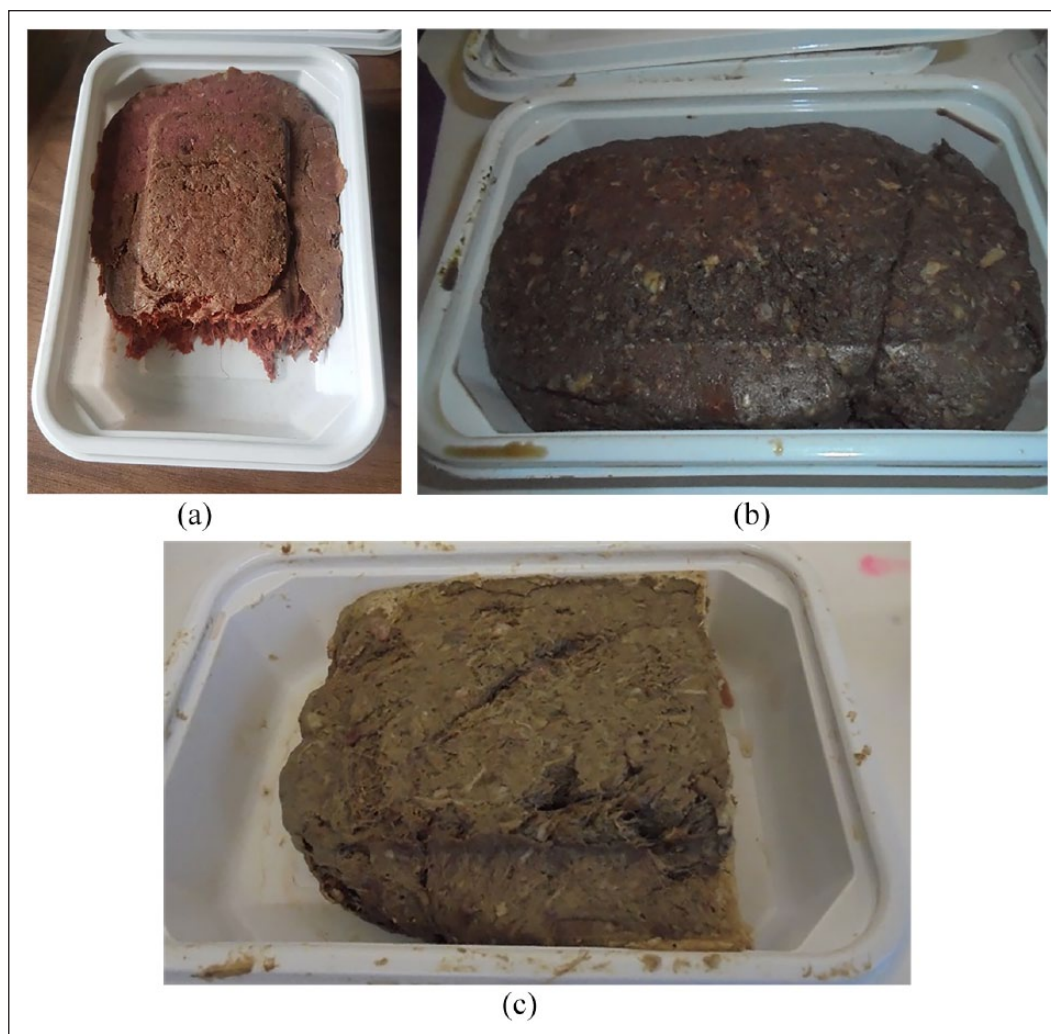


Figure 9 For three of the five clusters, owners had retained the food product that the affected cats were being fed at the time that they became ill. (a) One owner sent photos taken by themselves; (b,c) the other two owners sent the food to the University of Edinburgh by refrigerated courier and the photos were taken inside the Containment Level 3 facility prior to further testing

were fed a variety of the products from the same company, including the venison product.

As such, the evidence we have collected during our investigations of these cases provides compelling, if only circumstantial, evidence of an association between the diet of these cats and their *M bovis* infections.

The company was alerted, and an internal investigation by it led to the voluntary withdrawal of the venison version of their food from sale, 'because some of the ingredients were not inspected in line with EU requirements. The absence of inspection means the safety of the product cannot be confirmed and may therefore carry a potential risk'. The batches withdrawn are those dated as best before between March 2019 and August 2019, and pre-date those submitted for testing at the University of Edinburgh. The FSA is now undertaking this area of the outbreak investigation independently.

Discussion

This outbreak currently includes 13 cats, in five separate clusters, that were found to be infected with *M bovis*; six cats were clinically unwell at diagnosis (five of these are now dead), while seven were found to be positive by IGRA (ie, they had evidence that they have been infected with *M bovis*), although how many of these latter cats are likely to become ill is unclear. Three have not been assessed further by their veterinarian (cluster 2), and one (cluster 4) has already lost a great deal of weight, potentially related to (this cat has been treated for *M bovis* infection and has since recovered all its previously lost weight). While still circumstantial, the only probable source of the infection is that the cats were all exposed to *M bovis* via the same commercially available raw food. New cases are still occurring and further data collection is in progress at the time of writing, but the authors intend to make readers aware of the details and the scale

of this problem as soon as practically possible. The cases reported here appear to be limited to cats that have been fed this feline-specific food (ie, no dogs have yet been affected) and no owners are self-reporting as unwell.

The feeding of commercial raw meat-based diets (RMBDs) to companion animals has increased substantially in popularity in recent years, not just in the UK, but globally.^{27,28} In the USA, sales of RMBDs doubled in the 5 years to October 2017 and have expanded by 15.9% in the past year alone, making the industry worth an estimated US\$195 million to the US economy.²⁹ In Europe, a study from the Netherlands found that 51% of dog owners fed their animals either completely or partially an RMBD.³⁰ A similar increase has been seen in the UK, with what were only a handful of companies registered as producers of RMBDs a few years ago having increased to a current excess of 80 registered with the Department for Environment, Food & Rural Affairs.²⁷ Shapiro et al³¹ found in a survey of Australian cat breeders that raw meat was fed as an integral constituent of the diet by 89% of respondents. The reasons behind so many people adopting RMBD feeding are numerous and complex. Some owners feel that it is physiologically more 'natural' for their pet to consume food similar to that they would have eaten at an earlier stage in their evolution (ie, pre-domestication).^{31–33} There have been speculative suggestions that RMBD feeding may be beneficial in preventing the onset of dental disease, food-responsive inflammatory bowel disease and atopy,^{31–33} and a recent paper has shown that the microbiome of dogs fed RMBDs is more diverse than when fed processed diets.³⁴

The question of what the veterinary profession should be advising clients with respect to RMBDs is highly emotive on both sides of the debate, and the evidence for and against is frequently either low quality or simply anecdotal reports with a lack of rigorous, properly controlled trials.^{31–35} There is, however, consensus that home-prepared RMBDs should be generally avoided as it is extremely difficult to make these nutritionally balanced.³⁵ This concern has been overcome by the introduction of commercially available complete RMBDs that comply with the strict legislative requirements, overseen by the European Pet Food Industry Federation, to ensure nutritional and energy requirements are met if any given product was provided as the sole ration for the lifetime of the animal in question.^{36–38}

Unfortunately, statutory regulations governing RMBDs only relate to the presence or absence of a limited number of infectious organisms.³⁹ It is one of the few areas regarding RMBDs on which there is agreement: that there is a significant increase in the risk of infectious diseases to both pets and owners through the consumption and handling of such products on a regular basis.^{40–46}

Almost all RMBDs are sold frozen in order to extend their expiry dates and, in the case of some organisms (eg,

nematodes such as *Trichinella* species), reduce or eliminate contamination.⁴⁷ In studies of the zoonotic infectious agents present within thawed RMBDs, *Salmonella* species have received the most attention.^{44–46} Although subclinical infections occur frequently in animals, *Salmonella* species can cause gastroenteritis and even septicaemia.^{48–50} For example, Stiver et al reported fatal septicaemic salmonellosis after RMBD feeding in two cats.⁵¹ Other studies have successfully recovered a variety of viable zoonotic pathogens, including *Escherichia coli* serotype O157:H7, *Listeria monocytogenes*, *Sarcocystis* species, *Campylobacter* species and *T gondii*, from RMBDs.^{44–46,52} At least one such RMBD contaminant, Shiga toxin-releasing *E coli*, has been linked to human deaths in the UK, and PHE is attempting to educate owners about the risks associated with RMBDs.⁵³ In the USA, raw cat food has similarly had to be recalled due to contamination with *L monocytogenes*.⁵⁴

At this stage, as cases are continuing to be diagnosed, there is insufficient evidence to ascertain if this is a single, one-off event or an ongoing problem. However, in either instance this outbreak raises concerns over the strength of current regulations with regard to meat inspection procedures and may necessitate an increase in trained veterinary meat inspection of a carcass before it is passed as fit for animal consumption. With particular reference to wild venison, one such development could be that the entire gralloch (all viscera) be brought to the slaughterhouse for veterinary inspection.

Our study is the first to report infection of companion animals with an MTBC pathogen, which appears to be associated with feeding an RMBD, with 13 cats in five households infected, as identified to date. Not only does this imply that the owners were handling contaminated material, but the gastrointestinal presentation means that the cats may have been shedding *M bovis* into their home environment, creating the potential for even greater exposure to owners. Of further concern, the Pet Food Manufacturers' Association guidance to owners handling RMBDs is that they should clean surfaces, utensils and animal food bowls with 'soap and hot water'.⁵⁵ Pasteurisation requires milk to be heated to 71.5°C and held at that temperature for a minimum of 15 s to inactivate *M bovis*;⁵⁶ it is highly unlikely that owners would be able to achieve this in a domestic setting, so *M bovis* is very likely to be able to persist on kitchen surfaces even after the owners feel that the surfaces have been 'cleaned'. Furthermore, *M bovis* is able to survive for at least 60 days in standing water,⁵⁷ such as might be found in or around a kitchen sink, over which time further organisms may be added with each prepared meal, making it highly plausible that the small dose needed to infect a human could be reached.

The severity of clinical disease that was seen in the cats we report here is much greater than we would typically expect to see through infections acquired from

hunting infected rodents. The clinical signs of pyrexia, anaemia and leukocytosis (caused by a mature neutrophilia) are not typically present in feline TB cases and the young age of many of the cats affected is also striking, as is the fact that the cats were indoor only, and not from areas of England known to have endemic *M bovis* in rodents, badgers, other wildlife and cattle.^{58–60} These factors may mislead clinicians presented with future cases to not consider TB as a potential differential diagnosis, delaying the time until correct diagnosis and thus exposing owners to possible infection for extended periods.

Many of the cats presented in these clusters were either too sick to attempt treatment, or clinically deteriorated despite attempted therapies, giving a case fatality rate, to date, of 83% compared with our more usual 70–80% successful treatment rate.² The severity of infection was similar to that which we previously reported in an outbreak of *M bovis* TB in a pack of working Foxhounds, where it was concluded that the source of infection was most likely the feeding of contaminated raw meat.⁶¹ That outbreak resulted in a higher-than-typical clinical attack rate for TB (approximately 10% of the group) and when the hounds started to display clinical signs, they deteriorated so rapidly that euthanasia was required on welfare grounds within hours to days.⁶¹ Additionally, feline abdominal *M bovis* infections acquired in a nosocomial setting and cats with disseminated *M bovis* infection that appeared to have followed extensive ingestion also displayed rapid clinical progression and severe signs such that euthanasia on welfare grounds was quickly required.^{62,63}

The parallels between these fulminant outbreaks suggest that there may be an underlying difference in the immunopathogenesis of the disease, whereby gastrointestinal/peritoneal challenge of companion animals with *M bovis* results in more severe disease that is less amenable to treatment. Thus there may be implications for the clinical management of gastrointestinal TB cases in comparison with the more typical dermal presentation. Alternatively, or additionally, it may be that strains of *M bovis* that are capable of establishing infections in companion animals following gastrointestinal/peritoneal challenge may have or utilise additional virulence factors which make these infections clinically more aggressive. The latter scenario would have profound implications as regards the zoonotic risk to owners if they were being exposed to *M bovis* with inherently greater virulence.

The IGRA was used to detect *M bovis* infection in all six of the unwell and a further six of the in-contact cats. However, one in-contact cat (cluster 3) was IGRA tested and found to have a greater response to PPDA than PPDB (ie, a pattern not consistent with that expected of *M bovis* infection). It may be that this cat has not been challenged with *M bovis* but has coincidentally and/or

previously been sensitised to antigens of the *M avium-intracellulare* complex. However, studies in calves sensitised to environmental mycobacteria and infected with *M bovis* have concluded that *M bovis* infection may be concealed for some time in this situation.⁶⁴ It is therefore possible that this cat may develop a different response (ie, that typical of *M bovis*) if it were to be retested in the future.

Work by the team at the University of Edinburgh is ongoing to develop a rapid PCR assay, which we hope can be performed on tissue lysates, (unstained) cytology slides and formalin-fixed biopsy tissues. Where this was used in these cases it proved extremely useful; however, its use outwith a research capacity will require more extensive evaluation before this is made available to clinicians. Similarly, the team is continuing to work with Biobest Laboratories to validate the use of the feline IGRA in the detection and monitoring of TB in cats.

Urgent work is now needed to establish the extent of any contamination of the RMBD pet food chain in the UK, and a full epidemiological investigation is under way to establish where lessons can be learned in order to safeguard animals being fed RMBDs.

Management of suspect cases

The team at the University of Edinburgh is always keen to hear from clinicians who may have, or have had, suspicions about similar cases, and can offer our experience in both diagnosing and managing these patients. Currently, we are advising that animals known to have been exposed to the recalled food, or which have been exposed to a confirmed case but are displaying no clinical signs, should be tested by IGRA at least 4 weeks after the last known exposure. If these tests are positive then diagnostic imaging should be undertaken to assess for structural (ie, active) disease (full-body CT or radiographs and, ideally, abdominal ultrasonography).

Where present, active disease should be treated appropriately, as outlined in O'Halloran and Gunn-Moore.¹ There is currently no evidence on which to base recommendations for the appropriate course of action for animals that are IGRA test positive but lack evidence of structural disease. It may be appropriate in these cases to closely monitor body weight, BCS and resting respiratory rate, and to investigate further if these decline. Alternatively, some owners may elect to give prophylactic therapy; isoniazid (which is occasionally used in humans for this purpose) can be used for 6 months but experience of its use in cats is limited and toxicities may occur. Instead, some owners may choose to treat using the triple combination antituberculosis therapy for 3 months, given that the use of this protocol is well established and potential side effects are well documented.

Conclusions

This report follows our initial alert regarding an ongoing outbreak of *M bovis* TB in pet cats across the UK. It describes six clinically sick cats and seven in-contact cats with a mortality rate of 83%. Lifestyle investigation revealed the common factors between clusters were that the cats were exclusively indoor only and fed a commercially available raw food product produced by a single manufacturer. The FSA, APHA, PHE and the food manufacturer concerned have been notified/informed. Our results provide compelling, if circumstantial, evidence of an association between the commercial raw diet of these cats and their *M bovis* infections.


Addendum Since submitting this article in November 2018, the outbreak has continued across the UK. The number of cases has now increased to include at least 30 clusters involving >90 cats. Information about the continuing outbreak will be published as soon as is practically possible.

Acknowledgements We wish to thank all of the veterinarians and owners of these cats for facilitating the investigation and giving permission for the findings to be published.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding Conor O'Halloran is supported by a Biotechnology and Biological Sciences Research Council (BBSRC) studentship (BB/M014894/1). Jordan Mitchell is supported by a BBSRC studentship (BB/M010996/1). Jayne Hope is funded by BBSRC Institute Strategic Programme funding (BB/P013740/1 & BBS/E/D/20002174).

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