Feline mycobacterial infections

Author : DANIÈLLE GUNN-MOORE

Categories : Vets

Date : June 9, 2014

DANIÈLLE GUNN-MOORE BVM&S, BSc, PhD, MACVS, MRCVS considers diagnostic approaches, such as radiography and PCR techniques, in the second of three-part series

THIS is the second of three articles on feline mycobacterial infections. The previous article (*VT*44.10) covered epidemiology, pathogenesis, predisposition and clinical signs; this one covers diagnosis, and the third one will cover management.

Diagnosis

Unfortunately, many cases of feline mycobacteriosis look very similar, regardless of which species of mycobacteria is involved (that is tuberculosis, feline leprosy syndrome [FLS] or non-tuberculous mycobacteriosis [NTM]). Since different mycobacteria have different sources and zoonotic risks, respond differently to antibiotics and have dissimilar prognoses, further investigation is needed to determine which infection is present. Unfortunately, diagnosis is always challenging. This is because many of the mycobacteria do not grow in culture (classically only about 50 per cent grow), and even those that do, such as *M microti*, can take three months to be identified. In addition, serological tests have historically proved unhelpful and molecular diagnostics are not always available and can be very expensive.

Non-specific tests

A thorough evaluation of the patient is necessary to assess the extent of local infection and the degree of systemic involvement.

Changes in serum biochemistry and haematology, if present, are non-specific and vary with the severity of disease. For example, hypercalcaemia appears to correlate with disseminated disease.

Radiography can be useful in the appraisal of lung involvement. However, changes are very variable and include tracheobronchial lymphadenopathy, interstitial or miliary lung infiltration, localised lung consolidation or pleural effusion. Since pulmonary involvement is usually via haematogenous spread, this leads to diffuse interstitial (later becoming bronchial) changes being seen most commonly (Bennett et al, 2011). Bone lesions tend to consist of areas of bony lysis and sclerosis, osteoarthritis, discospondylitis or periostitis.

Abdominal radiography and ultrasound examination may reveal hepatomegaly or splenomegaly, abdominal masses, mineralised mesenteric lymph nodes or ascites.

Specific tests

The recently developed interferon-gamma (IFN?) test can detect members of the tuberculosis complex (such as *M bovis* and *M microti*) and *M avium* (see useful contacts at end for availability; Rhodes et al, 2011). Other specific tests have been investigated, but generally proved less helpful, although newer tests for serum antibody responses are being developed. Unlike other species, cats do not react strongly to intradermally administered tuberculin and the results from intradermal skin testing are unreliable.

Identification of mycobacteria

Aspirates and/or biopsy samples should be Ziehl-Neelsen (ZN) stained. Histopathology typically reveals granulomatous inflammation, with foamy macrophages containing variable numbers of acid-fast bacteria (AFB). The number of AFB depends on:

• the species and strain of mycobacteria involved (for example, large numbers are typically seen with *M avium* infections);

• the location of the granuloma; and

• the nature of the cat’s immune response. Where the immune response is poor, lepromatous changes are often seen with large numbers of AFB (for example, lepromatous FLS), while when the immune response is more robust a tuberculous response is more likely, and AFB will be few (tuberculosis, or tuberculous FLS due to *M lepraemurium* or *M* sp strain Tarwin; Davies et al 2006; Malik et al, 2013).

It can be difficult to identify NTM within (pyo)granulomatous panniculitis because these lipophilic organisms are lost from within the lipid droplets during processing. The use of modified Fite’s or rapid ZN stains may improve detection. Demonstration of organisms within panniculitis is more

readily achieved looking at Romanowsky-stained cytology samples; this may reveal large numbers of macrophages containing negatively stained bundles of organisms. Similar negatively stained bundles of mycobacteria may also be seen within macrophages and giant cells with *M avium* or lepromatous FLS.

Specialist culture is needed to determine which species of mycobacterium is involved. Unfortunately, many samples that contain AFB fail to culture, including all those with FLS and even some with *M microti*, particularly when there are few bacteria present.

Molecular PCR techniques can be used to identify mycobacteria. However, they are often expensive and have limited availability (see later for details).

Correct handling of biopsy material

In practice, correct handling usually involves taking a biopsy from a case where mycobacterial disease is only one of a large number of possible differential diagnoses. If in-house facilities are available for ZN staining, this can be performed on aspirates or biopsy impression smears. However, in most cases biopsy material must be sent to a veterinary diagnostic laboratory. Collect the biopsy, cut it into three or four pieces, fix one in formalin for histopathology and ZN staining and, pending results, place two in a sterile container and freeze them. Where other bacterial infections are suspected, the fourth sample should be sent unfixed for routine bacterial culture and ZN staining. If the sample is found to have ZN positive organisms, one of the frozen pieces can be sent for specialist culture (by the AHVLA and/or a mycobacterial reference laboratory; see end for details), while the last sample is kept in case further investigation is needed. This is advisable for all enlarged lymph nodes and cutaneous/ subcutaneous lesions in cats.

Remember: in the UK, approximately one per cent of feline tissue samples submitted to diagnostic laboratories for routine histopathology have changes consistent with mycobacterial infection (Gunn-Moore et al, 2013).

Until the organism is identified it should be considered a potential human pathogen.

When dealing with a potentially tuberculous case, wear gloves and use routine aseptic practices when handling the biopsy and the biopsy site. Under the Tuberculosis Orders in England, Wales, and Scotland, the identification of *M bovis* in clinical or pathological samples taken from any mammal (except humans) is notifiable to the AHVLA. The orders impose a duty on any veterinary surgeon who even suspects tuberculosis in a domestic pet to immediately notify the divisional veterinary manager at the local office of the State Veterinary Service (Defra, 2013). When a confirmed case is euthanised, it is advisable to have the body cremated.

References

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Useful contacts:

DANIÈLLE GUNN-MOORE

Contact the author to discuss cases in more detail. We are trying to collate all cases in cats in the UK to gain a better understanding of the presentation, causes, and treatment responses of the condition. We are also investigating the possible role of faulty vitamin D metabolism in these cats and would love to receive 1ml to 2ml of serum and 0.5ml EDTA, to look at genetic predisposition.

Professor of Feline Medicine, University of Edinburgh Small Animal Hospital, Easterbush Veterinary Centre, Roslin, Midlothian EH25 9RG. Tel: 0131 650 7650 / 0131 650 6182; Email: danielle.gunn-moore@ed.ac.uk

AHVLA

All potential cases should be reported to the AHVLA. It is willing to undertake mycobacterial culture free of charge in cases where the history, clinical signs and/or histopathological findings are suggestive of mycobacterial infection. Contact the TB diagnostic laboratory prior to sending samples to ensure appropriate samples are submitted, and enclose case details when submitting samples:

TB Diagnosis Section (SEB2), AHVLA (Defra), Weybridge, Wood Lane, New Haw, Addlestone, Surrey KT15 3NB. Tel: 01932 357471

BIOBEST

For IFN-? blood testing, 2ml of heparinised blood is needed. Contact the laboratory first as the test is only run every two weeks. The test costs about £200 unless the case fits the inclusion criteria for a study by the author, in which case the cost is £100. Send sample in a well-padded envelope marked with “Do not refrigerate” to:

Biobest Laboratories Ltd, 6 Charles Darwin House, The Edinburgh Technopole Milton Bridge, Nr Penicuik EH26 0PY. Tel: 0131 440 2628 Fax: 0131 440 9587 www.biobest.co.uk

REFERENCE LABORATORIES

For culture of samples that have failed to grow for AHVLA or for more extensive mycobacterial culture, the reference laboratories in Cardiff and Leeds have extensive experience of handling samples from cats. There will be a charge (generally £100 to £150, but it varies on how difficult the organism is to grow). Please contact the laboratory to ensure appropriate samples are submitted:

Regional Centre for Mycobacteriology (PHLS), Llandough Hospital, Penlan Road, Penarth, Cardiff CF64 2XX. Tel: 02920 716408

Director: Francis Drobniewski, National Mycobacterium Reference Laboratory (NMRL), HPA National Mycobacterium Reference Laboratory, Abernethy Building Institute of Cell and Molecular Science (ICMS), 2 Newark Street, London E1 2AT. Tel: 020 7377 5895 Fax: 020 7539 3459 Email: f.drobniewski@qmul.ac.uk

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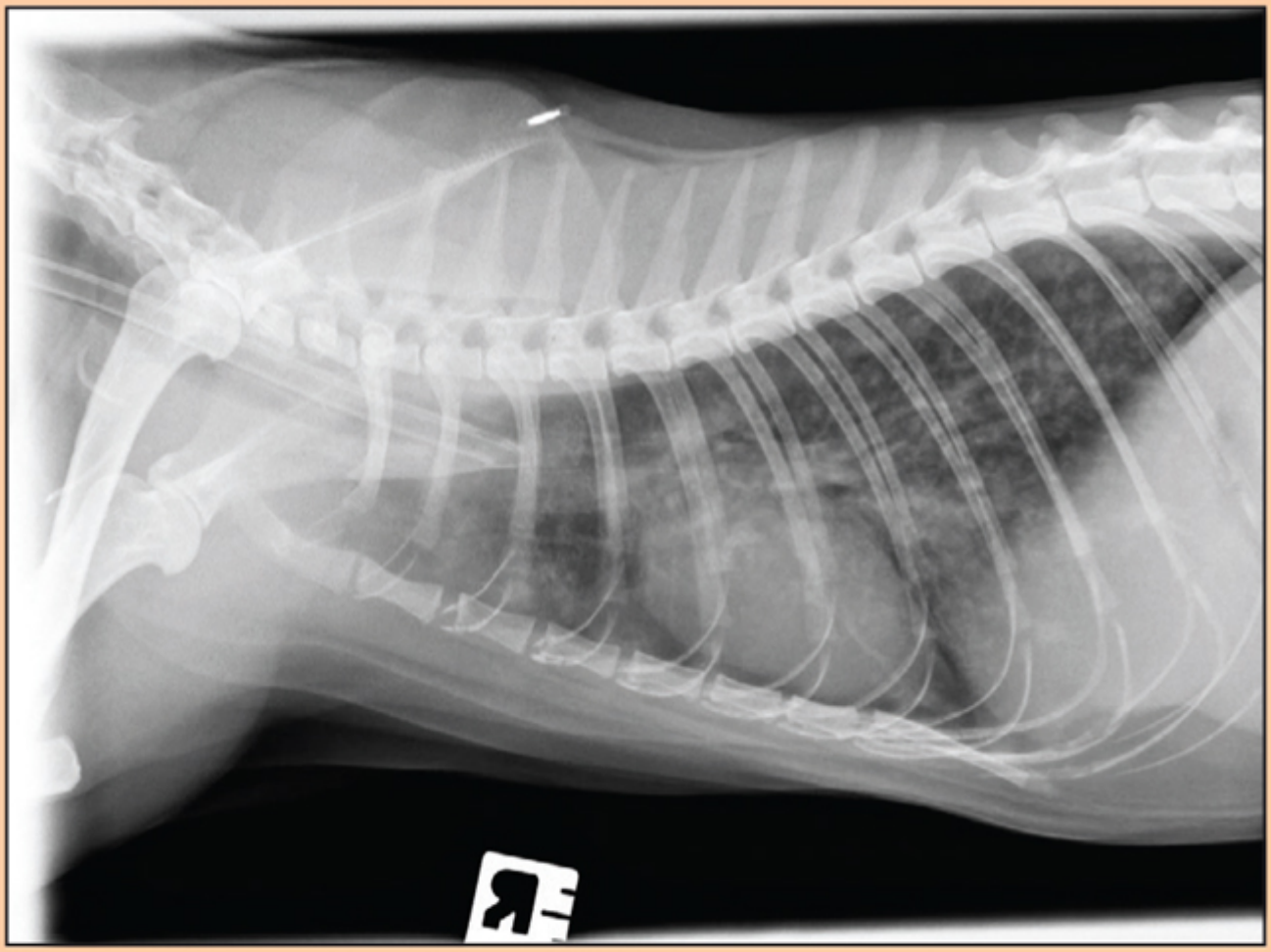
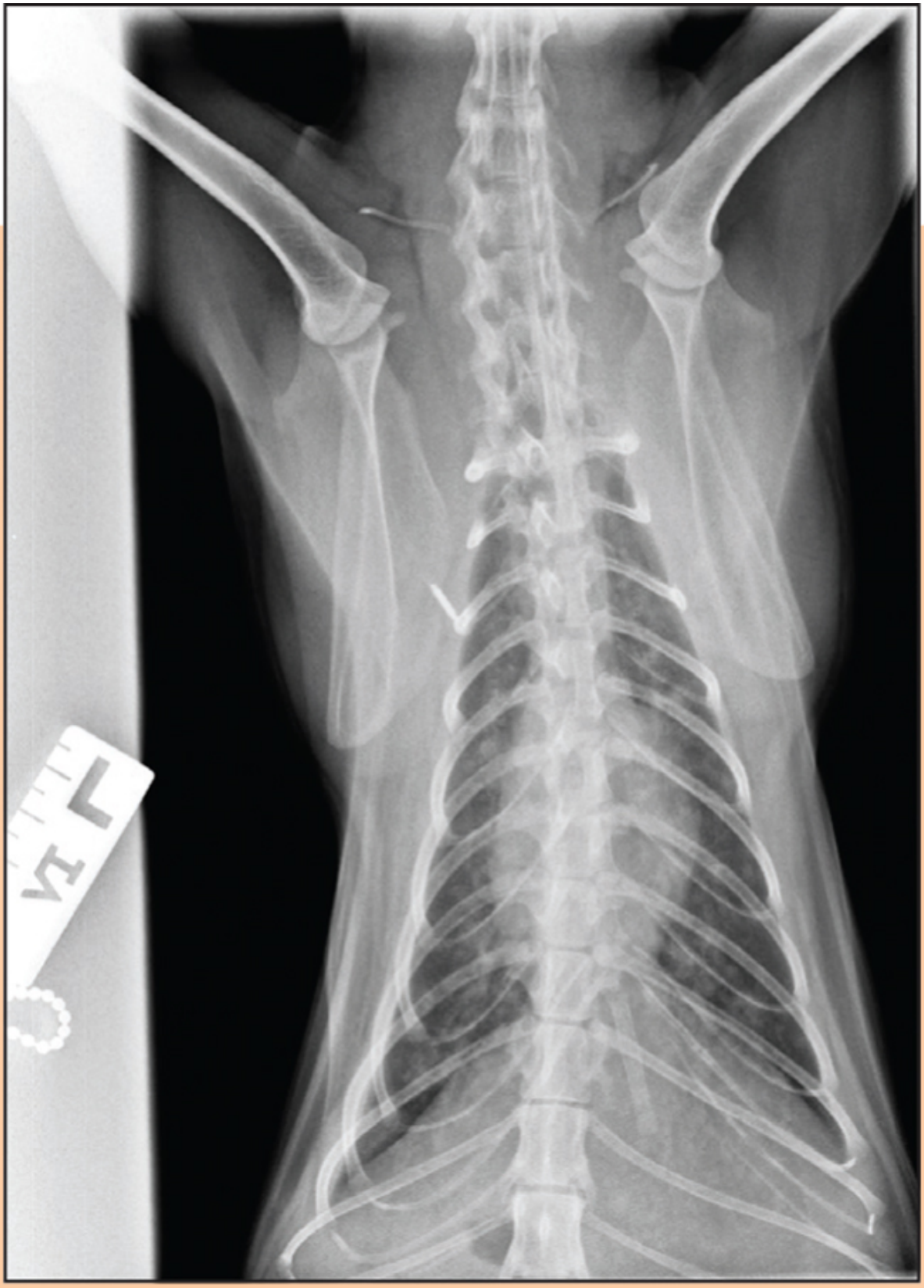
Where it is impossible to collect a sample for culture, it may be possible to confirm the presence of mycobacteria and whether the organism is a member of the tuberculosis complex by PCR test performed on formalin-fixed tissue (although a fresh unfixed tissue sample is always preferred). Costs as of July 2013: routine mycobacterium detection and Tb PCR £117+vat, plus a further £233+vat for differentiating members of the tuberculosis complex. Please contact the laboratory below prior to sending samples to ensure appropriate samples are submitted:

Deborah Gascoyne-Binzi, principal clinical scientist, Leeds Teaching Hospitals Trust, Department of Microbiology, The General Infirmary, Great George Street, Leeds LS1 3EX. Tel: 0113 392 3929 (Laboratory: 0113 392 8797) Fax: 0113 343 5649; Email: deborah.gascoyne-binzi@leedsth.nhs.uk



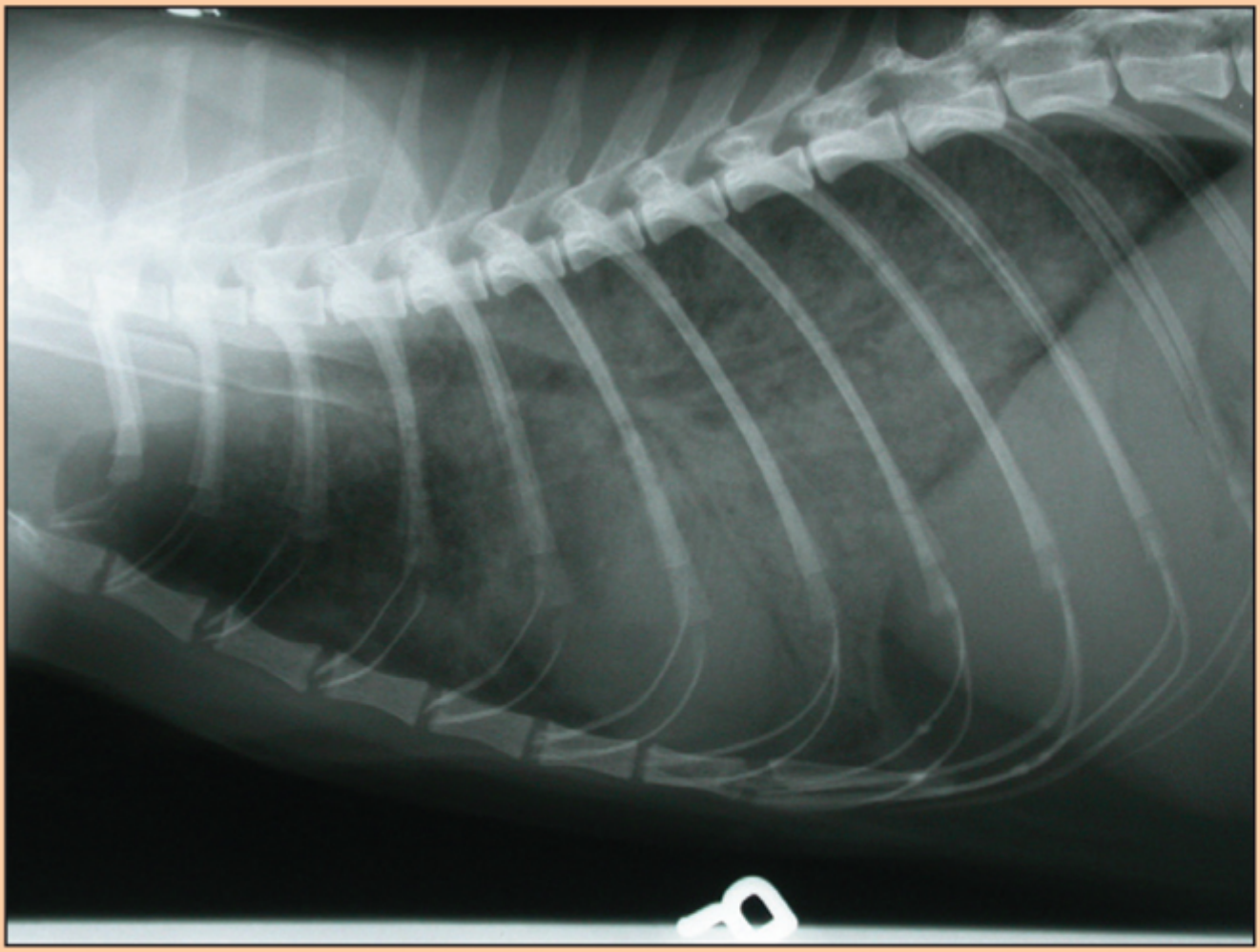
Lateral chest radiograph, *M microti* in an adult male neutered cat.

IMAGE: John Wood.



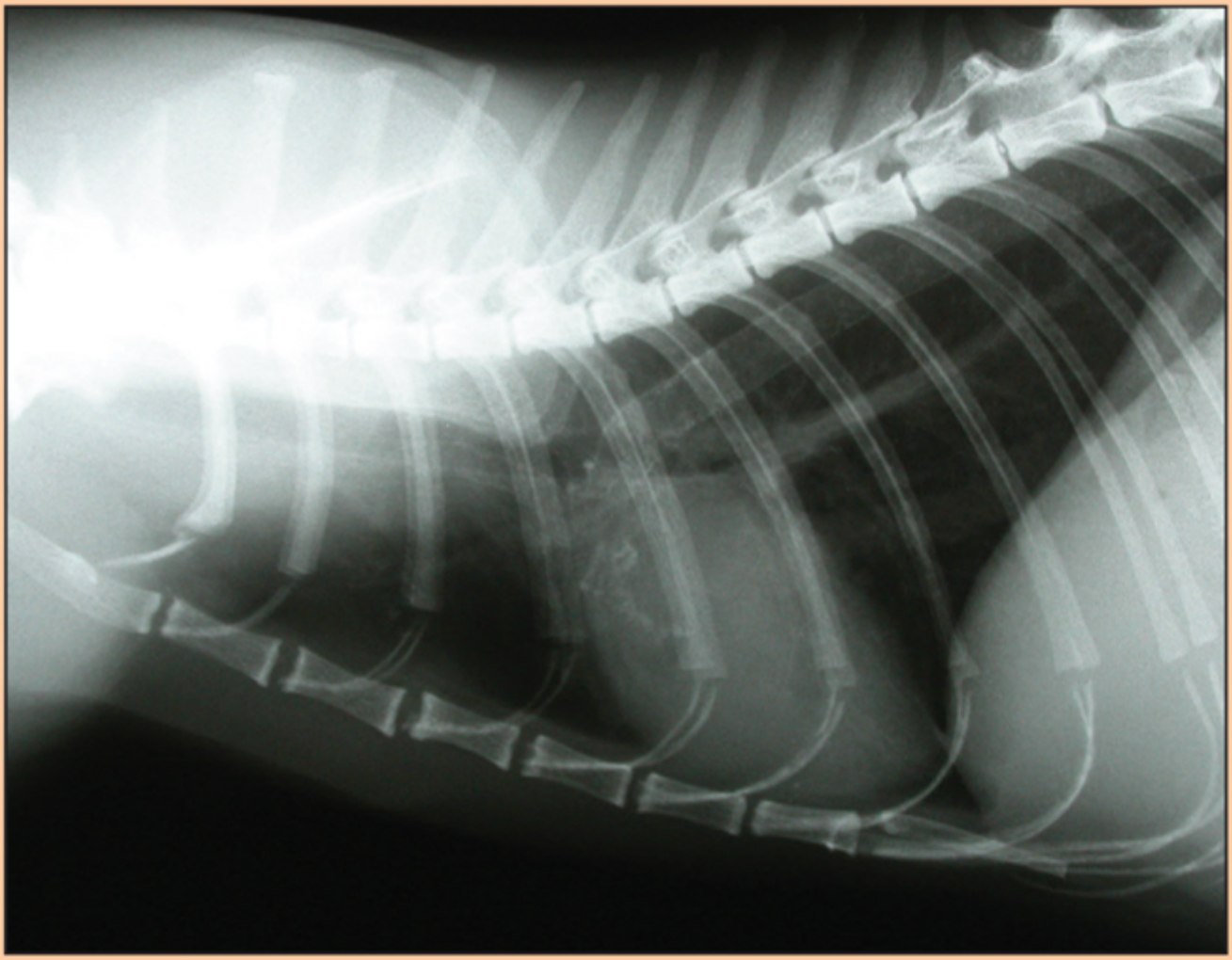
Ventrodorsal chest radiograph, *M microti* in an adult male neutered cat.

IMAGE: John Wood.



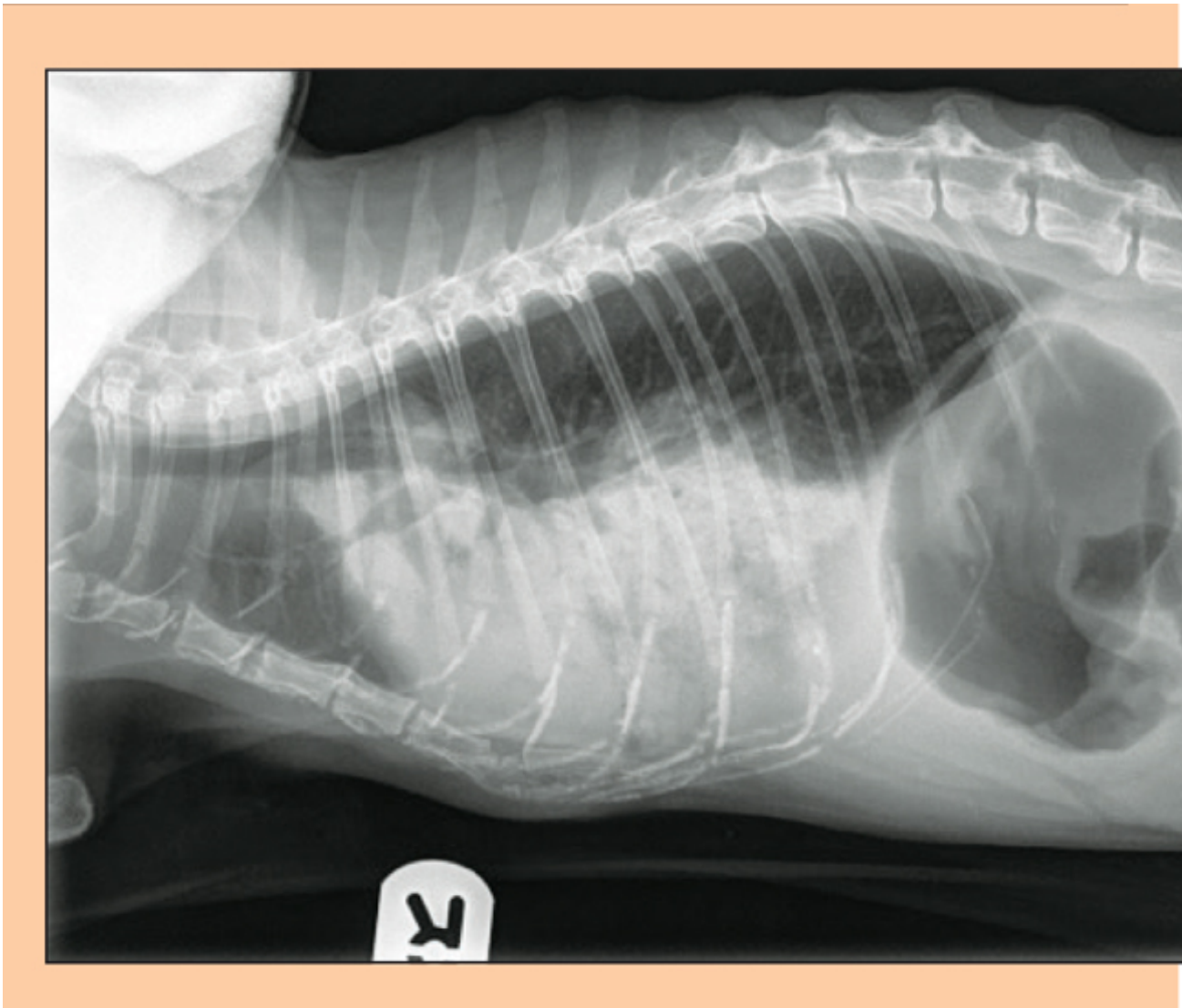
Lateral chest radiograph, *M bovis* in an adult male neutered cat.

IMAGE: John Wood.



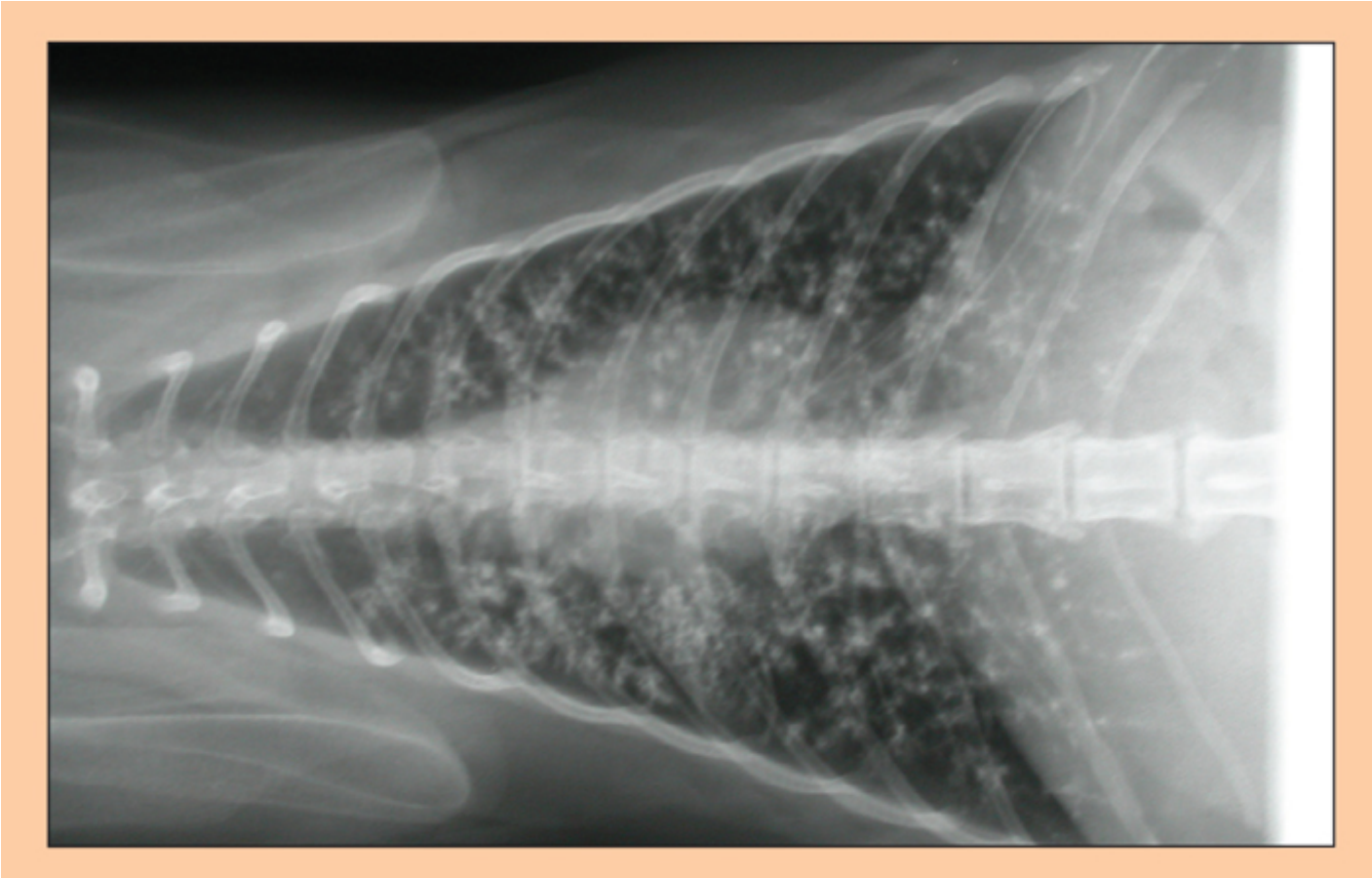
Lateral chest radiograph, *M bovis* in an adult male neutered cat with hypercalceamia.

IMAGE: Susannah Brown



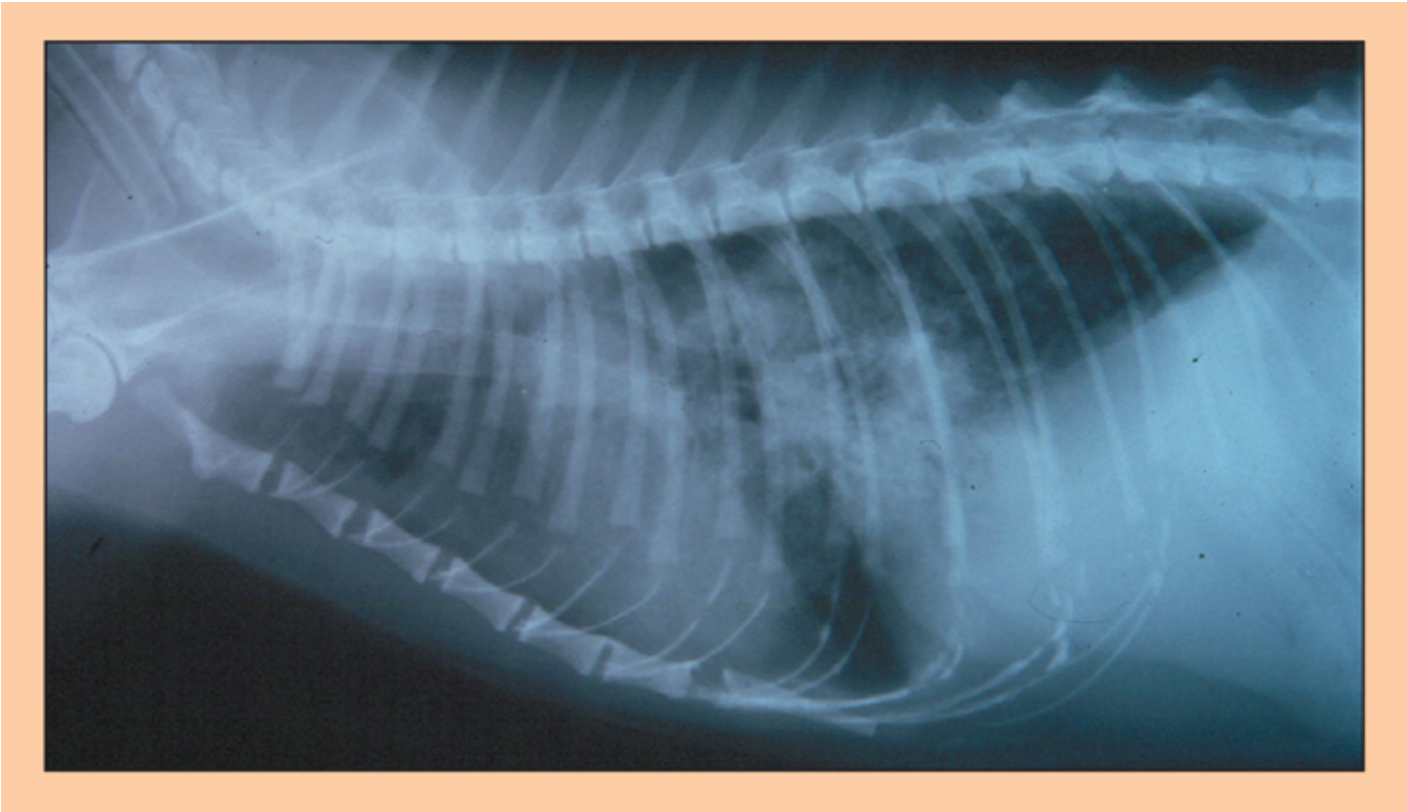
Lateral chest radiograph, *M bovis* in an adult male neutered cat.

IMAGE: Susannah Brown

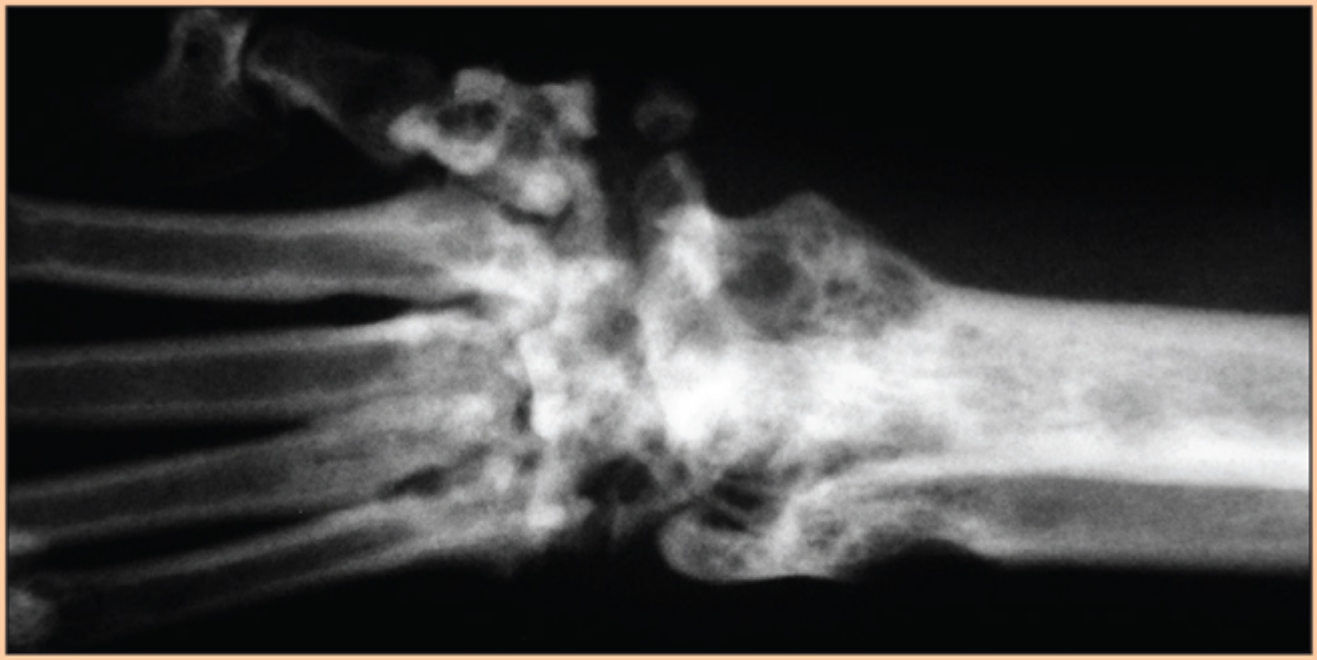


VD chest radiograph, *M bovis* in an adult male neutered cat.

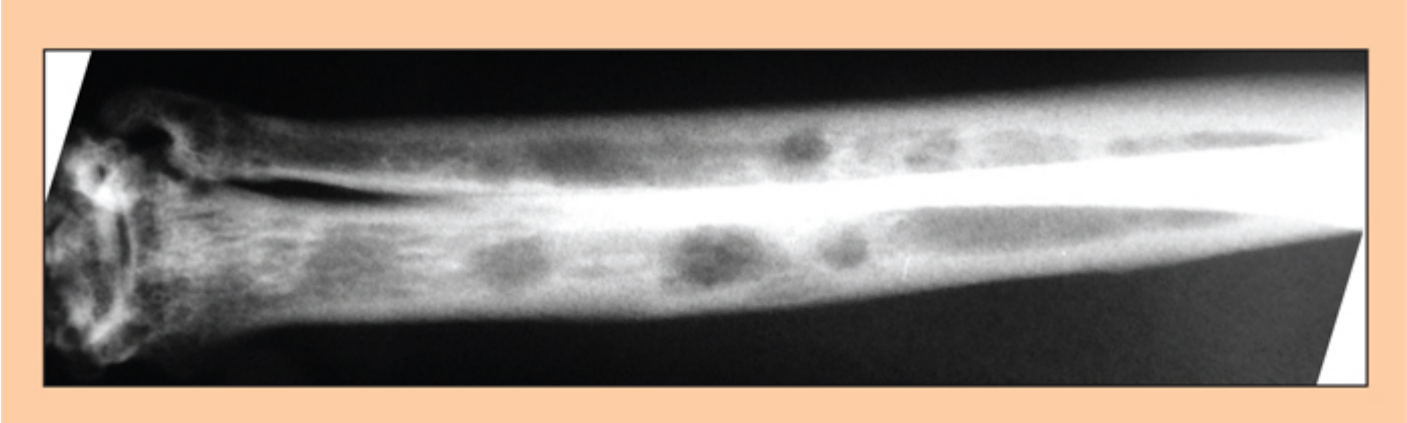
IMAGE: Pam Meldrum



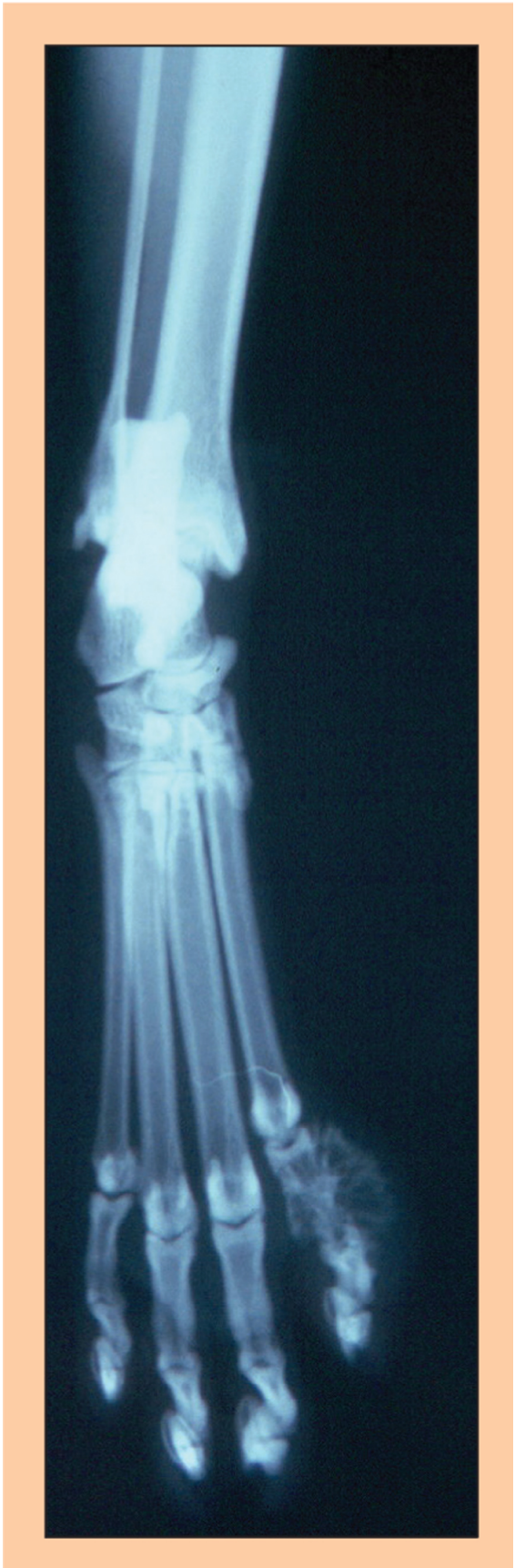
Lateral chest radiograph, *M microti* in an adult female neutered cat.



Dorsoventral carpal radiograph, *M bovis* in an adult male neutered cat.

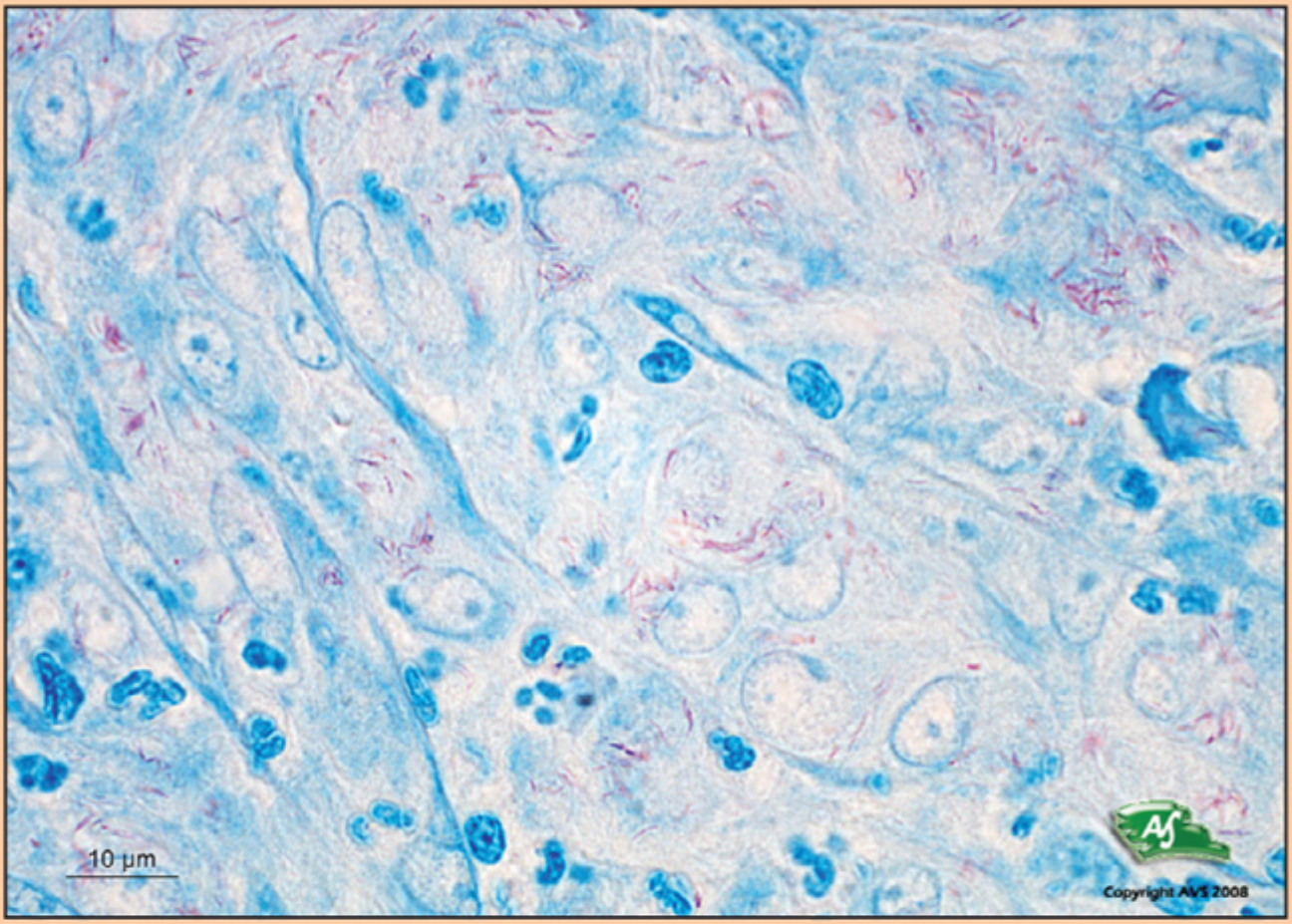


Dorsoventral radius and ulna radiograph, *M bovis* in an adult male neutered cat.



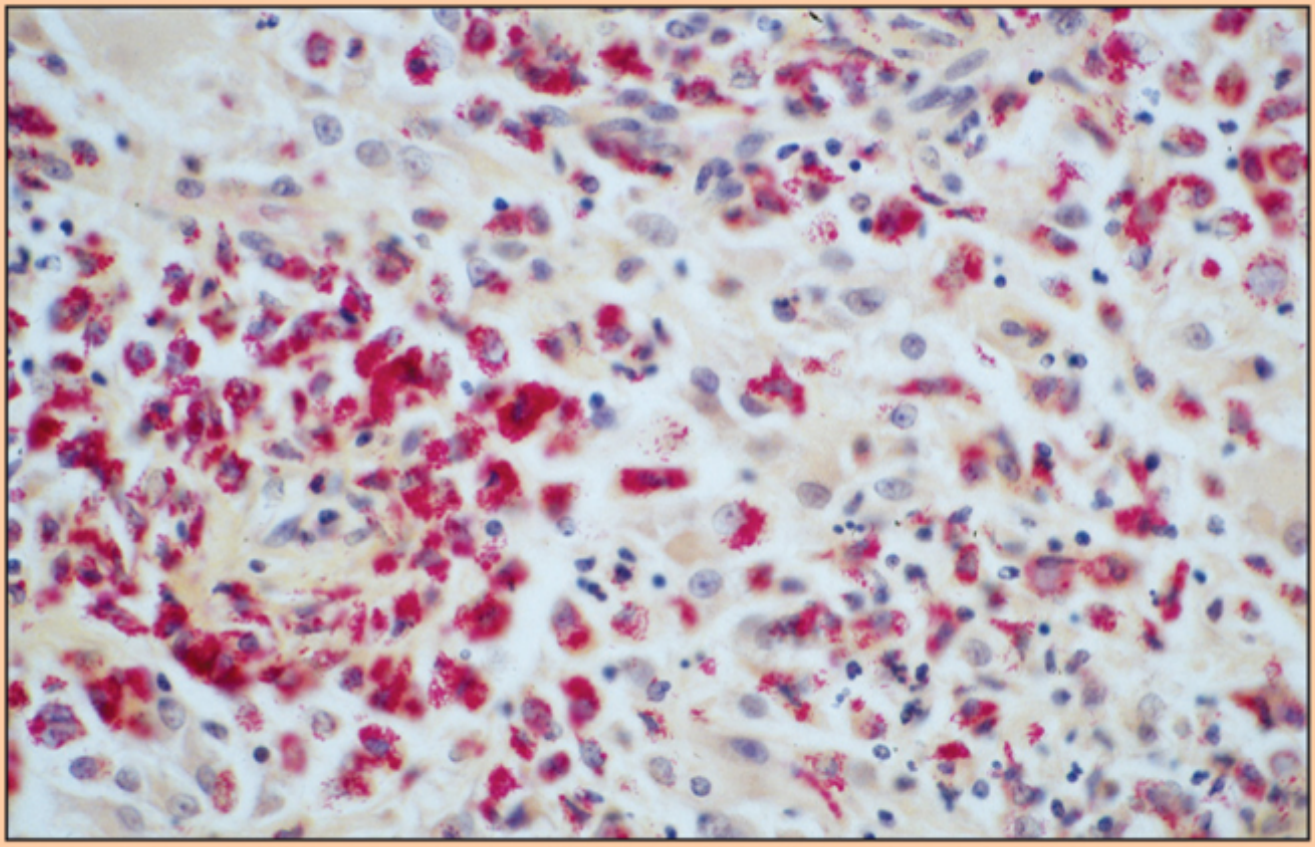
Dorsoventral hind foot radiograph, *M mictoti* in an adult male neutered cat.

IMAGE: Mike Hollywood.



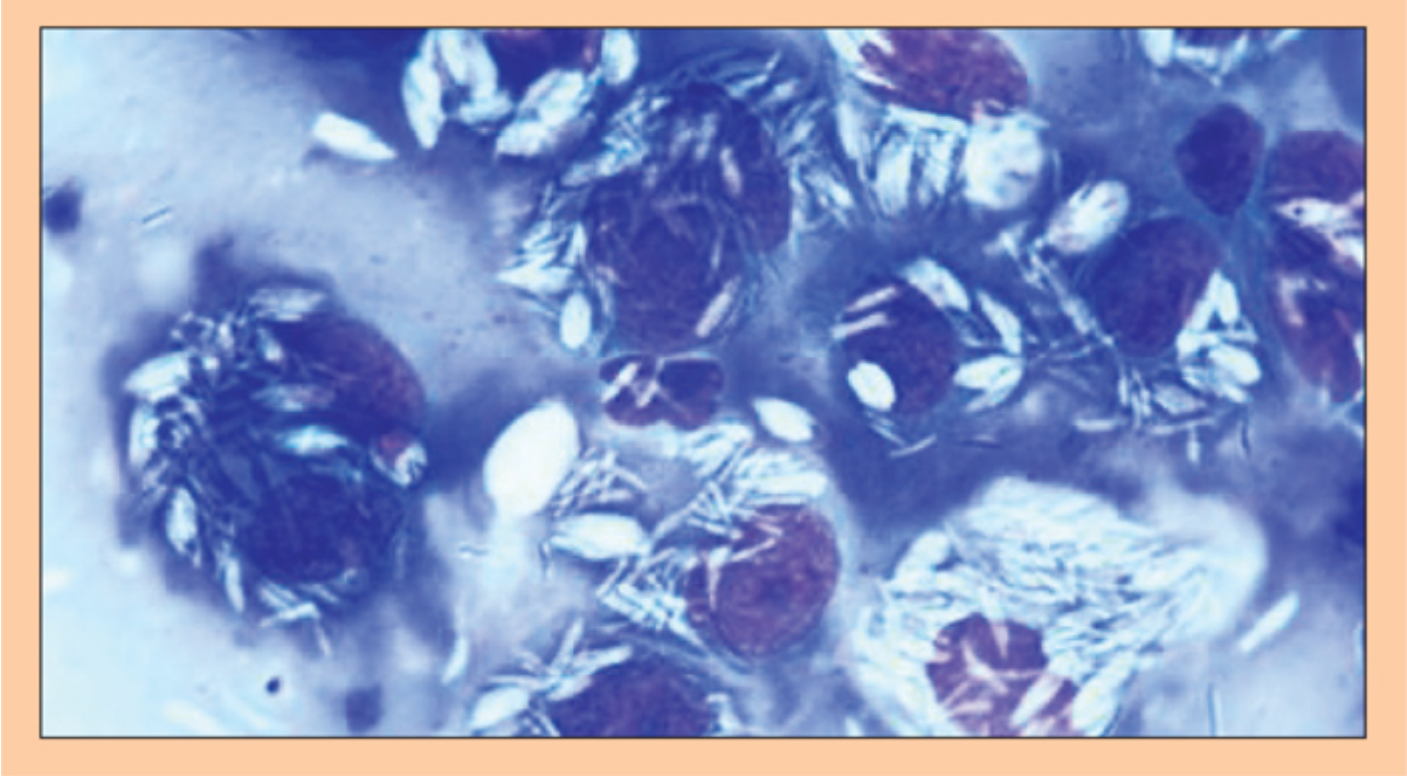
Moderate numbers of intrahistiocytic AFB, ZN stain, *M microti*.

IMAGE: Jorge Del Pozo and Richard Fox.



Massive numbers of AFB in granulomatous tissue in the brain, ZN stain, *M avium*.

IMAGE: Jorge Del Pozo and Richard Fox.



Massive numbers of AFB within granulomatous skin tissue, Diff-Quik stain, culture failed to grow.

IMAGE: Richard Malik.