



Leproid granulomas: a unique mycobacterial infection of dogs

History and epidemiology

In 1973, a Rhodesian veterinarian, Richard Smith, documented a mycobacterial skin infection in a Doberman and a Rhodesian Ridgeback dog that was characterised by variably sized subcutaneous nodules¹. It was thought at first that these dogs may have had tuberculosis and, because of public health considerations, they were euthanased and subjected to postmortem examination. There was no internal organ involvement at necropsy and, although acid-fast bacilli (AFB) were abundant in the lesions, culture was negative for the tubercle bacillus.

Similar cases were recorded soon thereafter in an Australian veterinary discussion forum², although the infection was not subjected to systematic investigation until the end of the 20th century³. The disease was given the name 'canine leprosy' by some veterinary dermatologists, but the alternative name canine leproid granuloma syndrome (CLGS) was later suggested by an Australian veterinarian David Watson. It is now clear that leproid granulomas are not uncommon and indeed are by far the most frequently encountered mycobacterial disease of dogs in Australia.

Clinical signs

Primary lesions consist of single or multiple, well circumscribed subcutaneous nodule(s). These lesions can appear anywhere on the dog, although they are usually located on the head and typically on the dorsal fold of the ears. The nodules are hard, painless, and vary in size from 2mm up to 5cm in diameter (Figure 1: A&B). Small nodules are detected as hard subcutaneous lumps, while larger nodules may show superficial hair loss. Very large lesions may ulcerate (Figure 1: C). Lesions can be disfiguring

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and cause irritation, especially when they are multiple and secondarily infected.

Leproid granulomas are confined to the subcutis and skin, and do not involve regional lymph nodes, nerves or internal organs. Affected dogs suffer no apparent systemic ill effects. Subsequent studies have shown that this infection has a worldwide geographic distribution, with cases recorded from coastal and inland regions of all states of Australia, New Zealand, Zimbabwe, Brazil and at least four States of the United States^{4,5}. The condition seems to be especially common in Australia and Brazil for reasons that are currently unclear.

There is a strong propensity for short-coated breeds to be affected, with Boxer and Boxer-cross dogs accounting for nearly half of reported cases. Despite the fact that CLGS was first reported over 30 years ago, its aetiopathogenesis has not been fully elucidated. It is thought that the causal organism may have a low inherent virulence and/or special growth prerequisites, such as a requirement for low temperature, which permits them to survive and multiply only in superficial tissues.

Smith's original report stated that "lesions appear suddenly, and are usually seen on dogs pestered by biting flies". This, and other epidemiological evidence suggests that flies or some other biting arthropod inoculate mycobacteria from an environmental niche into susceptible tissues. Lesions then develop following an undefined incubation period. The predilection for lesions to develop in regions favoured by biting insects is consistent with this hypothesis, as is the preponderance of short-coated, large breed dogs, which are housed outdoors generally.

Diagnosis

Diagnosis is usually straightforward, as the distribution of lesions, coupled with the tendency for nodules to be multiple, is suggestive of this aetiology, particularly in an at-risk breed. Diagnosis can be confirmed by obtaining specimens for cytological or histological examination⁶. DiffQuik-stained smears from aspirates demonstrate numerous macrophages with variable numbers of lymphocytes and plasma cells and lower numbers of neutrophils. Usually few to moderate numbers of negatively-stained, medium-length bacilli can be detected within macrophages or extracellularly.

From a histological perspective, lesions in the subcutis and dermis consist of pyogranulomas composed of epithelioid macrophages, Langerhans-type giant cells (Figure 2) with scattered neutrophils, plasma cells and small lymphocytes. The number and morphology of AFB in Ziehl-Neelsen (ZN)-stained sections are highly variable (Figure 3: A&B) and probably depends on the maturity of the lesion and the corresponding host immune response.



Figure 1. Appearance of leproid granuloma lesions in dogs.
A: solitary lesion on the ear of a Mastiff.
B: solitary lesion on the dorsal ear fold of a British bulldog.
C: multiple, ulcerated lesion on both ear folds of a Boxer dog.

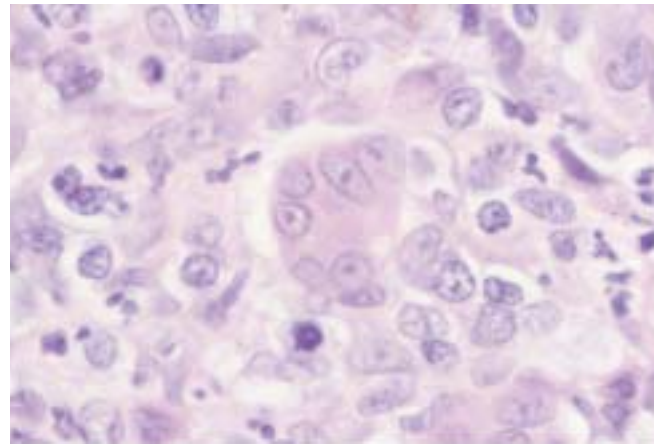


Figure 2. Histology of a leproid granuloma lesion in the subcutis of a dog. Note the Langerhans-type giant cell. Haematoxylin & eosin; x 560.

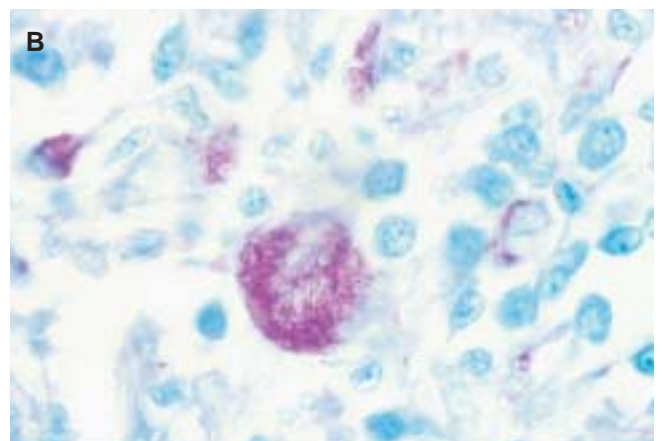
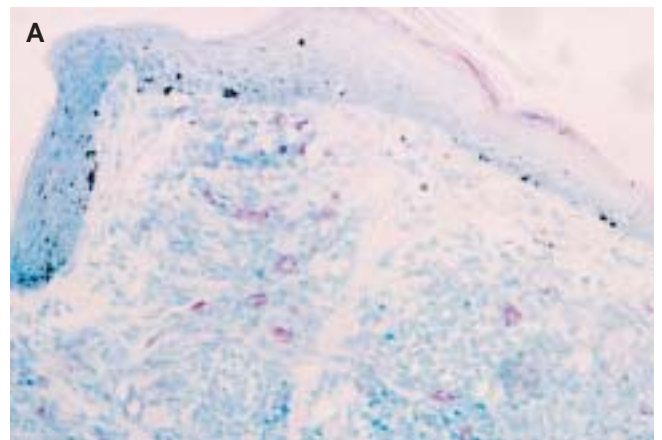


Figure 3. Low (A) and high (B) powered photomicrographs of CLG histology. Mycobacteria stain positively (i.e. acid-fast) with the carbol fuchsin (pink stain), and tend to be located intracellularly within macrophages and giant cells. ZN; x 85 and 560, respectively.



Currently, it is impossible to confirm the diagnosis by culture, as the *in vitro* growth requirements for this fastidious organism have not been determined, despite concerted attempts in at least two Australian mycobacteria reference laboratories. A negative culture, however, can exclude some other mycobacterial aetiologies.

Molecular analyses

Polymerase chain reaction (PCR) methodologies, using genus specific primers to amplify variable regions 2 and 3 of the bacterial 16S rRNA gene which are of value for mycobacterial species identification, have been performed on DNA extracted from canine leproid granuloma specimens⁷.

The same, novel partial 16S rRNA gene sequence was identified by three different laboratories, using at least two different DNA extraction and PCR methodologies. This unique and identical sequence has been observed in all specimens investigated irrespective of the country of origin of the specimen.

Three discordant specimens, whose PCR amplicons did not hybridise to the probe developed for the novel sequence, were reported previously⁷. However, subsequent research supported a single mycobacterial species being associated with CLGS. In two of the discrepant cases, it was confirmed that lack of hybridisation resulted from insufficient PCR product, and in the other case another novel mycobacterial species was identified, but the specimen was actually of feline origin.

Sequence analysis of the unique partial 16S rRNA gene amplicons from cases of CLGS revealed high nucleotide identity with the *M. simiae* clade of slow growing mycobacteria, and the presence of a short helix 18. This characteristic, although normally associated with rapidly growing mycobacterium, is an unusual feature of

the slow growing, fastidious mycobacterial species *M. simiae*, *M. interjectum* and *M. genavense*. Interestingly, the species represented by this sequence has never been recorded in mycobacterial granulomas of cats, horses, people, or other non-canine mammalian species. Hence, there is thought to be no public health risk to the owners of affected dogs.

Treatment

Most dogs with leproid granulomas have self-limiting disease, with spontaneous regression of lesions over time, typically within 1-3 months of the lesions appearing. This 'self cure' occurs presumably as a result of an effective cell-mediated immune response. Indeed, it is likely that many cases with inconspicuous or few lesions are never presented for examination.

In patients presented for veterinary attention with a limited number of lesions, surgical excision can be curative, and provides material with which to confirm the diagnosis histologically and by PCR analysis. In a minority of cases, the infection progresses to produce chronic, disfiguring lesions that may persist indefinitely. Treatment with β -lactam drugs, enrofloxacin or doxycycline fails to have an impact on the course of infection, although these drugs may be of some benefit by eliminating secondary pyogenic infections.

Refractory lesions respond to therapy with combinations of antimicrobial agents known to be effective against nontuberculous mycobacteria, including rifampicin, clarithromycin, clofazimine and doxycycline. A combination of rifampicin and clarithromycin is currently recommended⁸. A topical formulation of clofazimine in petroleum jelly has been used as an adjunct to systemic drug therapy, and currently, we are evaluating a topical preparation containing both rifampicin and clofazimine.

There is much yet to be learnt about this fascinating mycobacterial infection. Determination of the full 16S rRNA gene sequence should provide a valuable taxonomic insight into this novel species and should facilitate determination of the *in vitro* growth requirements of this likely saprophyte.

Many important questions remain unanswered. For example, what is the organism's normal environmental niche? Is an insect vector involved, and is it merely a mechanical vector or does the organism multiply in the arthropod? Why are dogs infected, but not other species, and why is the boxer breed especially susceptible? Can the disease be reproduced experimentally? Clearly, there is a lot of work to do for veterinary mycobacteriologists interested in this 'new' bug!

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