MYCOBACTERIUM AVIUM COMPLEX; AN UPDATE

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INTRODUCTION

Mycobacterium avium complex (MAC) infections have been recorded in a wide range of different mammalian species, including deer (Lepper and Corner, 1983). Most of these infections are asymptomatic and often do not lead to the development of gross lesions. On rare occasions MAC infections progress to a stage which results in severe clinical disease. Clinical cases are thought to occur principally in immunocompromised hosts such as humans infected with the human immunodeficiency virus (Kiehn et al., 1985). This paper will review the results of investigations carried out at the Central Animal Health Laboratory on MAC infections in deer

The Mycobacterium avium complex (MAC)

The complex contains two closely related species, Mycobacterium avium and Mycobacterium intracellulare. They were differentiated into separate species principally on the basis that M.avium but not M.intracellulare strains are fully virulent for chickens. These species are not readily distinguished using standard bacteriological methods(Kent and Kubica, 1985). The majority of human and veterinary diagnostic laboratories, including the Central Animal Health Laboratory at Wallaceville do not routinely identify MAC isolates beyond the level of belonging to the complex.

Serotyping and biochemical analysis of cell wall components have been used to subtype members of MAC. There are three serotypes of M.avium (serotypes 1-3) and 25 serotypes of M.intracellulare (serotypes 4-28). Some MAC strains can not be serotyped because they either autoagglutinate or they are serologically unreactive.

Recent studies indicate that Mycobacterium paratuberculosis the agent of Johne's disease and Mycobacterium lepraemurium the cause of rat and feline leprosy are also members of MAC (McFadden et al., 1987). Mycobacterium paratuberculosis is principally distinguished from other members of the complex by its requirement for the iron chelator, mycobactin for in vitro growth. However, the taxonomy of M.paratuberculosis is complicated by the presence of strains of M.avium whose primary isolation is enhanced by mycobactin and by closely related, but distinct mycobacteria recovered from the European wood pigeon. Both these varieties of mycobacteria have been isolated from deer (Jorgensen and Clausen, 1976, Matthews et al., 1981).

Isolation of MAC strains from deer in New Zealand

A total of 35 strains of MAC and 504 strains of M.bovis were isolated from deer between 1970 and 1983 (de Lisle and Havill, 1985). A further 100 strains of MAC and 442 strains of M.bovis have been isolated from deer in the following five years. These figures need to be interpreted with considerable caution. A large proportion (46/97 47%) of the recent MAC strains came from only three small groups of animals. It is expected that with the widespread use of the comparative test, coupled with a decrease in culturing pooled lymph nodes from skin test reactors with "no visible lesions", there will be a decrease in the number of MAC isolates from deer.

Serotyping and DNA restriction endonuclease analysis of MAC deer isolates

MAC have been isolated from a wide range of species in New Zealand, including pigs, cattle, deer, cats, possums, wallaby, humans, poultry, and horses. There is insufficient data to determine whether or not MAC infections are more common in deer than cattle in New Zealand. Serotyping of many strains by G.Meissener, Germany revealed that New Zealand isolates were principally serotypes 1, 2, 8, and 9. Small numbers of serotypes 10, 14 and 21 were also found. Studies at the Central Animal Health Laboratory revealed that examination of DNA by restriction endonuclease analysis (REA) revealed that this technique could be used to classify strains into similar groups as that which could be achieved by serotyping (Wards et al., 1987).

Serotyping and REA have been used to examine MAC isolates from three groups of deer which reacted to the tuberculin skin test. A total of 29 of 80 fawns from a Nelson property reacted to bovine purified protein derivative (PPD) in the cervical skin test (ST). The reactors were subsequently examined with the comparative skin test with all animals having reactions to the avian PPD greater or equal to that of the bovine PPD. No gross lesions consistent with a mycobacterial infection were found at necropsy in any of the ST reactors. MAC strains were isolated from lymph node pools (head, thorax, abdomen, body) of 21 of the 29 animals. They were recovered from more than one lymph node pool in 9 animals. All isolates were examined by REA and serotyping. Twenty five of the 31 isolates could be classified into the same three major groups by both REA and serotyping.

Group 1	Serotype 1	12 isolates
Group 2	Serotype 8	3 isolates
Group 3	Serotype 9	10 isolates

Six isolates, including one serotype 3, one serotype 4 and four untypable strains could not be classified into groups.
Similar examinations were done on 13 MAC isolates recovered from the lymph node pools of 12 of 24 imported deer which reacted to the ST. A gross lesion consistent with a mycobacterial infection was found in only one of these animals. In a separate group of imported animals two further MAC strains were isolated from skin test reactors. The imported strains could be classified into two groups, one corresponding to serotype 2 (7 isolates) and the other to serotype 8(8 isolates).

Apart from one exception, the MAC strains from these three groups were recovered from grossly normal lymph nodes. Similar results were reported by Matthews et al. (1981) who isolated MAC strains from 23.7% (187/797) of grossly normal lymph nodes from British deer. These results indicate that there is no deer to deer spread of MAC infection. Possible sources of infection include birds and environmental niches such as soil and water. One should note that birds are only possible sources of infection for M.avium (serotypes 1-3) and that these serotypes have also been found in the environment. An extensive search for an environmental source of infection on the Nelson property was unsuccessful (Prosser, 1989). The finding of different serotypes and DNA restriction types in the Nelson and imported animals raises the possibility of multiple sources of MAC infection.

Very few cultures have been attempted of lymph nodes with no visible lesions from deer which have not reacted to the tuberculin skin test. Although a few MAC isolates were obtained from these cultures some caution must be exercised in assuming that all MAC isolates from skin test reactor deer are the cause of tuberculin sensitivity. Conversely spurious isolations of MAC can occur if samples are contaminated during their collection.

Clinical disease caused by M.avium - intracellulare

Clinical disease caused by M.avium - intracellulare has been reported to occur sporadically in British deer (Hopkins and McDiarmid, 1964, Hime et al., 1971). Recently a mycobacterium was isolated from a deer in New Zealand which died from an overwhelming mycobacterial infection. This animal had a granulomatous enteritis containing large numbers of acid fast staining bacteria - lesions consistent with a diagnosis of Johne's disease. Examination of the mycobacterial isolate serologically and by REA revealed it to be M.intracellulare serotype 8. Strains with virtually identical DNA restriction patterns to this isolate have been recovered from other animal species in New Zealand. The characteristics of this case are consistent with a disease in a deer with a seriously compromised immune system rather than a highly virulent MAC.

Johne's disease in deer

Johne's disease is still a rare condition in deer in New Zealand with fewer than 20 reported cases (Gumbrell, 1987). Mycobacteria have been isolated from four cases of presumptive Johne's disease in New Zealand deer. One of these isolates was a MAC serotype 8 and has been described above. Two of the remaining isolates have been examined by REA. One of the isolates was identical to strains of M.paratuberculosis isolated from cattle (Collins and de Lisle, 1986). The other strain was different from the "cattle" strains but was identical to isolates of M.paratuberculosis which have been recovered from sheep with Johne's disease. These findings indicate that both sheep and cattle are potential sources of infection for deer.

In marked contrast to what has been observed in New Zealand, there has been a serious outbreak of Johne's disease in red deer in Scotland (Reid, 1988). Multiple, fatal cases of Johne's disease occurred in yearling deer. The different manifestations

of Johne's disease in deer in the two countries may be due to strain differences of M.paratuberculosis or the conditions under which deer are raised.

Considerable concern has been raised over the requirements for determining the Johne's disease status of deer entering or leaving New Zealand. Currently available diagnostic tests for Johne's disease have severe limitations in detecting the preclinical stages of infection. The situation is further complicated by the lack of proven cases of Johne's disease in deer which can be used to evaluate the various diagnostic tests.

REFERENCES

de Lisle, G.W. and Havill, P.F. (1985) Mycobacteria isolated from deer in New Zealand from 1970-1983. N.Z. vet. J. 33:138-40.

Collins, D.M. and de Lisle, G.W. (1986) Restriction endonuclease analysis of Mycobacterium paratuberculosis isolated from cattle. Am. J. Vet. Res. 47: 2226-9.

Gumbrell, R.C. (1987) Johne's disease in deer in New Zealand. Deer Branch Course No 4, New Zealand Veterinary Association 174-80.

Hime, J.M., Keymer, I.F., Boughton, E. and Birn, K.J. (1971) Tuberculosis in a red deer (Cervus elaphus) due to an atypical mycobacterium. Vet. Rec. 88:616-7.

Hopkinson, F. and McDiarmid, A. (1964) Tuberculosis in a free-living red deer, Cervus elaphus in Scotland. Vet. Rec. 76:1521-2.

Jorgensen, J. and Clausen, B. (1976) Mycobacteriosis in a Roe-Deer caused by wood-pigeon mycobacteria. Nord. Vet.-Med. 28:539-46.

Kent, P.T. and Kubica, G.P. (1985) Public Health Mycobacteriology A Guide for the level III laboratory. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, Georgia

Kiehn, T.E., Edwards, F.F., Brannon, P., Tsang, A.Y., Mario, M., Gold, J.W.M., Whimbley, E., Wong, B., McClatchy, J.K. and Armstrong, D. (1985) Infections caused by Mycobacterium avium in immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroagglutination characteristics. J. Clin. Microbiol. 21:168-73.

Lepper, A.W.D. and Corner, L.A. (1983) Naturally occurring mycobacterioses of animals. The Biology of Mycobacteria, Volume 2. ed. Ratledge, C. and Stanford, J. Academic Press, 417-521.

McFadden, J.J., Butcher, P.D., Thompson, J., Chiodini, R. and Hermon -Taylor, J. (1987) The use of DNA probes identifying restriction fragment length polymorphisms to examine the Mycobacterium avium complex. Molecular Biology 1:283-91.

Matthews, P.R.J., McDiarmid, A. and Collins, P. (1981) Mycobacterial infections in various species of deer in the United Kingdom. Br. vet. J. 137:59-66.

Prosser, B.A. (1989) Methods used to investigate a possible environmental source of Mycobacterium avium-intracellulare-scrofulaceum (MAIS) infection in farmed deer. J. Appl. Bact. 66:219-26.

Reid, H. (1988) Mycobacteria in deer - the UK experience. NZ Microbiological Society, Dunedin.

Wards, B.J., Collins, D.M. and de Lisle, G.W. (1987) Restriction endonuclease analysis of members of the Mycobacterium avium-M.intracellulare - M.scrofulaceum serocomplex. J. Clin. Micro. 25:2309-13.