ADIASPIROMYCOSIS IN SOUTH AUSTRALIAN HAIRY-NOSED WOMBATS (Lasiorhinus latifrons)

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ADIASPIROMYCOSIS IN SOUTH AUSTRALIAN HAIRY-NOSED WOMBATS *(Lasiorhinus latifrons)*

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Abstract: Spherical organisms, with an average diameter of about 22 μm, were detected in the lungs of adult and pouched young hairy-nosed wombats *(Lasiorhinus latifrons)*. Although infections of up to 640×10^3 organisms per cubic centimeter were detected, their presence produced only limited pathological change. In-vitro growth was obtained at 30 C but not at 37 C or 40 C. However, at the higher temperatures, typical chlamydospore spherules were produced by colonies initially grown at 30 C. This report presents the first record of adiaspiromycosis in Australia and in wombats.

INTRODUCTION

Although pulmonary adiaspiromycosis has been reported from most countries of the world, it has not been reported previously in Australia. The detection, in the lungs of hairy-nosed wombats *(Lasiorhinus latifrons)*, of spherules resembling chlamydospores of *Chrysosporium (Emmonsia) parvum*, prompted an investigation into their identity.

MATERIALS AND METHODS

Animal material. Six adult hairy-nosed wombats *(Lasiorhinus latifrons)* were shot near Blanche Town in South Australia. Lung tissue removed immediately after death was placed on wet ice and forwarded by air to Mt. Pleasant Laboratories. The transit time from collection to processing at Mt. Pleasant Laboratories was 18-24 h. Pieces of fixed lung from 22 pouch young *L. latifrons*, (S.A. Museum specimens), 12 rabbits *(Oryctolagus cuniculus)*, a feral cat *(Felis catus)*, and a fox *(Vulpes vulpes)* also were examined. Twelve of the pouch young wombats came from the Nullarbor Plain and 10 from near Blanche Town while the rabbits, the cat and the fox were caught in or near wombat burrows in the Blanche Town area.

Culture. Supernatant fluid, obtained after grinding fresh lung with sterile sand and nutrient broth containing 1000 IU/ml penicillin and 1000 μg/ml streptomycin, was streaked onto Sabourauds dextrose agar (SDA), and SDA containing 1000 IU penicillin and 1000 μg/ml streptomycin, SDA containing chloramphenicol 50 μg/ml and cycloheximide 500 μg/ml and also SDA containing 50 μg/ml chloramphenicol. Plates were incubated aerobically, in the inverted position at room temperature, 30 C, 37 C and 40 C.

Histology. Material for histologic examination was fixed in 10% neutral formol saline, cut at 6 μm and stained with haematoxylin and eosin (H & E), periodic acid Schiff haematoxylin - tartrazine (PAS) or Southgate's mucicarmine.

RESULTS

Although there was no clinical or gross pathologic evidence of respiratory disease in the adult wombats, numerous spherules were detected in the lung

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homogenate supernatant fluid. In H & E stained sections of these lungs the spherules stained dark blue with a generally faintly granular interior, often containing pleomorphic paler areas, and a peripheral capsular zone about 1.5 μm in thickness which stained faintly pink. In PAS sections spherules were deep purple to almost black while in mucicarmine sections the out layer stained a vivid pink and surrounded a blue-black central core. Spherules thought to be degenerating stained less intensely.

Details of the sizes of the spherules detected in the adult and pouch young wombats are shown in Table 1.

The mean diameter of the spherules in the six adult wombat lungs ranged from 20.8 μm to 28.8 μm but in two of these animals populations of smaller spherules, with average diameters of 15.7 μm and 7.9 μm, were also detected. Spherules similar to, but generally smaller than those seen in adult wombats were detected in the lungs of 8 of 22 pouch young wombats. Three of these pouch young came from the Nullarbor Plain and 5 from the Blanche Town area. The earliest infection was detected in an animal approximately 50 days of age and as pouch young increased in size, so did the mean diameters of the spherules detected. No spherules were detected in the lungs of the rabbits, the feral cat or the fox.

Calculated spherule densities in adult wombat lungs (see Table 1) ranged from 55 × 10^3/cm^3 to 640.5 × 10^3/cm^3; however, even though the levels of infection were high the host pulmonary response was remarkably mild. Spherules were observed free in alveoli and within alveolar walls where they were often associated with an interstitial reaction involving mild fibroplasia and collections of leucocytes and macrophages. The most marked pulmonary reaction invoked by the spherules was the production of small focal granulomata and multinucleate giant cells, or local fibrosis and mononuclear cell infiltration about the spherules as shown in Figures 1 and 2. The giant cells were generally associated with the more degenerate looking spherules. No evidence of budding or intrapulmonary mycelial growth was observed.

Primary growth *in vitro* occurred only at room temperature (approximately 15-20 C) and 30 C and it was inhibited by cycloheximide but not by penicillin, streptomycin or chloramphenicol. Growth at room temperature was slow. Young colonies grown at 30 C were similar to those classed as white cottony[1] in appearance and they often had a

TABLE 1. Diameters (μm) of spherules in the lungs of pouch young hairy-nosed wombats *(Lasiorhinus latifrons)* and diameters (μm) and calculated density of infection of spherules in the lungs of adult *L. latifrons*.

Pouch Young Wombats			Adult Wombats			
Approx. age in days	Mean diam. ± SD	Range	Animal No.	Mean diam.* ± SD	Range	Spherules/ $cm^3 \times 10^3$
50	4.8 ± 1.9	1.8 - 7.2	1	22.0 ± 3.9	16.2 - 28.8	55.34
60	9.5†	9.0 - 9.9	2	21.6 ± 3.6	16.2 - 28.8	376.80
90	10.5 ± 1.2	9.0 - 13.0	3	20.9 ± 2.4	16.2 - 27.0	91.00
120	13.3 ± 3.8	7.2 - 18.0	4	21.5 ± 3.4	16.2 - 28.8	190.00
120	13.8†	11.2 - 16.4	5	20.6 ± 3.1	16.2 - 25.2	384.66
120	15.8†	11.2 - 19.8	6	28.8 ± 2.2	20.3 - 36.0	640.50
160	12.6 ± 4.2	1.8 - 21.6		Smaller forms		
160	11.4 ± 3.1	8.1 - 18.0	2	15.7 ± 1.9	12.6 - 16.2	
			6	7.9 ± 1.7	5.4 - 10.8	

*Mean of 20 measurements †Less than 5 spherules detected in slide

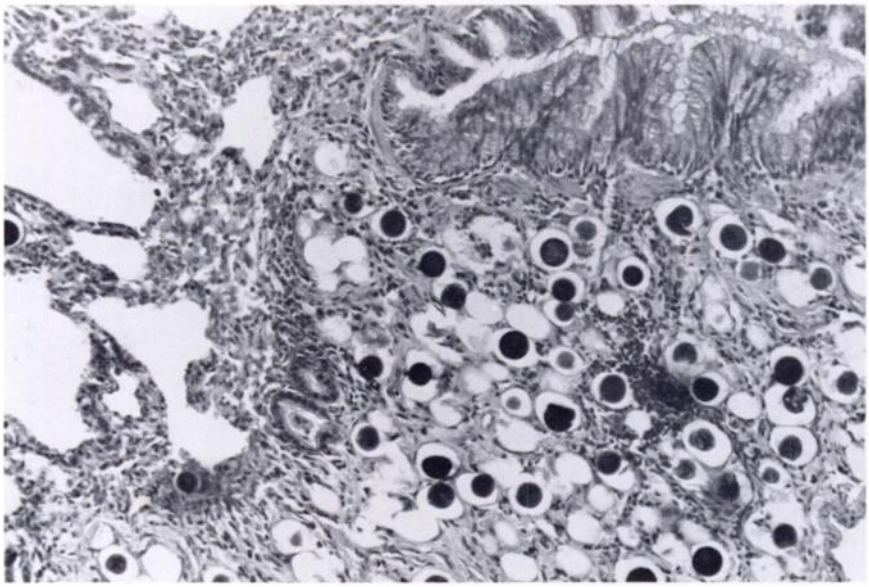

FIGURE 1. Focus of spherules associated with a mild fibrogranulomatous reaction in the lung of an adult *L.latifrons*. H & E 440×.

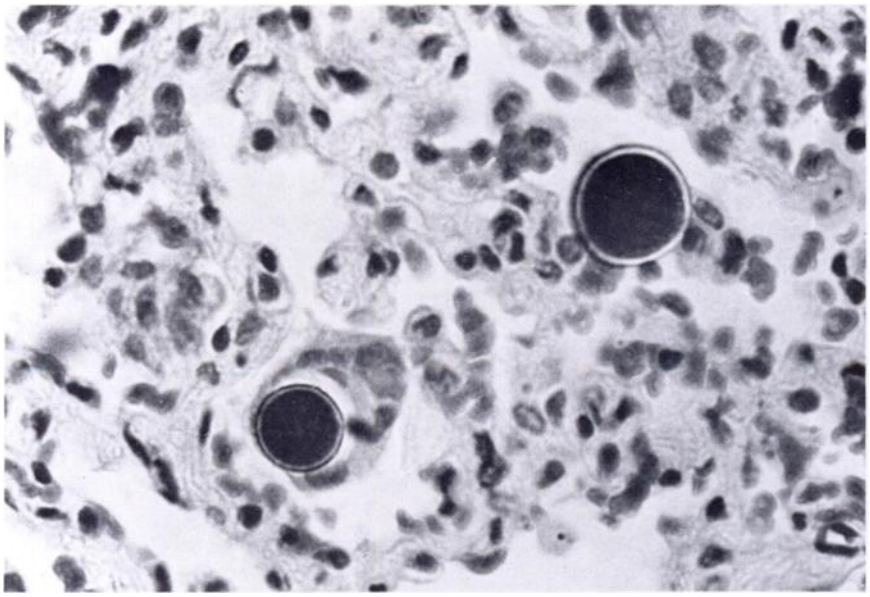

FIGURE 2. Higher power view of spherules showing the peripheral capsular zone and phagocytosis by multinucleate giant cell. H & E 1600×.

raised centre and a glabrous margin; however, on aging, the colonies became more granular due to folding, which was often very marked, and they generally changed to a light brown colour at this time. At 30 C the microscopic appearance of colony growth was that of a mass of septate mycelia producing numerous intercalary or terminal conidium-like bodies. In older degenerating cultures or in cultures exposed to temperatures of 37 C or 40 C chlamydospore-like spherules were produced, although most soon collapsed. Some of these spherules measured over 25 μm in diameter but they did not stain as intensely with H & E or PAS stains as did spherules in the lungs.

DISCUSSION

The pulmonary mycosis, adiaspiromycosis, is characterised by the intrapulmonary growth of spores to adiaspores (chlamydospores). These can reach diameters of 20 μm to 40 μm in one species, *Emmonsia parva,* or 200 μm to 700 μm in a second species, *E. crescens.*[6] However, as there are no discernable differences between the two types of fungi with regard to colony growth or the microscopic pathology they produce,[5] Carmichael[2] considers that the organisms be regarded as two varieties of the same species which he called *Chrysosporium parvum* var. *parvum (E. parva)* and *C. parvum* var. *crescens (E. crescens).*

The epidemiology of adiaspiromycosis is not known with certainty but the preponderant occurrence of this disease in the lungs of burrowing rodents indicates that *C. parvum* is a soil inhabitating fungus and that contaminated soil is the source of infection; indeed *C. parvum* var. *crescens* has been isolated from soil by Ciferri and Montermartini.[6] Sharapov[10] suggested that *C. parvum* var. *crescens* is part of the rhisospheric root-associated microflora of certain shrubby and herbaceous plants and the finding of Leighton and Wobeser[8] tends to support this. However, Krĭvanec and Otčenášek[7] propose that a prey-predator relationship may exist in which carnivors eat infected animals. This is thought to result in the passage of adiaspores through the digestive tract with their elimination in the faeces in which they subsequently grow. That this is in fact possible has been demonstrated in a weasel *(Mustela nivalis)* by Doby and Boisseau-Lebreuil[3] and in the domestic cat by Krĭvanec and Otčenášek.[7] In addition, Doby and Boisseau-Lebreuil[3] also have shown that adiaspores will germinate from the pellets of predatory birds. However, adiaspores of *C. parvum* will not germinate under anaerobic conditions and consequently the likelihood of spread of *C. parvum* from infected decomposing cadavers is considered to be low.[7]

The spherules seen in the lungs of pouched young wombats are typical of *Haplosporangium parvum* isolated from the lungs of desert rodents.[4] *H. parvum* was the original name for *C. parvum* var. *parvum.* In the adult wombats the spherules were also similar to *C. parvum* var. *parvum,* and, although a little larger than generally seen in natural infections[6] they were of similar size to *C. parvum* var. *parvum (E. parva)* detected in a fox *(V. vulpes).*[9] From histologic material supplied to them, both Carmichael (pers. comm.) and Krivanec (pers. comm.) consider the organisms in the wombat lungs to be similar to, if not identical with, *C. parvum* var. *parvum.*

The fact that the spherule sizes in pouched young wombats increased linearly with their ages and that some of the spherules in wombats of 120 days or more in age were in the same size range as the larger forms of adult spherules indicates that the smaller and larger forms are probably different growth stages of the same organism. In both the adult and pouched young wombats the lung pathology was mild and typical of that seen in pulmonary adiaspiromy-

cosis[6,13] while colony morphology at 30 C and spherule or chlamydospore production at 37 C and 40 C was also typical of *C. parvum* (Carmichael, pers. comm.). The reason that typical conidia did not develop in culture is not known, although factors such as suboptimal culture conditions for initial isolation or a virus infection of the organism may be responsible. It is also possible that the Australian isolate represents a new variety or species (Carmichael, pers. comm.).

In New Zealand adiaspiromycosis, caused by *C. parvum* var. *crescens*, is not uncommon in the brush possum *(Trichosurus vulpecula)*[11] and it may be relevant that in New Zealand many brush possums have adapted to sleeping at ground level[12] probably because of population pressures and limited suitable arborial locations (Hathaway, pers. comm.).

The fossorial habits and the fact that there are no common predators of the wombat suggests that the prey-predator cycle is not important in the epidemiology of the disease in South Australia. Rather, it would seem more likely that the infection is acquired by the inhalation of spores in the dust of soil in wombat burrows, although we also postulate that a direct cycle may exist in which spherules are coughed up, swallowed and pass out in the wombats own faeces.

That pouched young wombats are infected at such an early age indicates that actual physical contact with the source of infection is not necessary and this further supports the hypothesis that infection comes from spores in the dust of burrow soil.

This report therefore presents details of the detection and isolation of adiaspiromycosis and *C. parvum* in Australia. In addition, it would appear that, in the hairy-nosed wombat, infection is acquired from the inhalation of spores probably from the dust in wombat burrows. A prey-predator cycle is not considered to be involved.

A similar although smaller organism to the one described here has been observed in Tasmania in the common wombat *(Vombatus ursinus)*. This organism has a mean diameter of 9 ± 1.4 (S.D.) μm, with a range of 3 to 23 μm. Like the organism in *L. latifrons* it produces only mild lung pathology characterised by some localised increase in interstitial cellularity and also by localised intra-alveolar macrophage and multinucleate giant cell accumulation.

It is believed that this organism is also *C. parvum* although possibly a different variety to that in *L. latifrons* (Mason, unpubl.).

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