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## *Mycobacterium intracellulare* INFECTION IN A WATER MONITOR

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**Abstract:** Mycobacteriosis caused by *Mycobacterium intracellulare* serotypes Davis (8) and Altman (18) is described in a water monitor (*Varanus semiremex*). Infection with this organism has not been reported previously in reptiles in Australia.

### INTRODUCTION

Tuberculosis has been reported in many poikilothermic animal species, viz. snakes, turtles, lizards and crocodiles.<sup>14,16,24</sup> Validly described species of mycobacteria associated with naturally-occurring infections in lower vertebrates include *M. chelonae*,<sup>5</sup> *M. fortuitum*,<sup>11</sup> *M. thamnophaeos*,<sup>1</sup> *M. marinum*,<sup>7</sup> *M. xenopi*<sup>21</sup> and *M. avium*.<sup>21,22</sup> Mycobacteriosis in turtles, snakes, lizards and crocodiles has been characterized by a granulomatous inflammation with involvement of skin, kidney, liver, spleen and lung.<sup>2,14,16,25</sup> This report describes a miliary granulomatous mycobacterial infection caused by *M. intracellulare* serotypes Davis (8) and Altman (18) in a water monitor (*Varanus semiremex*).

### CASE HISTORY

A young male water monitor, a large Australian lizard, was acquired from Queensland in June, 1976, and housed with several other species of lizard in a large enclosure. It became ill shortly thereafter, refused food and began to show abnormal postural positions. It was presented live to the Veterinary Research Institute for examination and was observed to circle to the left and rest in a tightly wound position. The lizard was killed and examined at necropsy.

### NECROPSY FINDINGS

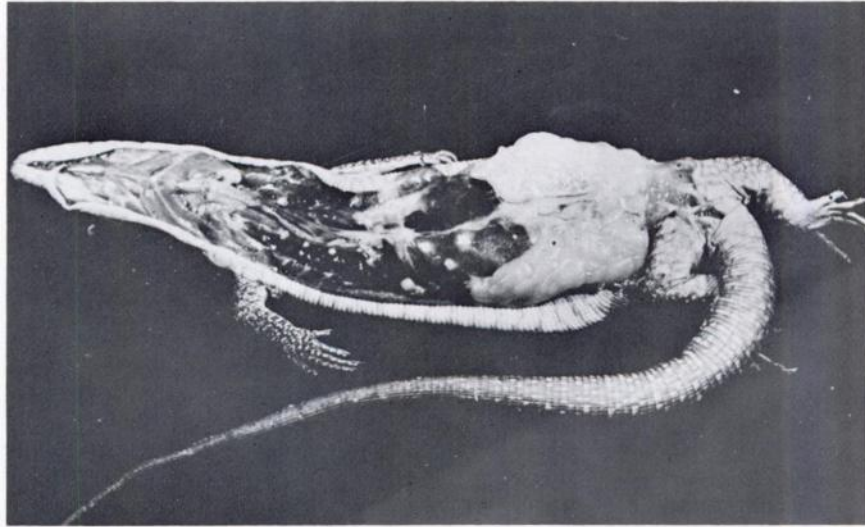
The lizard was thin and the bones were prominent. Hard yellowish-white nodules, 2 to 10 mm in diameter, with dry caseous centres were present throughout the mesentery, kidneys, liver, lung, under the fascia of the thigh muscles, retroperitoneally along the spine and beneath the periosteum of the sacrum (Fig. 1).

### MICROSCOPIC RESULTS

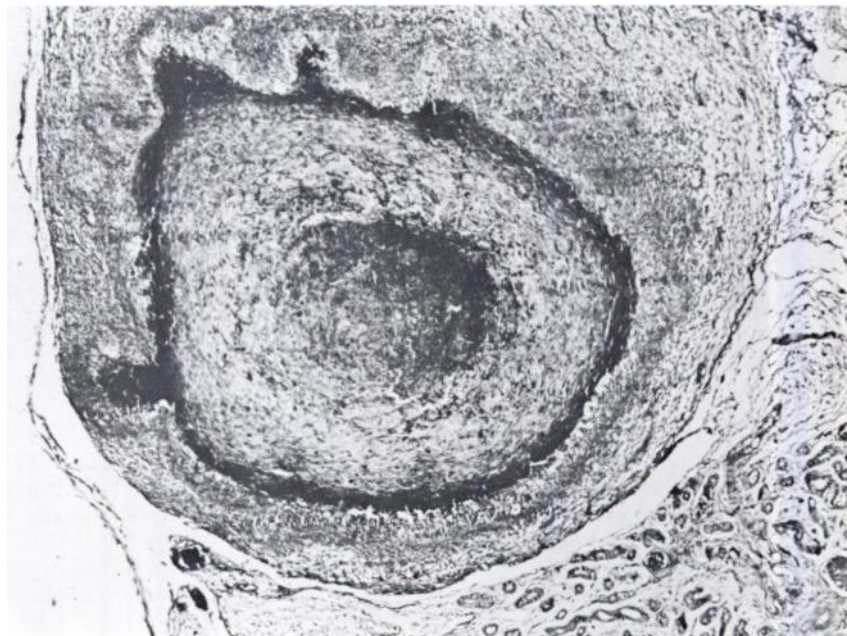
Tissues for histologic examination were fixed in 10% buffered formalin, sectioned at 6 µm and stained with haematoxylin and eosin and by the Ziehl-Neelsen (ZN) and Auramine Fluorescent<sup>13</sup> method for acid-fast bacteria.

Microscopically, the nodules consisted of intensely eosinophilic, amorphous, caseous necrotic debris enclosed by a mantle of epithelioid cells and heterophils (Fig. 2). Fibrin and granulation tissue often were associated with these areas, but there was no evidence of giant cell formation. Acid-fast organisms were not found in tissues or on impression smears with routine or modified ZN staining methods, but were present in sections stained by the fluorescent method.

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**FIGURE 1.** Multiple white nodules visible in lung, liver, kidney and mesentery of a water monitor with *M. intracellulare* infection.



**FIGURE 2.** Caseous necrotic nodule within the kidney enclosed by a mantle of epithelioid cells and heterophils (ZN  $\times 160$ ).

## MICROBIOLOGIC RESULTS

Portions of liver and samples of faeces were homogenized in phosphate-buffered saline (PBS) and then shaken in an equal volume of 4% sodium hydroxide for 15 min. The resultant suspension was neutralized with 10% hydrochloric acid and centrifuged at  $250 \times g$  for 5 min to remove debris. The supernatant was then centrifuged at  $1600 \times g$  for 30 min. The pellet was resuspended in a small quantity of PBS and streaked onto slopes of Lowenstein-Jensen, Stonebrink and modified Herold with mycobactin media.<sup>23</sup> Slopes were incubated at 22 C and 37 C for at least 12 weeks.

After 6 weeks, a pure growth of pale yellow pigmented colonies was observed on Lowenstein-Jensen slopes inoculated from the liver specimen and incubated at 22 C and 37 C. Later, growth occurred on other slopes. The isolate was acid-fast, negative in the Tween 80 hydrolysis, nitrate reduction and semiquantitative catalase tests<sup>26</sup> and positive in the tellurite reduction test.<sup>10</sup> Inoculation of  $1.4 \times 10^5$  organisms into the wing vein of a 6-week-old chicken produced lesions characteristic of avian tuberculosis.

A suspension was tested by Schaefer's seroagglutination test,<sup>19</sup> as modified by Reznikov and Leggo<sup>15</sup> and found to consist of a mixture of *Mycobacterium intracellulare* serotypes Davis (8) and Altman (18).

*M. intracellulare* serotype Davis was recovered from tissues of experimentally infected chickens.

## DISCUSSION

Reptiles debilitated by injury, malnutrition or other disease are susceptible to mycobacterial disease.<sup>14</sup> Isolation of mycobacteria alone does not necessarily indicate disease, as they are commonly found in aquaria, water in contact with animals, and water from lakes and streams.<sup>6</sup> Furthermore, frogs experimentally infected with *M. intracellulare* serotype Davis shed bacteria into the environment without showing signs of disease.<sup>8</sup> However, the granulomata found in the viscera, mesentery and bone of this monitor are consistent with those lesions described in reptiles with tuberculosis.<sup>2,3,7,12,14,16</sup> Involvement of fascia and musculature of the thigh, and the adjacent tissues and periosteum of the spine and sacrum may have resulted in the circling behavior shown by the lizard.

The distribution of *M. intracellulare* is widespread and it has been isolated from soil,<sup>27</sup> house-dusts<sup>4</sup> and water.<sup>6,18</sup> In Australia, infection by *M. intracellulare* (Battey disease) is a serious and important medical problem.<sup>17</sup> Both serotypes isolated have been implicated in human pulmonary and lymph node infections.<sup>20</sup> *M. intracellulare* serotype Davis is frequently found in water<sup>9</sup> and it seems very likely that the environment was the source of the causative organisms of this infection. Lizards may, therefore, act as a possible reservoir and a health hazard to humans, wildlife and domestic animals.

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