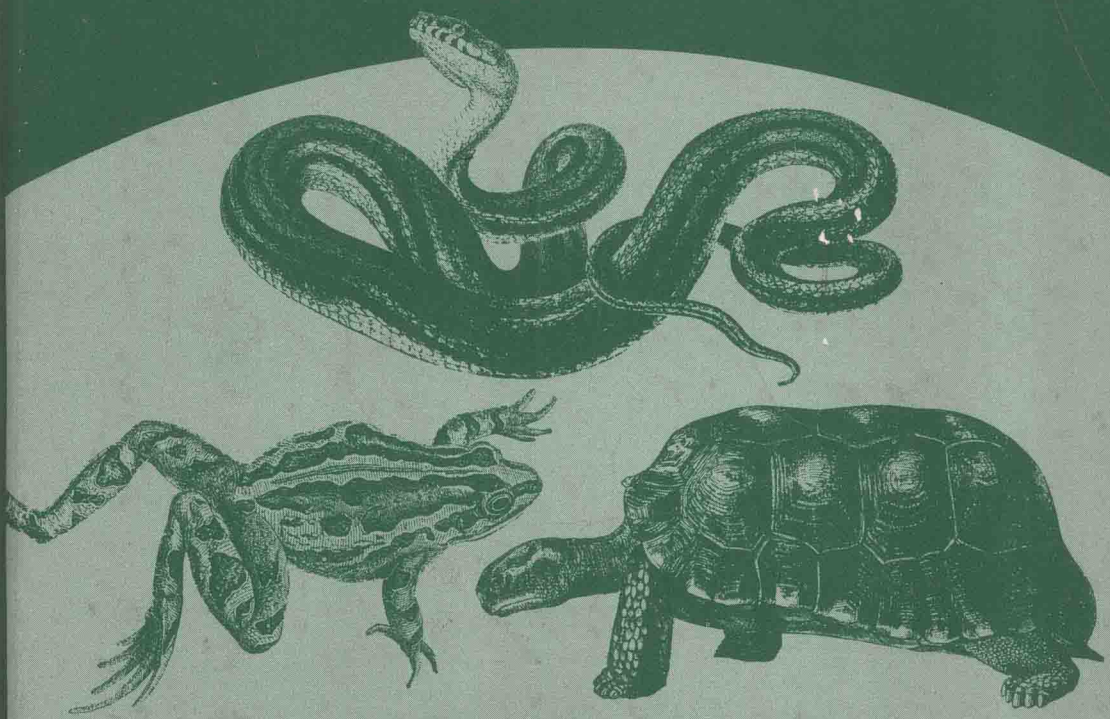
Diseases of Amphibians and Reptiles



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PREFACE

While diseases of free-ranging and captive mammalian and avian wildlife species have received considerable interest in the past 25 years, those of amphibians and reptiles (collectively, the herptiles) generally have been assigned lesser importance. The literature concerning disease in herptiles is widely scattered, consisting chiefly of case reports and prevalence surveys, and with heavy emphasis on captive reptiles. The dynamics of the host-agent-environment relationship have been studied for only a few diseases. This diverse data base is primarily a function of the paucity of investigators whose chief interest is in diseases of herptiles.

This first edition represents an effort to bring together some of the diffuse knowledge on infectious and non-infectious diseases of free-ranging and captive herptiles. Issue may be taken with the choice of topics; however, predominant diseases, as well as some diseases of lesser prominence, are presented. The editors were forced to accept certain omissions, particularly with amphibian diseases, simply for lack of contributors. The resulting text, however, we hope will be of value to veterinarians, herpetologists, wildlife disease investigators, wildlife managers, zoo curators, and university students.

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MYCOBACTERIOSIS

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INTRODUCTION

Mycobacteriosis of amphibians and reptiles is a group of infectious diseases caused by bacteria of the genus Mycobacterium. While insidious chronicity is the rule, peracute and acute cases occur; reflecting the heterogeneous biological behavior of the various mycobacterial species as well as the heterogeneity of host responses.

Mycobacteriosis is the oldest known infectious disease of amphibians and reptiles with a history dating back to the latter part of the nineteenth century. Seven years after Robert Koch isolated Mycobacterium tuberculosis in 1882, Sibley reported the first case of spontaneous mycobacteriosis in a poikilotherm (Sibley, 1889). The distinction went to a snake, Natrix natrix, necropsied at the London Zoological Society Gardens several months after its capture in Italy. It was not until 1905 that amphibian mycobacteriosis emerged with a report on hepatic mycobacteriosis in three frogs (Kuster, 1905). Until 1953, these sporadic reports were regarded as interesting novelties. However, with the recognition of human susceptibility to some of the mycobacteria isolated from poikilotherms and subsequent confirmation of their importance as human pathogens came a broader interest in mycobacteriosis of amphibians and reptiles (Marks and Schwabacher, 1965; Moore and Frerichs, 1953). Several recent reports have proposed herpeteofauna as reservoirs for mycobacteria of public health significance based on

experimental evidence (Kazda and Hoyte, 1972; Marcus et al., 1975,1976). However, there has been no subsequent confirmation that reptiles or amphibians are true reservoirs for those mycobacteria of public health concern.

Mycobacteriosis has been most frequently reported among exhibited reptiles and amphibians as well as laboratory maintained amphibians utilized for research purposes. This reflects more diligent observation of confined specimens rather than a lack of spontaneous mycobacteriosis among free-living populations. When attention has been directed towards these free-living animals, mycobacteriosis has been identified as an enzootic disease, at least in amphibians. Such enzootics may be important modulators of population densities through the culling of debilitated individuals since some form of host impairment appears to be important in predisposing individuals to mycobacteriosis. Incidences as high as 20% have been reported for mycobacteriosis among South American anurans (Machicao and LaPlaca, 1954). In well managed captive populations of amphibians and reptiles, incidences of 0.1 to 0.5% have been reported (Brownstein, 1978; Griffith, 1939).

ETIOLOGY

Mycobacteriosis is caused by a ubiquitous and versatile group of bacteria belonging to the order Actinomycetales, gram-positive aerobic or facultatively anaerobic bacilli that tend to form filaments in culture. The members of the family Mycobacteriaceae are distinguished from other families within the order by a cell wall rich in lipids, arabinose, and galactose. The cell wall is resistant to decolorization by acidified organic solvents once stained by basic dyes.

Mycobacteria are ubiquitous within the environment and may be divided into obligate vertebrate parasites, obligate or accidental vertebrate commensals, and saprophytes. The parasitic forms include the agents of the classical mycobacterioses; tuberculosis (human, avian, bovine and vole), leprosy (human, armadillo and murine), and bovine paratuberculosis. In recent years, attention has been directed towards the commensal and saprophytic mycobacteria with the recognition that they are occasionally capable of initiating disease among vertebrates. It is these commensal and saprophytic mycobacteria that account for the majority of cases of mycobacteriosis in amphibians and reptiles. These usually innocuous mycobacteria generally have optimum growth at temperatures

below those encountered within the bodies of higher vertebrates. It is, therefore, among the poikilotherms that they most frequently emerge as pathogens.

There are currently 26 mycobacterial species of accepted taxonomic status (Runyon et al., 1974b). As previously mentioned, seven of these species are primary pathogens of homeothermic vertebrates. The remaining 19 species include 6 species isolated from amphibians and reptiles with spontaneous mycobacteriosis. Several other mycobacteria are capable of inducing mycobacteriosis in amphibians and reptiles under experimental conditions, although their status as spontaneous pathogens is unknown. Mycobacterial pathogens of amphibians and reptiless are listed in Table 1.1.

Due to the low incidence of mycobacteriosis among captive populations of amphibians and reptiles, few investigations have encompassed sufficient cases to draw conclusions about the relative frequencey of mycobacterial isolates. The largest series of cases was reported by Griffith at the London Zoological Park (Griffith, 1939). Twenty of the 28 cases of mycobacteriosis of reptiles included in the report were caused by M. marinum with the remainder caused by M. chelonei and M. thamnopheos. However, among anurans, M. fortuitum (ranae, giae) has been most frequently reported (Darzins, 1952; Gonzales, 1938; Kuster, 1905).

The mycobacteria recovered from spontaneously diseased amphibians and reptiles represent 3 of the 4 Runyon groups used to characterize atypical mycobacteria (Runyon, 1970). The criteria used include growth rate and pigment production in culture. M. marinum, a group I slow growing photochromogen, is a ubiquitous inhabitant of fresh and salt water (Aronson, 1926; Linell and Norden, 1954). Epizootics among humans have been associated with aqueous environments from which M. marinum was isolated (Linell and Norden, 1954).

In contrast to M. marinum, M. xenopi, usually classified as a group III slow growing non-chromogen (actually a delayed scotochromogen), appears to have limited environmental distribution other than as a vertebrate commensal (Marks and Schwabacher, 1965; Runyon et al., 1974b). However, the presence of saprophytic sources is suggested by a report of M. xenopi isolated from water taps (Bullin et al., 1970). A second group III non-chromogen, M. avium, was recently isolated from a turtle and represents the first confirmed case of spontaneous mycobacteriosis

Table 1.1. *Mycobacterium* Species Reported to Produce Disease in Amphibians and Reptiles.

Mycobacterium Species	Category[a]	Reference
Amphibians		
M. fortuitum	1	Gonzales, 1938; Kuster, 1905
M. fortuitum	2	Darzins, 1952
M. fortuitum	3	Gonzales, 1938
M. marinum	1	Aronson, 1957; Shively et al., 1981
M. marinum	3	Clark and Shepard, 1963
M. thamnopheos	3	Aronson, 1929a
M. xenopi	1	Schwabacher, 1959
Reptiles		
M. avium	1	Thoen et al., 1977
M. avium	3	Griffith, 1941
M. chelonei	1	Friedmann, 1903; Griffith, 1939
M. fortuitum	3	Darzins, 1952; Gonzales, 1938
M. intracellulare	1	Friend and Russell, 1979
M. marinum	1	Griffith, 1919, 1939
M. marinum	3	Clark and Shepard, 1963
M. thamnopheos	1	Aronson, 1929a,b
M. thamnopheos	3	Aronson, 1929a
M. tuberculosis	3	Bertarelli, 1905; Sibley, 1892
M. ulcerans	3	Marcus et al., 1975; 1976

[a]1=spontaneous disease among captive animals;
2=spontaneous disease among free-living animals;
3=experimentally induced disease

in a poikilotherm caused by the avian organism (Thoen et al., 1977). Ophidian susceptibility to experimental *M. avium* infection has been known since 1941 (Griffith, 1941). Early investigators had implicated avian sources for spontaneous mycobacteriosis among exhibited snakes, but cultural confirmation was lacking (Aronson, 1929b; Gibbes and Shurley, 1890).

M. fortuitum, M. chelonei (abscessus) and M. thamnopheos are rapid growers of Runyon group IV. Both M. fortuitum and M. chelonei have wide environmental distribution, being found in soil, dust and water and as vertebrate commensals and opportunistic pathogens (Darzins, 1952; Runyon et al., 1974; Smith, 1972). M. thamnopheos has only been recovered from reptiles with mycobacteriosis (Aronson, 1929a,b; Griffith, 1939). The status of M. thamnopheos as a legitimate member of the genus has been questioned on the basis of the small carbon skeleton of the cell wall mycolic acids which more closely resemble those of Nocardia (Lechevalier and Gerber, 1971). Indeed, M. thamnopheos is the least acid-fast of all the mycobacteria.

Several mycobacteria isolated from spontaneous cases in reptiles have not been given species status in the eighth edition of Bergey's Manual of Determinative Bacteriology. M. schlangen, a non-chromogen, caused disseminated mycobacteriosis in several snakes at the Berlin Aquarium (Rabinowitsch-Kempner, 1938). M. tropidonotus was recovered from several cases of oophidian mycobacteriosis at the London Zoological Park (Griffith, 1970).

PATHOGENESIS

The ubiquity of saprophytic and commensal mycobacteria and the generally low incidence of mycobacteriosis in amphibians and reptiles attests to the high degree of innate resistance possessed by these two classes of vertebrates. This is in contrast to the classical mycobacteriosis of higher vertebrates where pathological changes are the rule when host and bacterium interact. Disease production in amphibians and reptiles not only requires contact with these potential pathogens but also some form of host defense impairment to augment the infectious process (Reichenbach-Klinke and Elkan, 1965; Schwabacher, 1959).

Host impairment may include acid-fast bacillary access to tissues through breaches in surface epithelium and failure of specific and non-specific defense mechanisms to contain acid-fast bacilli in tissues. Disruption of epithelial barriers such as skin or respiratory or alimentary mucosa by physical, chemical, microbiological or nutritional injury may create a conduit for mycobacterial invasion. Cutaneous trauma is believed to be important in certain cases of atypical mycobacteriosis of man (Linell and Norden, 1954).

There is some evidence that surface epithelium may not be an effective mycobacterial barrier in normal hosts. In an experiment involving intragastric inoculation of Anolis carolinensis with M. ulcerans, 3 of 17 lizards became chronic hepatic carriers. Two of these lizards had identifiable acid-fast bacilli within the wall of intrahepatic bile ducts unaccompanied by pathological alterations. The authors concluded that ascending biliary infection accounted for the presence of acid-fast bacilli in the liver since M. ulcerans reaching the liver embolically produces a granulomatous response (Marcus et al., 1976). It therefore appears that, in certain apparently healthy individuals, some degree of host invasion does occur and that latent mycobacterial infections of the liver may not be uncommon. Alimentary (biliary) epithelium is not a barrier and bacterial progression is inhibited by other host factors. Other investigators have found a high incidence of chronic hepatic carriers among frogs, Rana temporarin, in an environment contaminated with M. intracellulare serotype Davis (Kazda and Hoyte, 1972).

Arguing against the activation of latent hepatic infections in the pathogenesis of mycobacteriosis are two observations: 1) acid-fast bacilli in the liver of cases of hepatic mycobacteriosis are not demonstrable outside granulomas, including intrahepatic bile ducts, and 2) a diligent search of the gastrointestinal tract in cases of hepatic mycobacteriosis will usually confirm the presence of micro- or macrotubercles, supporting hepatic portal embolization rather than ascending biliary infection as the source of acid-fast bacilli in the liver (Fig. 1.1). Such tubercles characteristically occur within the lamina propria of the small intestine beneath intact epithelium. Whether this represents invasion of intact epithelium or regenerated, previously disrupted epithelium is not known.

The skin and respiratory tract may also serve as portals of mycobacterial entry in amphibians and reptiles. Both of these portals have been implicated in chelonian mycobacteriosis (Friedmann, 1903; Rhodin and Anver, 1977). Cutaneous mycobacteriosis has been reported in Xenopus laevis infected with M. xenopi (Schwabacher, 1959).

There is no evidence that species susceptibility to mycobacteriosis exists among amphibians and reptiles although individual susceptibility certainly does occur. Current information based on experimentally produced mycobacteriosis suggests a broad host range for those mycobacteria isolated from spontaneously diseased amphibians and reptiles.

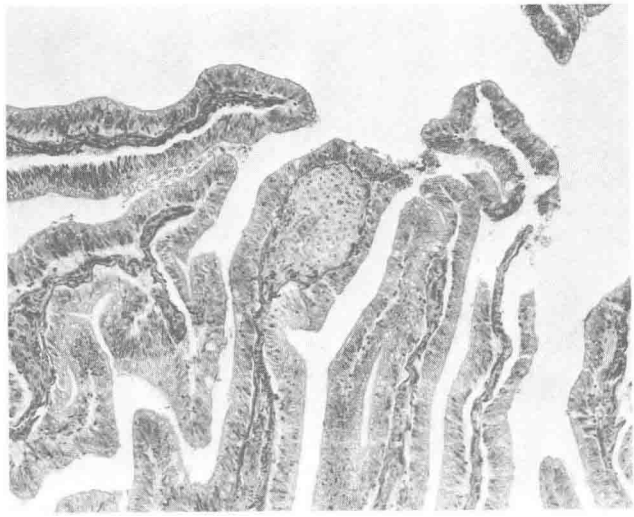

Fig. 1.1. Microtubercle in the lamina propria of a small intestinal villus from a snake with hepatic mycobacteriosis. H&E X100

TRANSMISSION

As a complex of diseases, poikilothermic mycobacteriosis exhibits a variety of transmission patterns. Contagion has not been a feature of reported cases in amphibians and reptiles, although it appears likely in certain epizootics among fish (Baker and Hagan, 1942; Besse, 1949; Winsor, 1946). More likely modes of transmission among amphibians and reptiles are contact with environmental sources. Infected insects have been implicated in alimentary mycobacteriosis of anurans (Machicao and LaPlaca, 1954). The importance of dietary sources of poikilothermic mycobacteriosis has been confirmed in salmon under intensive aquaculture (Wood and Ordal, 1958). When young fry and fingerlings are fed uncooked carcasses of adult salmon the incidence of mycobacteriosis approaches 100%.

The simultaneous isolation of mycobacteria from aquatic environments inhabited by amphibians and reptiles with spontaneous mycobacteriosis has been reported on several occasions (Darzins, 1952; Reichenbach-Klinke and Elkan, 1965; Schwabacher, 1959). The majority of cases of mycobacteriosis probably originate from contaminated water.

SIGNS AND PATHOLOGY

Mycobacteriosis of amphibians and reptiles may present as peracute, acute or chronic diseases. Peracute mycobacteriosis is strictly an experimental disease caused by parenterally administered rapidly growing mycobacteria of Runyon group IV (Aronson, 1929; Clark and Shepard, 1963; Gonzales, 1938). The disease is characterized by high acute mortality associated with massive intravascular proliferation of acid-fast bacilli and minimal host inflammatory response.

Acute mycobacteriosis is an uncommon disease in which bacterial proliferation is largely extracellular. The resulting inflammatory response is suppurative and therefore mimics both clinically and pathologically infections by more frequently encountered bacteria. This type of reaction is usually encountered in primary pulmonary infections of reptiles and nasopharyngeal infections of anurans (Machicao and LaPlaca, 1954). Reptiles with acute respiratory mycobacteriosis present with anorexia, depression, dyspnea and occasional rales. Gross necropsy findings include diffuse, edematous thickening of respiratory membranes and irregular, raised cream to yellow flocculent accumulations of exudate within the air sacs. Microscopically, the exudate is composed of heterophils infiltrating regions of caseous necrosis. Macrophages and fibroplasia are minimal. Within the inflammatory exudate are large numbers of extracellular acid-fast bacilli, measuring 1 to 10 microns, best demonstrated by Fite-Faraco method of acid-fast staining (Luna, 1968). Because this form of mycobacteriosis presents clinically and morphologically as an acute disease, the diagnosis is likely to be missed unless acid-fast stains are routinely performed on tissues with acute inflammatory reactions, especially lungs.

A variant of this acute type of inflammatory response was encountered by the author in the oviduct of a coachwhip snake, Masticophis flagellum, with chronic disseminated mycobacteriosis. There was an acute, diffuse necrotizing inflammation of the oviduct characterized by widespread inflammatory destruction of arteries and veins (Fig. 1.2). The vascular walls were edematous, indistinct and infiltrated with heterophils. Unlike the predominantly intracellular acid-fast bacilli that characterized the infection in other organs, those within the oviduct were extracellular. The association of acid-fast bacilli with necrotizing inflammation of arteries and veins has been described in erythema nodosum leprosum in leprosy patients

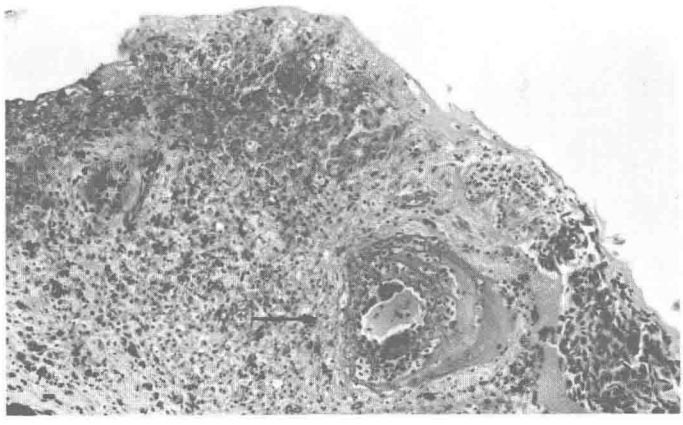

Fig. 1.2. Oviduct from a snake, Masticophis flagellum, with disseminated mycobacteriosis. Unlike the more typical granulomatous response, this acute response is characterized by vasculitis (arrow) and widespread tissue necrosis accompanied by massive extracellular proliferation of acid-fast bacilli. H&E X100

treated with sulfones (Wemambu et al., 1969). This side effect of leprosy therapy is believed to represent an Arthus reaction to mycobacterial antigens.

Anurans with acute nasopharyngeal mycobacteriosis usually have a cararrhal or suppurative nasal discharge with large numbers of extracellular acid-fast bacilli (Machicao and LaPlaca, 1954).

Chronic mycobacteriosis is the most frequent form of mycobacteriosis in amphibians and reptiles. The hallmark of this form of infection is granulamatous inflammation with tubercle formation. Like mammalian and avian tubercles, the tubercles of amphibians and reptiles undergo sequential changes as the tubercles age. However, there are a number of differences between tubercles of poikilotherms and those of mammals and birds.

The earliest event in spontaneous amphibian and reptilian tubercle formation is the accumulation of a compact nest of macrophages in various states of activation (Fig. 1.3 upper left). The macrophages are plump, polyhedral with distinct plasma membranes and abundant pink, often foamy cytoplasm. The vesicular nuclei are oval or indented and nucleoli are indistinct. Acid-fast