PATHOLOGIC CHANGES IN LIZARDS (Agama agama) EXPERIMENTALLY INFECTED WITH Dermatophilus congolensis

Authors: Chineme, C. N., and ADDO, P. B.

Source: Journal of Wildlife Diseases, 16(3) : 407-412

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-16.3.407

PATHOLOGIC CHANGES IN LIZARDS *(Agama agama)* EXPERIMENTALLY INFECTED WITH *Dermatophilus congolensis*

C. N. CHINEME and P. B. ADDO, Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Abstract: Captive laboratory-held lizards *(Agama agama)* experimentally inoculated with *Dermatophilus congolensis* by subcutaneous, intramuscular and intraperitoneal routes developed pyogranulomatous and necrotic lesions at and around the sites of inoculation. *D. congolensis* was consistently cultured from the lesions even at 75 days post inoculation. Histopathologic examination of selected organs and tissues showed granulomatous caseous abscesses in the dermis, subcutaneous tissue and liver, edema of the dermis and widespread muscular degeneration and necrosis. *D. congolensis* organisms were associated with these lesions. No lesions or organisms were seen in the epidermis of the skin.

INTRODUCTION

The lesions associated with *Dermatophilus congolensis* infection in domestic mammals have been widely and exhaustively described. [1,3,4,5,8,10] However, few reports of such lesions have been described in the lower vertebrates. The first report of isolation of *D. congolensis* from a lizard *(Amphibolurus barbatus)* and the results of transmission tests with lizards and a sheep appeared as recently as 1972.[11] Montali *et al.*[7] diagnosed dermatophilosis in three Australian bearded lizards *(Amphibolurus barbatus)* and described the gross and histopathologic lesions in the skin of the naturally infected animals. Anver *et al.*[2] isolated *D. congolensis* from cutaneous hyperkeratotic nodules of two marble lizards *(Calotes mystaceus)* and transmitted the isolate experimentally to other marble lizards and to mice by subcutaneous inoculation and by topical application after skin scarification.

The present report is concerned with description of pathologic changes that occurred in lizards *(Agama agama)* experimentally inoculated with bovine-derived *D. congolensis*.

MATERIALS AND METHODS

Ten lizards *(Agama agama)* (5 male and 5 female) were used for the study. Eight of these (4 male and 4 female) were inoculated with *D. congolensis* while two control animals (1 male and 1 female) were inoculated with sterile uninfected brain heart infusion (BHI) broth. Lizards were numbered on the ventral thoracic areas using Sanford's permanent waterproof, smearproof marker. Animals were fed insects, ground rabbit pellets and peanuts and given water *ad libitum* for the duration of the experiment. They were preconditioned for 10 days in cages before being inoculated.

The inoculum used was a first passage serum broth culture of bovine-derived *D. congolensis* incubated for 3 days at 37 C. Smears of the culture were prepared and stained with methylene blue and by Gram's method and determined to be a pure culture of *D. congolensis* before being used for inoculation. Three lizards each were inoculated with *D. congolensis* by the subcutaneous and intramuscular routes and 2 by the intraperitoneal route. Control animals received subcutaneous and intramuscular injections. Details of procedures and postmortem findings are shown in Table 1.

Sites for inoculation were first carefully cleaned with methanol and allowed to dry before the inoculum was given.

The lizards were observed and examined twice daily for appearance of gross lesions until they either died or were euthanized. Post-mortem examinations were carried out on all lizards and any gross lesions recorded. Smears and swabs were taken of the lesions and tissue samples were obtained for bacteriologic and histopathologic examinations. Swabs and tissue specimens taken from the skin, subcutaneous tissue, muscles and peritoneum were cultured on sheep blood agar. Specimens for histopathologic examinations were fixed in buffered neutral 10% formalin, embedded in paraffin, sectioned at 5μm and stained with hematoxylin and eosin. Selected tissue sections were stained with Brown and Brenn Gram, and Von Kossa methods.

RESULTS

Bacteriology

Direct smears from lesions stained with methylene blue or Gram's showed

TABLE 1. Inoculation routes and postmortem lesions in lizards experimentally exposed to *Dermatophilus congolensis*.

Lizard No.	Inoculation Procedure	Time of Death or Euthanasia (Days)	Postmortem Findings
1.	0.5ml Subcutaneous Left thigh	75+	Caseous abscess at inoculation site & muscle necrosis.
2.	1ml Subcutaneous Left back	35*	Multiple caseous abscesses at inoculation site & muscle necrosis.
3.	0.5ml Subcutaneous	45*	Multiple caseous abscesses extended from subcutis to underlying muscles.
4.	0.5ml Intramuscular Right thigh	27*	Caseous abscesses and necrosis in muscle.
5.	1ml Intramuscular Left back	35*	Multiple caseous abscesses and necrosis in muscle.
6.	0.5ml Intramuscular Left thigh	45+	Caseous abscess at inoculation site & muscle necrosis.
7.	0.5ml Intraperitoneal	4+	Multiple caseous abscesses on the peritoneum.
8.	1ml Intraperitoneal	21*	Multiple caseous abscesses on the peritoneum, stomach serosa and in the liver.
9.‡	0.5ml Subcutaneous Left back	21*	No lesions
10.‡	1ml Intramuscular Right thigh	45*	No lesions

+ Animals that died naturally.
* Animals that were euthanized.
‡ Controls

the diagnostic beaded branching filamentous rods and coccoid forms which divide both longitudinally and transversly. Direct culturing from the cutaneous, muscular and hepatic lesions resulted in the appearance of generally bright orange with occasional white to cream colonies of *D. congolensis* within 48 h. The colonies were dry, appeared pitted, and showed beta-hemolysis. No pathogenic microorganisms were cultured from similar tissues taken from control lizards.

Gross Pathology

All lizards inoculated with *D. congolensis* were mildly dehydrated and five showed serous atrophy of body fat. Approximately 8 days following subcutaneous and intramuscular inoculation with *D. congolensis,* raised nodules of various sizes appeared on the skin. Thirty-five days post-inoculation, the average measurement of the skin nodular lesions ranged from 2×1.5 cm to 3×2 cm. The intradermal and subcutaneous lesions consisted of several, small, firm, spherical, whitish to light-yellowish granulomatous caseated abscesses each measuring approximately 0.2 cm in diameter and rather firmly adhered to the adjacent tissues. The abscesses were mostly concentrated at and around the inoculation sites. The deep subcutaneous and muscular tissues were abnormally pale in color. The two lizards inoculated intraperitoneally had several granulomatous caseous abscesses attached to the serosal surfaces of the liver, stomach and the peritoneal wall, with the last structure appearing markedly hyperemic. Lesions in one of the subcutaneously inoculated lizards persisted up to 75 days post-inoculation.

Histopathology

The lizards inoculated subcutaneously with the bacteria showed no observable lesions in the epidermis, neither was any bacterial organism identified in this layer of the skin. However, the various layers of the dermis, subcutis and muscular layer were severely affected. Sections through the lesion showed loss of nuclear detail in the stratum compactum of the dermis, fibrinous edema of the stratum spongiosum and subcutis, edema and necrosis of the hypodermal muscle. Numerous *D. congolensis* were seen, especially in the sections stained by the Brown and Brenn Gram method. Deep in the muscle, there were degeneration and necrosis characterized by muscular clumping and fragmentation, loss of cross striations (Fig. 1) and in some areas proliferation of muscle nuclei. Von Kossa-stained sections of muscle demonstrated that necrotic muscle bundles had undergone dystrophic calcification. *D. congolensis* were abundant in the intermuscular connective tissue (Fig. 2). However, bacteria were not seen within muscle fibers.

The raised skin nodules seen grossly were due to presence of intradermal and subcutaneous caseous abscesses. The abscesses which were also seen in the liver and peritoneum showed similar histologic morphology. These consisted of a central zone of eosinophilic caseous necrotic material which was surrounded by a granulomatous layer consisting of large cells with foamy cytoplasm and round hyperchromatic nucleus. The epithelioid granulomas contained also numerous *D. congolensis* in their characteristic form and were encapsulated by a poorly developed fibrous tissue.

DISCUSSION

Even though the major lesions seen in the lizards were in the sub-epidermal tissues, and identical with those reported for *D. congolensis* in naturally- and experimentally-infected lizards,[11] conspicuously absent in the present study were foci of epithelial hyperplasia, branching filaments of *D. congolensis* within hyperkeratotic caps or the outer

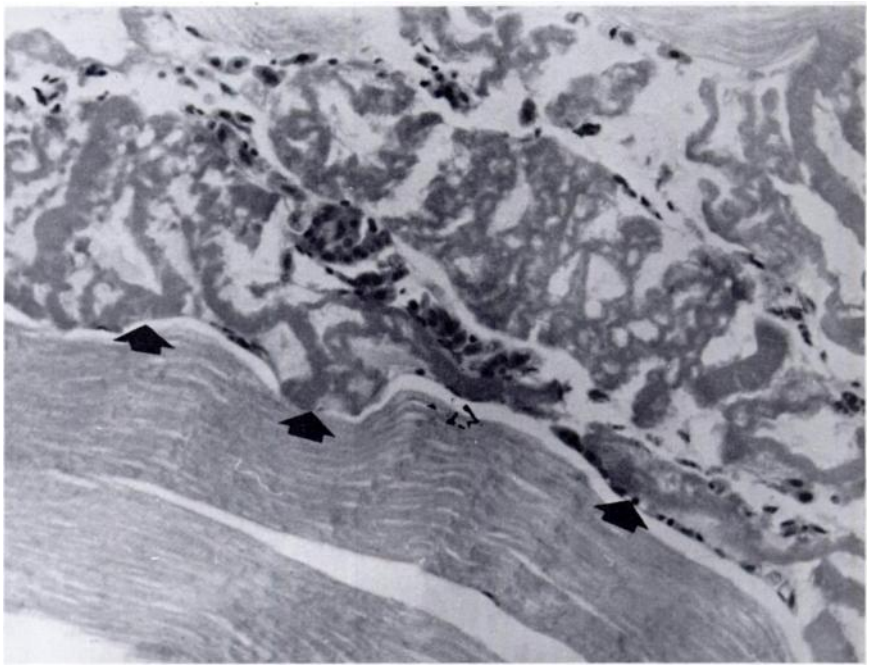

FIGURE 1. Histologic section of sub-dermal muscle from a lizard inoculated intramuscularly with *D. congolensis* demonstrating marked necrosis (arrow heads). H&E stain × 200.

epidermal layer of the skin lesions described in naturally-infected lizards.[2,7]

From the nature of the lesions, it would appear that inoculation with *D. congolensis* was followed by their early multiplication and dissemination within and around the inoculation sites. It is pertinent to observe here the major difference in the sites of lesion formation and presence of the etiologic agent between the bovine patients and our experimental animals. In mammals, particularly the bovine, the most striking microscopic feature was the superficial nature of the lesions which were usually restricted to the epidermis[4] whereas in the lizards, the most predominant lesions were subepidermal, mainly in the subcutis and muscles. In mammals inoculated subcutaneously, the most predominant lesions are epidermal and consist of laminated scabs overlying an acanthotic epidermis, intra-epidermal microabscesses and presence of *D. congolensis* in the cornified epithelium.[8] The microorganisms appeared to invade only the living epidermis and it has been suggested that inhibitory substances liberated by neutrophils may form a mechanical barrier to deeper invasion of *D. congolensis*.[3] Why such does not obtain in the lizard where the etiologic agent was not seen in the epidermis but only subepidermally is not readily known.

It has been stressed that if dermatophilosis is endemic in native fauna such as lizards, then there is a possibility that domestic animals could be infected from this source.[11] Observation from the present study shows that *D. congolensis* of bovine origin could survive and mul-

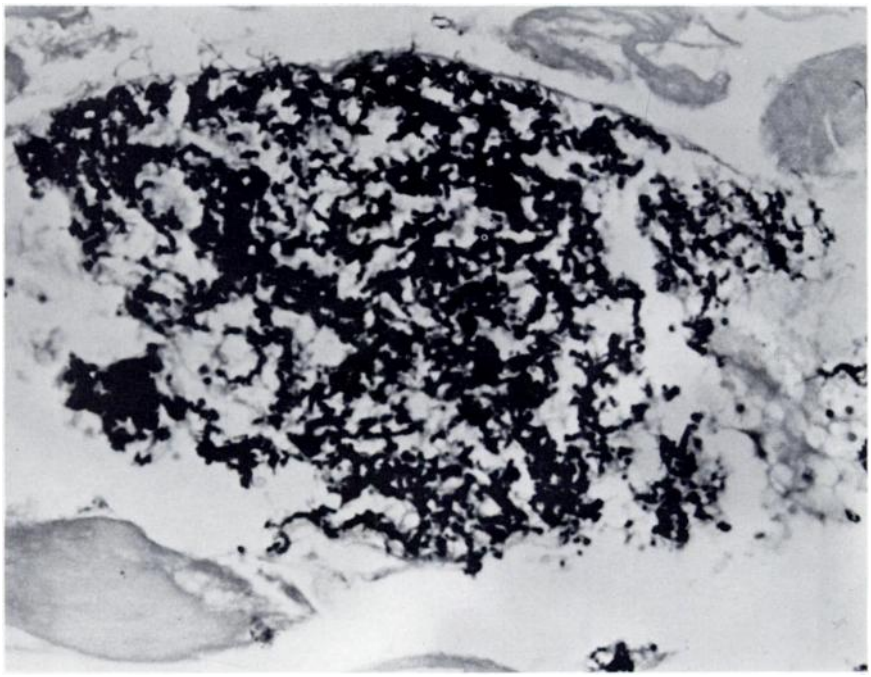

FIGURE 2. Focus of filamentous *D. congolensis* accumulation in intermuscular tissue of a lizard inoculated intramuscularly. Note adjacent degenerative muscles. Gram stain × 300.

tiply in the dermis, subcutis and muscles of lizards for up to 75 days. It is pertinent to speculate on the role of such reptiles which are ubiquitous in this part of the world in the epizootiology of dermatophilosis of domestic animals. Mechanical transmission of *D. congolensis* by insects has been experimentally demonstrated in rabbits[9] and insects feeding on lizards may serve as vectors for infection in mammals. *D. congolensis* has been isolated from ticks[6] and the occurrence of ticks on lizards also suggests a possible method of the bacterial transmission from lizards to domesticated animals. A major potential hazard of existence of dermatophilosis in such free-living widely-distributed animals as lizards would be the difficulty this would pose in its eradication from domestic food animals particularly cattle in which the disease is of immense economic importance in Nigeria. However, the fact that so few reports are available on natural infection in the wild suggests that reptiles may not be of much importance in the epizootiology of the disease in domestic livestock.

LITERATURE CITED

1. AMAKIRI, S.F. 1976. Anatomical location of *Dermatophilus congolensis* in bovine cutaneous streptothricosis. In: *Dermatophilus Infection in Animals and Man*. D.H. Lloyd and K.C. Sellers (Eds.) Academic Press, London, pp. 163-171.

2. ANVER, M.R., J.S. PARK and H.G. RUSH. 1976. Dermatophilosis in the marble lizard *(Calotes mystaceus)*. Lab. anim. Sci. 26: 817-823.
3. BIDA, S.A. and S.M. DENNIS. 1977. Sequential pathological changes in natural and experimental dermatophilosis in Bunaji cattle. Res. Vet. Sci. 22: 18-22.
4. BLANCOU, J.M. 1976. The treatment of infection by *Dermatophilus congolensis* with particular reference to the disease in cattle. In: *Dermatophilus Infection in Animals and Man.* D.H. Lloyd and K.C. Sellers (Eds.). Academic Press, London, pp. 246-259.
5. BRIDGES, C.H. and W.M. ROMANE. 1961. Cutaneous streptothricosis in cattle. J. Am. vet. med. Ass. 138: 153-157.
6. KUSEL'TAN, I.V. 1967. Abstr. Vet. Bull. (1968) 38: 590.
7. MONTALI, R.J., E.E. SMITH, M. DAVENPORT and M. BUSH. 1975. Dermatophilosis in Australian bearded lizards. J. Am. vet. med. Ass. 167: 553-555.
8. ODUYE, O.O. 1976. Histopathological changes in natural and experimental *Dermatophilus congolensis* infection in the bovine skin. In: *Dermatophilus Infection in Animals and Man.* D.H. Lloyd and K.C. Sellers (Eds.). Academic Press, London, pp. 172-181.
9. RICHARD, J.L. and A.C. PIER, 1966. Transmission of *Dermatophilus congolensis* by *Stomoxys calcitrans* and *Musca domestica*. Am. J. Vet. Res. 27: 419-423.
10. ROBERTS, D.S. 1965. The histopathology of epidermal infection with the Actinomycete *Dermatophilus congolensis*. J. Pathol. Bact. 90: 213-216.
11. SIMMONS, G.C., N.D. SULLIVAN and P.E. GREEN. 1972. Dermatophilosis in a lizard *(Amphibolurus barbatus)*. Aust. Vet. J. 48: 465-466.

Received for publication 6 November 1979