

AMOEBIASIS IN THE KOMODO
DRAGON

Varanus komodoensis

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AMOEBIASIS is an extremely severe disease entity in snakes and lizards. It often takes the form of an acute epizootic in reptile collections with high morbidity and mortality. The amoebae accompanied by bacteria invade the gut wall causing extensive caseous necrosis especially in the posterior segment of the intestine. The amoebae often invade the blood stream, particularly the portal circulation, and cause secondary necrotic lesions in the liver and other internal organs.

The most commonly recognised pathogenic amoebae of reptiles is *Entamoeba invadens* (Geiman and Ratcliffe, 1936, 1938) and is encountered in many species of the class Reptilia.

The following report of amoebiasis in Komodo dragons, *V. komodoensis*, at the National Zoological Park is presented to call further attention of zoo personnel to this serious problem in reptiles and to describe anatomic and pathologic features in this species.

On 4 March, 1964 the National Zoological Park received as a gift from the Government of Indonesia two *Varanus komodoensis*. The male was 9 ft long and weighed 200 lb, while the female was 6 ft long and weighed 125 lb. On arrival, the lizards were put in the Reptile House in a glass-fronted enclosure equipped with sun lamps, a warm-water pool and spot radiant heating. After a 10-day adjustment period, they were fed at 3- to 6-day intervals. The male consumed an average of 6 lb and the female 3 to 4 lb of meat per feeding. After two-and-a-half months (14 May) the

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male Komodo dragon stopped feeding and, on 1 June, suddenly showed evidence of abdominal pain by frequent colonic spasms. This condition was treated by the administration of 1/150 grain Atropine sulphate intramuscularly. Immediate relief was evident; however the dragon was found dead the following morning, 2 June 1964. Detailed gross and microscopic reports follow.

Routine stool examinations, including amoeba cultures, had been instituted on the Komodos one week before the male's death. Examination of smears, stained slides, and cultures showed the presence of amoeboid-like trophozoites. Reports of cultures were received on 1 June 1964, indicating two types of amoebae in culture, both of which could be grown *in vitro* at 25°C and 35°C. Exact species determination has not been made, but they apparently are different from I.P. and P.Z. strains of *Entamoeba invadens*, previously described (Meerovitch, 1958).

Treatment of the female Komodo dragon was complicated by a lack of knowledge as to the effectiveness of amoebicides in the lizard and the problem of administering the drug in her food, since she ate only once or twice a week.

Treatment of the female began immediately following the death of the male, using the adult dose of the human amoebicide Diodoquin® (diiodohydroxyquin, Searle), 650 mg in 150 cc of 0.9 per cent saline given daily in the form of a retention enema for 14 days. The lizard was netted and restrained by three keepers. The enema was administered by gravity and natural retention was observed lasting from 45 minutes to one hour.

Since reptilian amoebiasis commonly spreads from the gut to involve the liver and other internal organs and the Diodoquin® therapy is effective only against the enteric form, the senior author decided to treat the female dragon with Emetine hydrochloride USP (Lilly), a systemic amoebicide used in the human. To test the safety of this drug, the human dose of 65 mg was injected intramuscularly into the tail of a 40-lb *Varanus salvator* whose stool contained amoebae and two flagellates, tentatively identified as *Trichomonas* sp. The treatment was given once a day for seven days and no side-effects were

noticed. Ten days following this treatment, examination of the stool of the *V. salvator* was negative for amoeba, but remained positive for the flagellates.

After testing the safety of Emetine hydrochloride and the completion of the Diodoquin® enema series in the *V. komodoensis*, 65 mg of Emetine hydrochloride was injected intramuscularly into the tail muscles of the female dragon once daily for seven days.

During the course of treatment (14 daily enemas, 7 daily injections), it was possible to demonstrate amoebae and protozoa by stained slides and cultures. Fourteen days following the termination of treatment, the lizard was negative for amoebae and has remained so up to the present time. The lizard is very active at present and consumes about 4 lb of horse-meat twice a week.

The gross and microscopic post mortem examination conducted on the male Komodo dragon at the Armed Forces Institute of Pathology, Washington, DC, follows:

CLINICAL DIAGNOSES: Amoebiasis.

NECROPSY DIAGNOSES

1. Acute haemorrhagic proctitis, colitis and enteritis, severe.
2. Acute focal disseminated ulcerative gastritis and enteritis.
3. Liquefactive necrosis of liver, severe.
4. Cestodiasis, small intestine.

HISTOPATHOLOGIC DIAGNOSES

1. Amoebiasis, rectum, colon, stomach, small intestine and liver, severe.
2. Amoebic abscess, pelvic cavity.
3. Focal acute nephritis, possibly due to amoebae.
4. Cestodiasis, small intestine.
5. Parasitic (nematode) granulomas, stomach and colon, species undetermined.
6. Sero-fibrinous pneumonia, acute, moderate.
7. Parasitism, lung, type undetermined, mild.
8. Depletion of colloid and compensatory hyperplasia, thyroid(?).
9. Myopathy, skeletal muscle, type undetermined.
10. Blood clot, abdominal cavity.

GROSS FINDINGS

1. External examination: no external lesions were noted.
2. Primary incision: about a dozen irregularly rounded, smooth brownish masses about one inch in diameter were found randomly distributed in the thoracic and abdominal cavities. (Grossly they resembled lymph nodes, but microscopic examination of one proved it to be an encapsulated blood clot.)
3. Gastrointestinal tract: the gastric mucosa was divided into three distinct zones, i.e., cardiac, fundic and pyloric, and the mucosa was hyperemic throughout. Several small, raised whitish lesions, presumed to be necrotic foci ('button' ulcers) were present in the fundic and pyloric portions of the gastric mucosa. Several pieces of bone were in the stomach contents.

The first half of the small intestine contained tarry material that was separated with difficulty from the mucosa which appeared blackened. Raised necrotic lesions, as described in the stomach, above, were also present in the small intestine. Intestinal mucosa was very hyperemic. Numerous adult tapeworms, species undetermined, were present.

The colon appeared thickened and was thrown into coarse longitudinal folds. The rectum contained much black tarry material.

4. Liver: the liver was soft and extremely friable with semi-solid consistency and rounded margins.
5. Kidneys: several whitish foci, presumed to be urate deposits, were observed in the kidneys.
6. A solid brownish mass, about 1½ in. in diameter was found attached to the dorsal wall near the rectum.
7. No lesions were grossly observed in the mouth, pharynx, trachea, bronchi, lungs, diaphragm, heart, aorta, spleen, pancreas, adrenal, gall bladder, brain, pineal body, peripheral nerves, musculo-skeletal system, testes or hemipenes.
8. The thyroid, parathyroid and thymus were not identified grossly.
9. Ureters, vas deferens, spinal cord and bone marrow were not examined.

MICROSCOPIC PATHOLOGIC FINDINGS

1. Blood film: as this was a post mortem specimen, a differential count would have been of dubious value. Also, we could not identify many of the blood cells. Specific examination for amoebae, microfilariae and haemogregarines were negative. Numerous large rod-shaped bacteria were found, but they could have entered the blood stream agonally or post mortem.
2. Lungs: cartilage in a bronchial wall was undergoing ossification, presumably an ageing change. There was slight accumulation of a fibrino-serosanguinous exudate in air spaces and several foci of round cell infiltration in lung parenchyma. Pulmonary congestion was marked. A focus of drab olive-brown, homogeneous, largely non-birefringent pigment was found within macrophages. We could not identify this pigment. Several round, densely haematoxyphilic structures with a thin hyaline membrane were noted in air spaces and interpreted as parasite eggs, type undetermined. A single small granuloma consisting of eosinophilic granulocytes, multinucleated giant cells and an outer ring of fibrous tissue was noted in one of the septae. There was a suggestion of parasitic remnant in the centre of this lesion, but the exact aetiology of this granuloma could not be determined.
3. Stomach: a few amoebae were noted in the pyloric mucosa. Closely associated with them were masses of bacteria and local accumulations of lymphoid cells. Fragments of skeletal muscle were identified in the ingesta, indicating the lizard had eaten shortly before death.
 In the fundic area, multiple granulomata were found in the wall, particularly the lamina propria. Many of these discrete lesions contained cross-sections of small nematodes, species undetermined. Many epithelial cells were pyknotic and there was a light scattering of lymphoid cells and erythrocytes in the mucosa.
 In the cardiac stomach, a few focal areas of round cell infiltration were noted in the lamina propria and submucosa.
4. Small intestine: numerous sections of adult tapeworms were in the gut lumen. The epithelium was necrotic; occasional amoebae and numerous bacteria were found in the mucosa. Cellular response was primarily lymphocytic.
5. Colon: two more granulomata, similar to those found in the small intestine, were observed. A small nematode, species undetermined, was found in the centre of one of these. The mucosa was ulcerated and heavily infiltrated by amoebae and bacteria.
6. Rectum: The most intense inflammatory response in the gut was found in the rectum. There was extensive, diffuse ulceration; necrosis extended into the submucosa and inner circular muscle, serosal vessels were injected, and there was an intense cell response consisting of histiocytes, lymphoid cells and eosinophilic granulocytes. Many bacteria and amoebae had invaded the wall.
7. Pelvic mass (see Gross Findings, Item 6): this tissue consisted of a massive amoebic and bacterial infection of skeletal muscle. Bacteria and inflammatory cells freely invaded skeletal muscle bundles which were undergoing necrosis. The lesion was grossly circumscribed, but not encapsulated.
8. Liver: coagulation necrosis was diffusely evident and several foci of liquefaction necrosis were observed. Amoebae were very numerous throughout the hepatic parenchyma and in a branch of the portal vein.
9. Kidneys: occasional foci of round cell infiltration were noted and in one of these, cells that could possibly be amoebae were noted.
10. Thyroid: thyroid was not identified grossly. A tissue mass adjacent to the trachea was sectioned and is presumed to be thyroid. The structure in question consisted of a glandular organ composed of many variable sized follicles. The follicles contained varying amounts of a homogeneous eosinophilic amorphous mass, presumably colloid. Many follicles were very small and some appeared to be

- collapsed. The organ was very hyperemic. Some of the larger follicles seemed to branch.
11. Skeletal muscle: adjacent bundles showed considerable morphological variation. Some appeared vacuolated while others were amorphous and hyaline. In one area there were several closely grouped foci of large mononuclear cells interpreted as macrophages. While they were predominantly in interstitial tissue, they could possibly have been phagocytising necrotic muscle.
 12. Blood clot: one of the smooth brownish masses described under 'Gross pathologic findings, item 2, Primary incision', was sectioned and found to consist of a dense mass of extravascular erythrocytes completely surrounded by a fibrous capsule in which there was considerable yellow pigment (presumably haemosiderin). A peculiar finding was the presence of a few patent blood vessels within this clot.
 13. No significant lesions were found in sections of pancreas, spleen, adrenal, heart, aorta, carotid artery, testes, vasa deferens or abdominal fat.

COMMENTS

1. Numerous colonies of bacteria (often in massive numbers) were found in most of the organs examined, including peripheral blood. With the exceptions of liver, gastrointestinal tract and the pelvic abscess, there was generally no cell response to the bacteria, indicating they were an agonal or post mortem phenomenon.
2. The amoebae in the gut had abundant, readily stained cytoplasm and were probably trophozoite forms; amoebae in the liver generally had a large cytoplasmic vacuole and were probably in the cyst form, probably a defensive reaction by these organisms to the adverse environment (necrotic tissue) they were in.
3. Many anatomic features worthy of note were found and some of the more interesting findings are listed below (including some previously recorded in monitors or other lizards):
 - (i) Radiographs revealed plates of mature bone in the dorsal *skin*,
 - (ii) A membranous *diaphragm* separated abdominal and thoracic cavities.
 - (iii) The trachea branched into two *bronchi* midway between the larynx and hilus of the lungs.
4. Heart: there was a large interventricular septal (IVS) opening protected on both sides by 'flap' valves attached on the atrial side of the IVS that permitted blood to flow from the left ventricle into the right ventricle and then into the aortic outflow tract which arose from the right ventricle. A large muscle mass protruding into the right ventricular cavity helped shunt this oxygenated blood into the aorta and kept it from mixing with unoxygenated blood entering the right ventricle from the right atrium. The 'flap' valves previously referred to had a verrucous surface on the sides facing the IVS opening. The aortic outflow tract opened into a double aortic arch, the left aorta being about double the diameter of the right. The two aortas joined caudal to the cardiac apex to form a single artery.
5. The *spleen* was a rounded brownish red organ about 4 cm in diameter. At the hilus of the spleen was a solid, white, rounded mass about 1.5 cm in diameter. The latter tissue could not be identified grossly, but is believed to be *adrenal* on the basis of microscopic study. The white mass was found to be a highly vascularised glandular organ surrounded by a fibrous capsule which gave numerous septae into the parenchyma. The outer portion of this organ was composed of at least two different cell types. The predominant cell was large, with a clear, vacuolated cytoplasm and a round to oval pale basophilic nucleus containing fine chromatin threads; the cells were arranged in definite acinar pattern. The other cell in the outer zone of this gland was large with finely granular eosinophilic cytoplasm and a nucleus varying from vesicular in some cells to densely basophilic in others; these cells occurred in small to large groups, but it was difficult to ascertain their pattern of arrangement. This outer zone made up two-thirds the thickness of the organ. There was an inner zone containing numerous ducts lined by simple

cuboidal epithelium, some fat and a great number of cells that resembled lymphoid and R-E elements. Numerous macrophages in this inner zone were filled with a granular, yellowish-brown, non-birefringent pigment that was PAS and iron positive. This inner zone gave the general impression of an erythropoietic rather than a glandular area. The presence of ducts could not be explained.

The spleen contained numerous islands of tannish-pink polyhedral cells occurring in avascular sheets. These cells were PAS positive and iron negative. We were not able to identify these cells.

6. The *gastric mucosa* was grossly divisible into three glandular zones (cardiac, fundic and pyloric) from the oesophageal to the duodenal ends.
7. Two large hemipenes were found at the caudal aspect of the cloaca.
8. Active spermatogenesis was evident in both testes.
9. The brain was $2\frac{1}{2}$ in. in length and $1\frac{1}{4}$ in.

wide at the broadest part of the cerebrum. A *pineal* stalk extended from the diencephalon through the 2-in. thick *calvarium* to a depression in the dorsal mid-line of the skull forming the 'third eye'. The brain was much more accessible from the roof of the mouth because the ventral part of the cranium was much thinner than the dorsal part.

ACKNOWLEDGMENTS

The authors wish to thank Dr Louis S. Diamond, Laboratory of Parasitic Diseases, National Institute of Allergic and Infectious Diseases, Bethesda, Maryland, for his aid in cultural isolation of the amoebae. Dr Lynn Nelson of San Diego Zoological Garden and Dr C. P. Gandal of New York Zoological Park also contributed valuable information on the management of the *V. komodoensis*.

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Table 1. Weights and measurements of a male Komodo dragon, *Varanus komodoensis*, at post mortem examination.

Total weight	80 kg
Length	8 ft 11 in.
Length of skull	$10\frac{1}{2}$ in.
Circumference at jaw	$17\frac{1}{2}$ in.
Circumference at neck	8 in.
Length, withers to pelvis	$27\frac{1}{2}$ in.
Length of pelvis	4 in.
Length of tail	58 in.
Thoracic girth (behind front legs)	34 in.
Abdominal girth (largest portion)	42 in.
Pelvic girth (in front of hind legs)	26 in.
Tail girth	21 in.
Leg length (front)	$15\frac{1}{2}$ in.
Leg length (hind)	$20\frac{1}{4}$ in.
Mesenteric fat	1130 g
Right testis	43 g, $5 \times 1\frac{1}{4}$ in.
Left testis	41 $\frac{1}{2}$ g
Right kidney	220 g, $8 \times 2\frac{1}{4}$ in.
Left kidney	230 g, $8 \times 2\frac{1}{4}$ in.
Pancreas	71 g, $4\frac{3}{4} \times 2\frac{1}{2} \times 1\frac{1}{2}$ in.
Heart with great vessels	500 g
Brain	10 g
Stomach	20 in.
Intestines	60 in.
Liver	6.3 kg