



WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 11

9 January 2013

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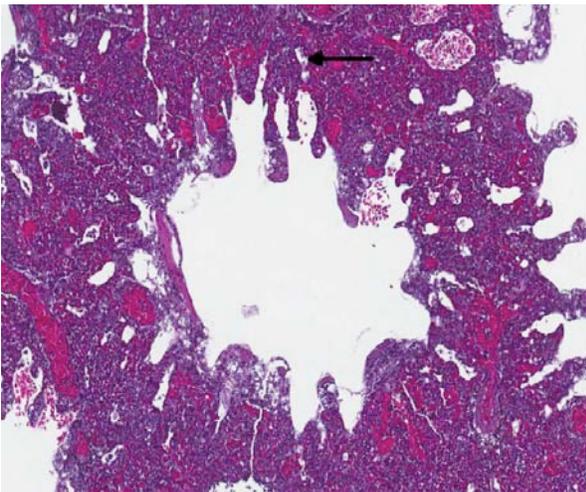
**CASE I: 508A (JPC 4017936).**

**Signalment:** 1-year-old male Montezuma quail (*Cyrtonyx montezumae*).

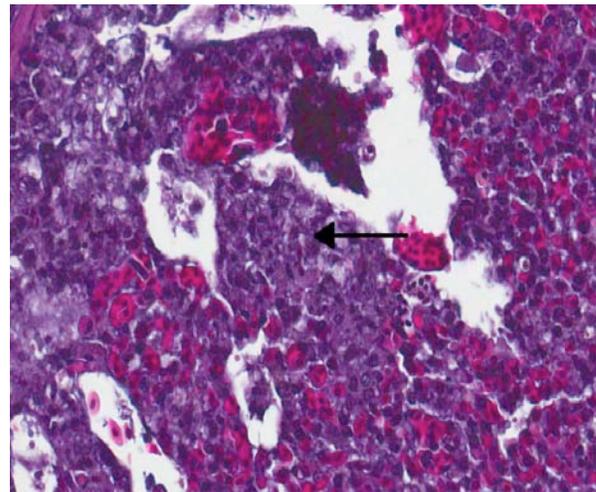
**History:** This animal was found dead with no premonitory signs. It was the second Montezuma quail death from this enclosure in a three-day period.

**Gross Pathology:** At necropsy, the quail was in good body condition with appropriate musculing and adipose tissue stores. The lungs were diffusely wet, dark red and slightly heavy. On section, they exuded a moderate amount of yellow-tinged, watery fluid.

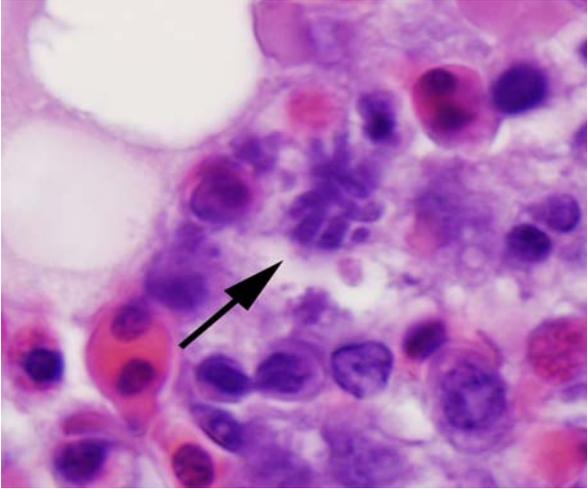
**Histopathologic Description:** Lungs: Cellularity is increased throughout the lungs, resulting in marked thickening of air capillary walls and mild thickening of air atria septa. Increased cellularity



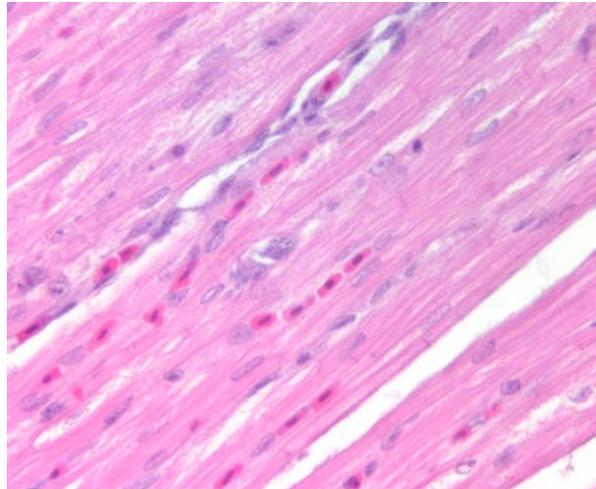
I-1. Lung, Montezuma quail: Diffusely, the lungs are hypercellular, with loss of air capillary architecture and compression of respiratory atria (arrow). (HE 80X)



I-2. Lung, Montezuma quail: Multifocally, there are numerous foci of necrosis (arrow) composed of cellular debris and necrotic histiocytes. (HE 400X)



1-3. Lung, Montezuma quail: Multifocally, endothelial cells contain protozoal meronts. (HE 1000X)



1-4. Heart, Montezuma quail: A focal mature meront containing numerous elongate merozoites is focally present. The affected cell type is not discernible, but is presumed to be an endothelial cell. H&E. (Photo courtesy of the Wildlife Conservation Society, Zoological Health, 2300 Southern Blvd., Bronx, NY 10460, www.wcs.org)

is the result of numerous infiltrating macrophages with fewer lymphocytes and rare plasma cells. Dense accumulations of leukocytes are occasionally present in perivascular spaces. Pale eosinophilic foci with loss of architecture and containing karyorrhectic debris are scattered randomly throughout the parenchyma (necrosis). Numerous protozoa-laden cells are scattered amongst the inflammatory population. Affected cells' cytoplasm contains large, immature, uninucleate meronts as well as mature meronts with numerous merozoites that are approximately  $2\mu \times 4\mu$ . Occasionally, meronts filling capillary lumens have a serpentine appearance. Foamy macrophages are present within airways, along with free erythrocytes and a small amount of pale eosinophilic wispy to homogenous material. There is frequent expansion of perivascular tissue by clear space (edema). The pulmonary vasculature is diffusely congested.

**Contributor's Morphologic Diagnosis:** Lungs: Pneumonia, interstitial, histiocytic to lymphoplasmacytic, subacute, diffuse, marked, with intracellular protozoa, scattered necrosis and pulmonary edema.

**Contributor's Comment:** This was one of two Montezuma quail to die within a three-day span at an urban zoological park in the spring of 2010. Both birds had similar gross and microscopic findings, the most significant of which was marked interstitial pneumonia with intralesional protozoa. Fatal pulmonary sarcocystosis was

presumed to be due to a *Sarcocystis falcatula*-like infection which has been responsible for multiple mortalities in thick-billed parrots (*Rhynchopsitta pachyrhyncha*) at the zoo. Affected parrots had identical pulmonary lesions as these quail and were housed in the same enclosure.

*Sarcocystis falcatula* (and *S. falcatula*-like organisms) is typical of the genus in that it utilizes a two-host life cycle. The only known definitive host in North America is the opossum (*Didelphis virginiana*) but unlike most *Sarcocystis* species it can utilize a number of avian species as intermediate hosts.<sup>3,5,6,7,8</sup> Susceptible birds ingest sporocysts shed in the feces of opossum, which release sporozoites once in the intestinal tract. Tissue invasion and local asexual reproduction ensues, followed by additional cycles of merogony in capillaries and venules throughout the body.<sup>6</sup> In the lungs, where merogony is often most pronounced, endothelial cells become swollen with cytoplasmic meronts. The presence of protozoa can lead to vascular obstruction, perivascularitis and vasculitis, and significant interstitial and airway edema.<sup>2,3,6</sup> Pulmonary lesions can be fatal in birds with heavy protozoal burdens, such as the quail in this case. In addition to the lungs, merogony has been noted to occur in the liver, spleen, pancreas, adrenal glands and heart.<sup>6</sup> Other than the liver, the protozoal presence is typically insignificant with mild or no inflammation.<sup>6</sup> In these quail, endothelial cells of the heart were the only extrapulmonary site of merogony and low numbers of organisms were

occasionally associated with mild lymphohistiocytic myocarditis. In birds that survive the initial reproductive cycles, sarcocysts typically develop in skeletal and cardiac muscle, where they remain until ingestion by the definitive host.<sup>2,3,6,7</sup> Sarcocysts were not identified in the case presented here, presumably an effect of how quickly the quail succumbed to pulmonary disease.

Multiple opossum were trapped on zoo grounds following initial diagnosis of this disease. In addition to direct infection via opossum feces, paratenic hosts or mechanical vectors, such as cockroaches and other insects, can cause infection indirectly.<sup>2</sup>

Confirmation of *S. falcatula*-like organisms in avian patients requires diagnostic evaluation beyond light microscopy. Individual merozoites can be mistaken for *Toxoplasma* or *Neospora* tachyzoites, and light microscopic morphology alone (of any stage) is not sufficient to differentiate individual *Sarcocystis* species.<sup>3</sup> Immunohistochemistry is available for *S. falcatula*, and organisms in previous thick-billed parrot sarcocystosis cases at our institution reacted positively to *S. falcatula* antisera. As *S. neurona* can also reactive positively to this antisera, *S. neurona*-specific immunohistochemistry was also performed and was negative.<sup>3</sup> Ultrastructural evaluation of *Sarcocystis* spp. reveals typical apicomplexan features, such as a conoid and micronemes; unlike *Toxoplasma*, however, *Sarcocystis* spp. are intracytoplasmic rather than within a parasitophorous vacuole. Rhoptries are likely absent in *S. falcatula*-like organisms, but this observation differs between authors.<sup>2,5,8</sup>

Recent molecular investigations into the genus have revealed differences between organisms previously grouped together as *S. falcatula*.<sup>3</sup> Such research suggests the presence of multiple species, thus prompting the use of “*S. falcatula*-like” rather than definitive identification.

**JPC Diagnosis:** Lung: Pneumonia, interstitial, histiocytic and necrotizing, diffuse, with numerous protozoal schizonts.

**JPC Comment:** The contributor provides a very good summary of *Sarcocystis falcatula*-like organisms. *Sarcocystis* species, like other

protozoal parasites in the phylum Apicomplexa, have a two-host life cycle; however, they differ from other genera in that asexual reproduction (schizogony and cyst formation) occur exclusively in the intermediate (herbivorous) host, and sexual reproduction (gametogony, fertilization, and sporulation) occur only in the definitive (carnivorous) host. *Sarcocystis* spp. usually do not cause illness in the definitive host, but schizogony in the endothelium of the intermediate host can result in serious and often fatal disease.<sup>1</sup> Clinical disease in the intermediate host may include fever, mucous membrane petechiation, edema, icterus, and macrocytic hypochromic anemia during the schizogonous phase or myositis during the encystment phase.<sup>9</sup> There are over 90 *Sarcocystis* species identified in mammals, birds and reptiles; some are associated with disease and others are considered nonpathogenic. The following species are pathogens of importance in domestic animals<sup>4,9</sup>:

<i>Sarcocystis</i> sp.	Intermediate host	Definitive host	Comments
<i>S. cruzi</i>	Cattle	Domestic and wild canids	Eosinophilic myositis; Hemolysis, abortion, lymphadenitis, tail tip sloughing, death
<i>S. gigantea</i>	Sheep	Cat	Only species of domestic animals visible to naked eye
<i>S. tenella</i> ( <i>S. ovicanis</i> )	Sheep	Dog	Encephalomyelitis; severe disease in lambs
<i>S. miescheriana</i>	Swine	Domesitic and wild canids	Diarrhea, myositis, lameness
<i>S. neurona</i>	Birds/horses*	Opossum	Equine protozoal myeloencephalitis

\*Horses are assumed to be dead end hosts, but may act as intermediate hosts.

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**CASE II: TVMDL 2012-01 (JPC 4018765).**

**Signalment:** Four-year-old, male red-tailed boa constrictor.

**History:** A four-year-old, male, 10-pound boa constrictor from a local zoo was submitted for necropsy following a 12-month duration respiratory tract disease. The illness was non-responsive to multiple antibiotics and, as the disease progressed, the animal exhibited increased respiratory effort, intermittent lethargy, and anorexia.

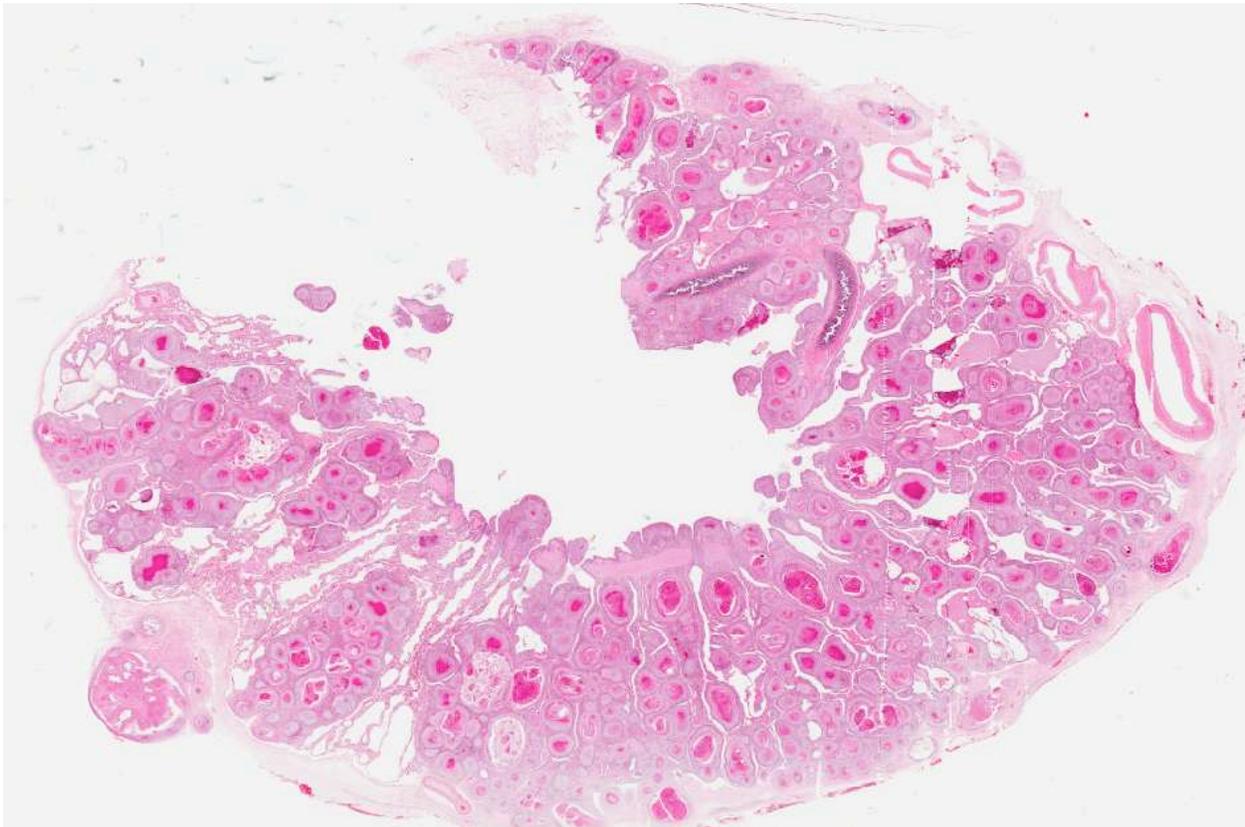
**Gross Pathology:** The animal was in thin body condition. There was extensive replacement of up to 80% of the normal pulmonary parenchyma by coalescing granulomas.

**Histopathologic Description:** The normal pulmonary architecture is predominately effaced by coalescing granulomas. The parabronchial submucosa and air capillary interstitium are moderately to markedly expanded by granulomas, which extend into and variably occlude air spaces and faveoli. The granulomas are composed of

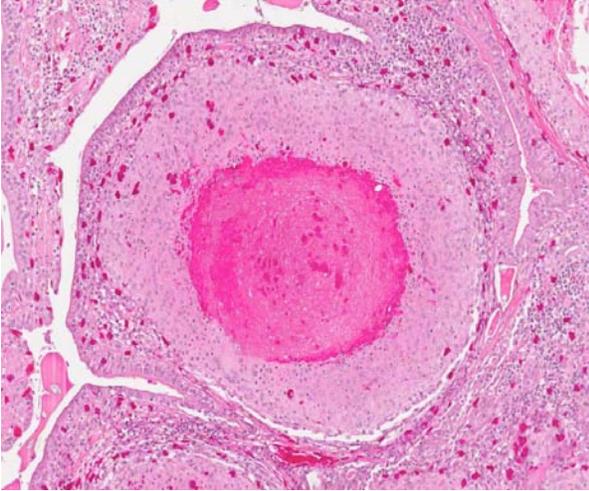
central necrotic debris surrounded by large numbers of epithelioid macrophages admixed with low numbers of heterophils and few lymphocytes and rare giant cells. Moderate numbers of macrophages, lymphocytes, and heterophils infiltrate the interstitium between granulomas with mild fibrosis. Ziehl-Neelsen staining revealed large numbers of acid-fast bacilli within macrophages consistent with *Mycobacterium* spp.

**Contributor's Morphologic Diagnosis:** Pneumonia, granulomatous, chronic, severe.

**Contributor's Comment:** Spontaneous mycobacterial infections have been reported in a wide variety of reptiles, including snakes, turtles, lizards, and crocodiles and descriptions of mycobacterial infections in reptiles have been reported since the early 1900s.<sup>2,3,4</sup> *Mycobacterium marinum* is a ubiquitous opportunistic pathogen and is the predominant mycobacterial pathogen reported in snakes. The original source of infection in captive reptiles is likely the environment, whether its introduction into the exhibit is via plant matter or other



2-1. Lung, snake: Diffusely, the faveolar interstitium is expanded by numerous granulomas. (HE 3.0X)



2-2. Lung, snake: Granulomas are up to 700  $\mu\text{m}$  in diameter and are centered on areas of cellular debris and degenerate heterophils. (HE 100X)

inanimate objects, or via a previously infected animal.<sup>2</sup> Infection is believed to be acquired by ingestion of infected material or via defects in the integumentary or respiratory systems.<sup>1,4</sup> Mycobacterial infections in reptiles can cause systemic illness accompanied by nonspecific signs such as anorexia, lethargy, and wasting.<sup>3</sup> Gross lesions present as grayish-white nodules present in the affected tissue. Histopathologic examination illustrates classic granulomas with a central core of necrotic debris surrounded by epithelioid macrophages.

Early diagnosis of mycobacterial diseases in captive reptiles is crucial to limiting the extent of disease transmission. The detection of mycobacteria among captive animals in zoos is concerning and treatment is often not advised considering the chronic and often advanced state of the disease, risk of infection to animals within the same and other exhibits, transfer of animals with unrecognized infection, and spread of infection to animal handlers.<sup>1,2</sup> Additionally, no successful treatment of infection with *Mycobacterium* spp. has been reported in reptiles.<sup>3</sup>

**JPC Diagnosis:** Lung: Pneumonia, interstitial, granulomatous, diffuse, severe.

**Conference Comment:** The contributor provides a concise review of mycobacterial diseases in snakes. Conference participants discussed the differential diagnosis for granulomatous inflammation in snakes. Reptiles typically respond to infectious agents with a granulomatous

inflammatory response, which is predominantly heterophilic or histiocytic, depending on both the etiologic agent and the host response.<sup>4</sup> Histiocytic granulomas are usually associated with intracellular pathogens, whereas heterophilic granulomas are generally associated with extracellular pathogens. In heterophilic granulomas, heterophil degranulation and central necrosis induce a strong histiocytic response; thus, both types of granulomas can progress to chronic granulomas.

*Mycobacterium* species including *M. avium*, *M. chelonae*, *M. fortuitum*, *M. intracellulare*, *M. marinum*, *M. phlei*, *M. smegmatis*, and *M. ulcerans* have frequently been reported to cause histiocytic granulomas in reptiles. Additionally, other intracellular bacteria from the family Chlamydiaceae, which sporadically infect reptiles, can also incite histiocytic granulomas. A retrospective study of ninety reptiles with granulomas (i.e., 48 snakes, 27 chelonians, and 15 lizards) showed that, although *Mycobacterium* species other than *Mycobacterium tuberculosis* (MOTT) are the most important infectious agents causing granulomatous inflammation in reptiles, *Chlamydia pneumoniae* and “Chlamydia-like” microorganisms (e.g., *Parachlamydia acanthamoebae* and *Simikania negevensis*) can also induce well-formed granulomas, and therefore should be considered in the differential diagnosis for granulomatous lesions in reptiles. Mycobacteria and chlamydia can be identified microscopically by Ziehl-Neelsen acid fast stain and immunohistochemistry for cLPS antigen, respectively; however, both of these methods are less sensitive than PCR.<sup>4</sup>

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## References

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**CASE III:** 08-72416 (JPC 3134858).

**Signalment:** A five-month-old male white-tailed deer (*Odocoileus virginianus*).

**History:** The fourth fawn to die from the herd.

**Gross Pathologic Findings:** The cornea of the right eye was partially cloudy. There was a 1.5 cm area of hemorrhage in the skin of the right ear. There was subcutaneous yellow edema fluid in the ventral neck, surrounding the trachea and esophagus. The right half of the thorax contained a large blood clot. Dorsal to the lungs and heart there was a blood clot approximately 20 cm long that surrounded the aorta and was attached to the musculature ventral to the spinal column. There was acute hemorrhage in one of the adrenal glands and the left testicle. There were small multifocal hemorrhages in the right testicle.

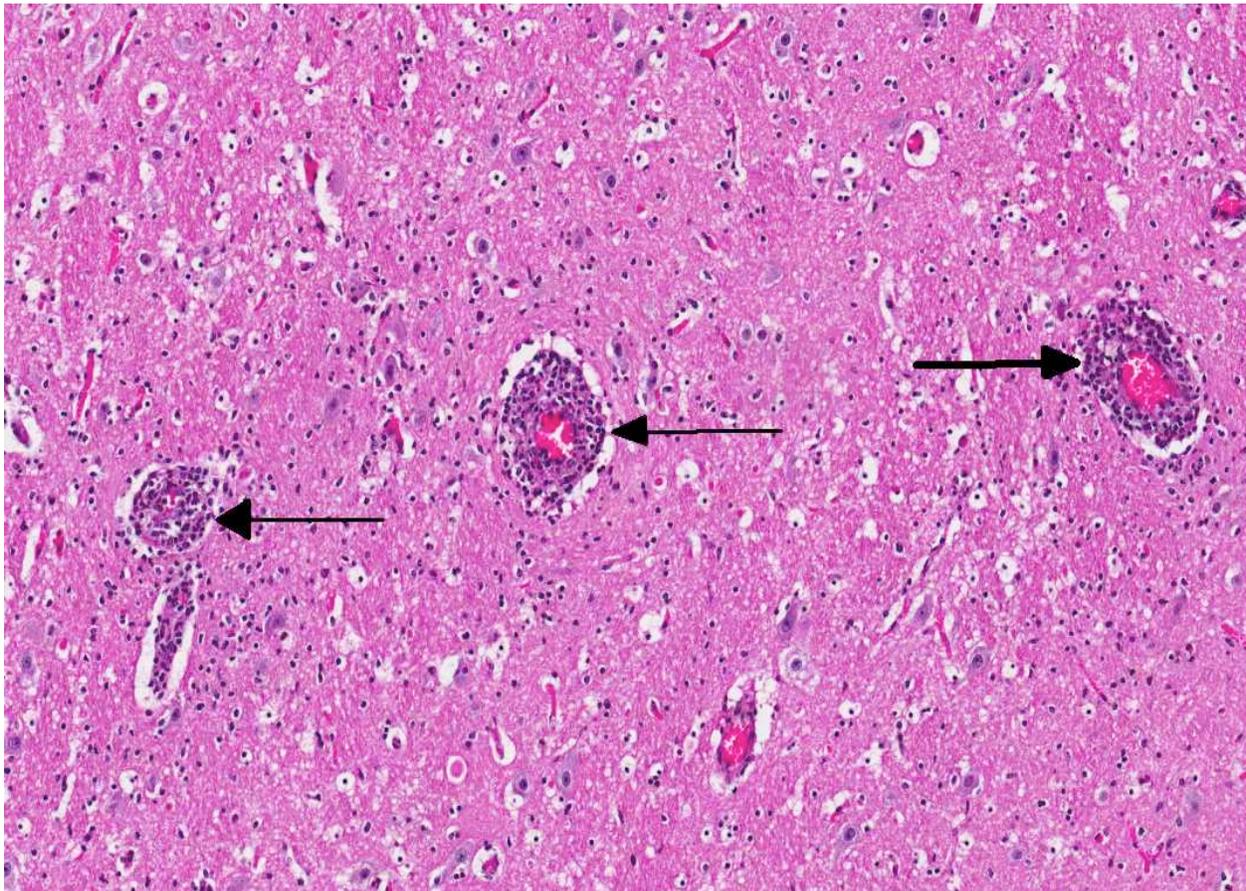
**Laboratory Results:** No anaerobic bacterial growth from lung, liver, or brain. Negative for

epizootic hemorrhagic disease virus by PCR. PCR positive for Caprine Herpesvirus-2.

**Histopathologic Description:** Cerebrum: Surrounding numerous meningeal and parenchymal vessels is an infiltrate composed of low to medium numbers of lymphocytes and neutrophils. The inflammatory cells often are equally present in the tunica media and tunica adventitia. There are variable numbers of macrophages, lymphocytes, and occasional plasma cells in the meninges. There are scattered glial cell infiltrates within the neuropil.

In most of the sections, there are several vessels in the leptomeninges that have fibrinoid necrosis of the vessel wall. Affected vessels often have adventitial to subintimal accumulation of neutrophils, lymphocytes, and macrophages.

In some sections, choroid plexus is present; it is heavily infiltrated by lymphocytes, plasma cells, macrophages, and neutrophils.

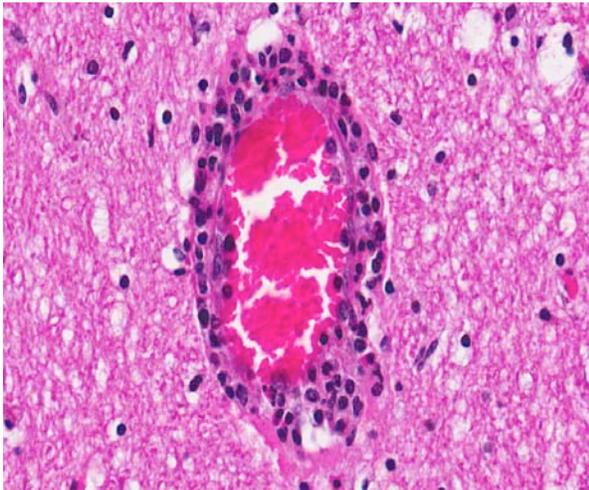


3-1. Cerebrum at level of lateral ventricles, deer: Within the neuropil, vessels are often surrounded by prominent cuffs of lymphocytes and plasma cells (arrows). (HE 100X)

**Contributor's Morphologic Diagnosis:** 1. Cerebrum: Vasculitis and perivasculitis, necrotizing, lymphocytic, multifocal, moderate.  
2. Cerebrum: Meningoencephalitis, lymphocytic, diffuse.

**Contributor's Comment:** Gross and histologic lesions were consistent with malignant catarrhal fever (MCF), and infection with caprine herpesvirus 2 was confirmed by PCR testing. MCF occurs in ruminant species and is caused by several herpesviruses, an enveloped, linear, doubled-stranded DNA virus. These viruses are in the *Rhadinovirus* genus of the subfamily *Gammaherpesvirinae*. There are currently four known members of the MCF virus group: alcelaphine herpesvirus 1 (AIHV-1), ovine herpesvirus 2 (OvHV-2), caprine herpesvirus 2 (CpHV-2), and a gammaherpes virus found in white-tailed deer with no known reservoir (MCFV-WTD).<sup>3,4</sup> Infection of white-tailed deer with CpHV-2 has been previously reported.<sup>3</sup>

Typical gross changes associated with MCF include conjunctivitis, cutaneous exanthema, crusting, and alopecia, nasal discharge, oral and esophageal ulcerations, urinary mucosal hemorrhages, and lymphoid enlargement.<sup>2</sup> Mortality in susceptible species approaches 100%; however, in the natural host, infection is latent or inapparent with intermittent virus shedding. These viruses are difficult or impossible to isolate in cell culture.



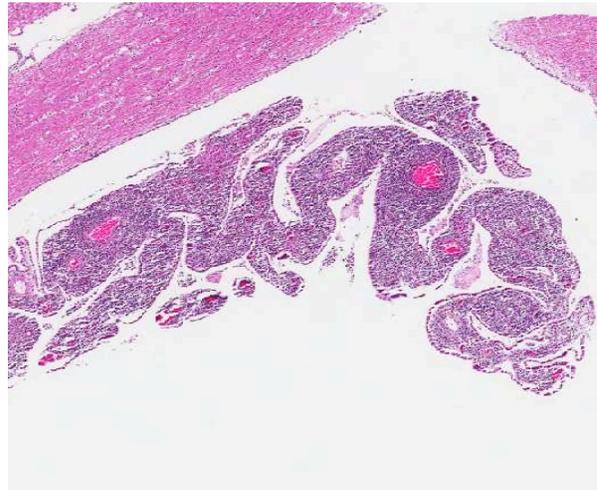
3-2. Cerebrum at level of lateral ventricles, deer: Occasionally, inflammatory cells are present within vessel walls, along with small amounts of necrotic debris and fibrin (vasculitis). (HE 400X)

The common histologic changes associated with MCF are lymphocytic perivasculitis and vasculitis with fibrinoid necrosis of medium sized arteries, and lymphoid hyperplasia.<sup>2</sup> Lesions are characterized by a proliferation of CD8+ T lymphocytes and tissue necrosis.<sup>5,7</sup> The mechanism of proliferation and vasculitis is unknown.<sup>5</sup>

MCF has been documented in white-tailed deer, along with other wild cervids, pigs, and cattle.<sup>1,2,8</sup> Raising bovids and deer around other small ruminants (sheep, goats) can be risky due to transmission of the MCF viruses from the host species that is not clinically ill. The incubation period is usually 2-10 weeks, but may on occasion be very much longer than this. Epizootic hemorrhagic disease (orbivirus) is a differential diagnosis for MCF in deer with acute hemorrhage.

**JPC Diagnosis:** Cerebrum: Arteritis and phlebitis, lymphoblastic and necrotizing, diffuse, moderate, with meningitis and choroid plexitis.

**Conference Comment:** The contributor provides an excellent summary of the gamma herpesviruses associated with malignant catarrhal fever in various species. In addition to the four members of the MCF virus group, there are several viruses that have been reported to cause MCF or MCF-like diseases in various species of hoofstock within zoological collections.<sup>6</sup> Recently, one such novel virus (MCFV-ibex) was identified as the etiologic agent for MCF observed



3-3. Cerebrum at level of lateral ventricles, deer: Diffusely, the choroid plexus is markedly expanded by a large numbers of lymphocytes and plasma cells. (HE 20X)

in bongo antelope. In this outbreak, a Nubian ibex was found to be the source of the virus. The presentation of MCF in bongos differs from the classic presentation in domestic ruminants and deer in the following ways: The bongos developed necrotizing cholangiohepatitis, neutrophilic necrotizing myocarditis, and they lacked the typical erosive or ulcerative oronasal lesions and enteritis often seen in other ruminants with MCF.<sup>6</sup>

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**CASE IV:** 1016743 (JPC 4020067).

**Signalment:** Adult (unknown age) female common northern boa constrictor (*Boa constrictor imperator*).

**History:** The patient was presented to the West Esplanade Veterinary Clinic (Metairie, LA) for a four-month history of anorexia and weight loss. Two other boa constrictors that were housed in close proximity to the patient had recently died after a period of anorexia of unknown duration. Also, there was a history of mite infestation in the household one year prior to presentation. Upon physical examination, the patient was thin with a body condition score of 2/5 and excess gas was noted on abdominal palpation. Heparinized blood was collected via cardiocentesis and submitted to the Louisiana State University Clinical Pathology Laboratory for a complete blood count. A blood smear from the patient is submitted for review. The patient died one week after initial presentation.

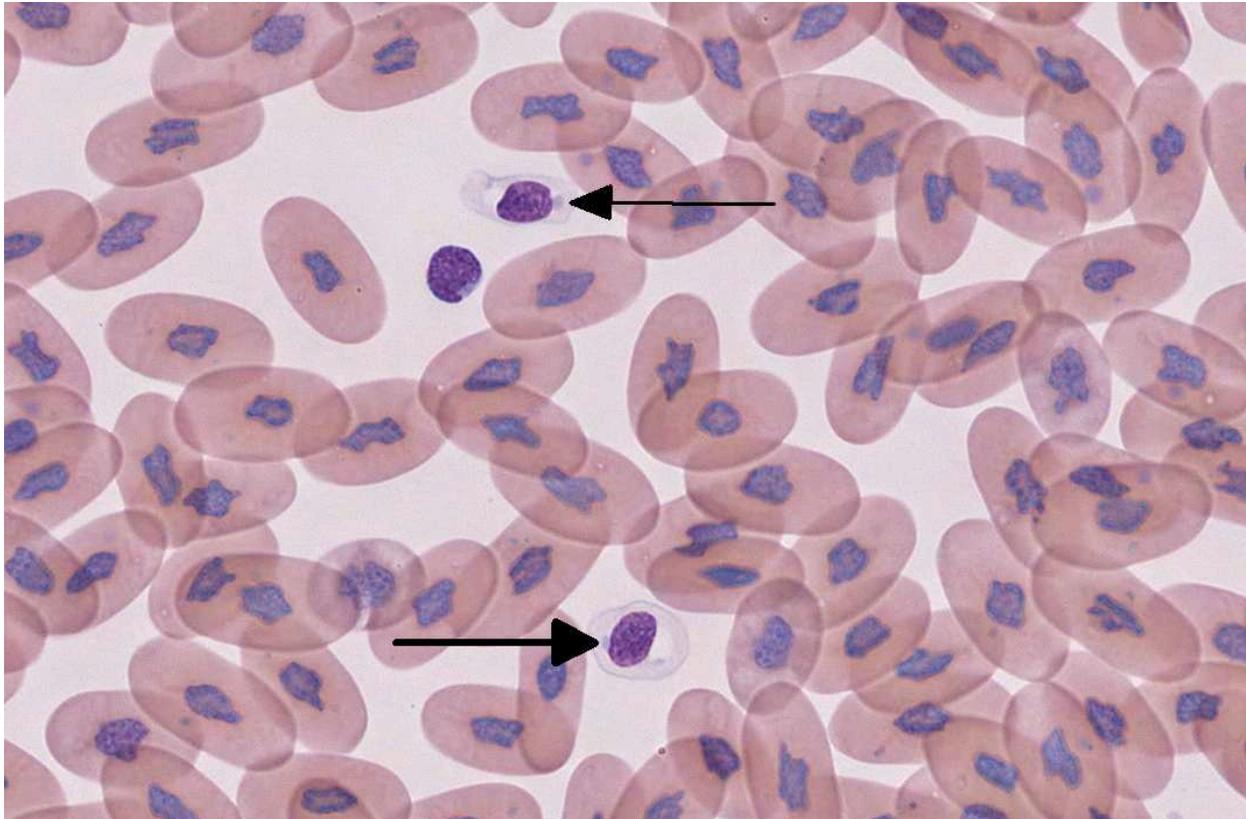
**Gross Pathology:** No gross lesions were noted.

**Laboratory Results:**

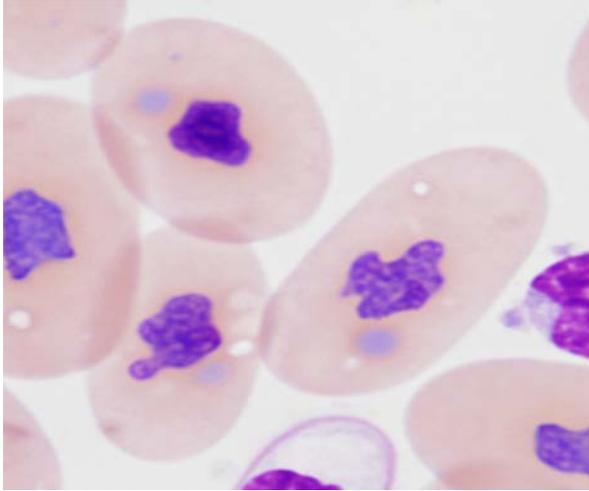
Complete Blood Count:

Parameter		Reference <sup>3</sup>
WBC (x103/ $\mu$ L)	<b>40 H</b>	4 – 10
Heterophils (x103/ $\mu$ L)	4	0.8 – 6.5
Lymphocytes (x103/ $\mu$ L)	<b>34.8 H</b>	0.4 – 6
Azuropils (x103/ $\mu$ L)	<b>1.2 H</b>	0 – 0.58
Eosinophils (x103/ $\mu$ L)	0	0 – 0.3
Basophils (x103/ $\mu$ L)	0	0 – 2
Thrombocytes	Adequate	N/A
PCV (%)	37	24 – 40
Total Protein (g/dL)	<b>9.7 H</b>	4.6 – 8.0

**Histopathologic Description:** A blood smear is submitted for review. The smear contains a marked increase in the number of leukocytes.



4-1. Blood smear; *boa constrictor*: Lymphocytes often contain a 2 $\mu$ m in diameter, round, cytoplasmic inclusion body (arrows). (Wright-Giemsa 1000X)



4-2. Blood smear, *boa constrictor*. Three erythrocytes (center) each contain a small (1-2  $\mu\text{m}$  in diameter) round light blue inclusion similar in color to those found in the lymphocytes. (Wright-Giemsa 1000x) (Photograph courtesy of Louisiana State University School of Veterinary Medicine. [www.vetmed.lsu.edu/pbs/](http://www.vetmed.lsu.edu/pbs/))

The leukocytes consist of predominantly small lymphocytes with rare lymphocytes that are intermediate in size. Many lymphocytes contain a single lightly basophilic intracytoplasmic inclusion of homogeneous texture that displaces the nucleus to the periphery of the cell. These inclusions vary in shape from round to oval to elongate with a diameter of 2-6  $\mu\text{m}$ . Smaller (~1-3  $\mu\text{m}$  in diameter) round inclusions of similar color are noted in moderate numbers of erythrocytes and rarely in heterophils. The erythrocytic inclusions were distinct from incidental basophilic punctate structures that are found frequently in erythrocytes from reptiles, believed to be degenerate organelles.<sup>1</sup> The erythrocyte density appears to be within normal limits. Mild polychromasia and rare earlier erythroid precursors are noted. Thrombocytes are adequate in number with normal morphology.

**Contributor's Morphologic Diagnosis:** Blood smear: Marked lymphocytosis with numerous basophilic intracytoplasmic inclusions consistent with boid inclusion body disease.

**Contributor's Comment:** Inclusion body disease (IBD) is a common and highly contagious disease seen worldwide in snakes of the families *Boidae* and *Pythonidae*.<sup>3,9,11</sup> The causative agent is unknown. Viral-like particles were observed with transmission electron microscopy in boid snakes with IBD.<sup>6,9,11,12</sup> Also, retroviruses have been isolated from boids with IBD.<sup>6</sup> However, a cause and effect relationship has yet to be proven.

Clinical signs are variable and include neurologic deficits and regurgitation.<sup>3,9,11</sup> Snakes may die within weeks; while others may remain asymptomatic for long periods of time.<sup>3</sup> The results of CBCs from boids with IBD vary depending on the stage of infection.<sup>9</sup> Lymphocytosis is more commonly seen in acutely infected boids, whereas chronically infected boids may have normal to low lymphocyte concentration.<sup>9</sup> The lymphocytosis is presumably secondary to antigenic stimulation. Chronic lymphoid leukemia or disseminated small cell lymphoma is considered less likely in the current case, since a neoplastic focus was not found on necropsy. However, bone marrow evaluation was not performed.

The diagnosis of IBD relies on identifying the characteristic intracytoplasmic inclusions. These inclusions can be found antemortem in blood films or buffy coat preparations.<sup>3,5,8,11</sup> In blood films, inclusions can be found in lymphocytes, erythrocytes, or heterophils.<sup>3</sup> IBD inclusions stained with Wright-Giemsa are intracytoplasmic and lightly basophilic with variable size and shape.<sup>3,8</sup> The diagnosis of IBD can be confirmed with H&E staining, in which case, these inclusions stain eosinophilic to amphophilic.<sup>3,5</sup> However, not all histologically confirmed IBD cases have easily identifiable inclusions in the circulating blood.<sup>5</sup> On postmortem examination, the inclusions can be found in glial cells and neurons of the central nervous system, lymphocytes in lymphoid organs, and epithelial cells of internal organs (gastrointestinal tract, liver, pancreas, etc).<sup>3,7,11</sup> In the current case, histopathology revealed inclusions in lymphocytes in the spleen, in hepatocytes, and pancreatic epithelium.

There are several reports of C-type retrovirus-like budding particles in IBD inclusions with TEM.<sup>6,7,9,11,12</sup> However, a recent report suggests that the inclusions are instead composed of aggregates of a unique, non-viral 68 kD IBD protein.<sup>3</sup> To the author's knowledge, there are no TEM descriptions of inclusions in circulating lymphocytes. The inclusions described here consisted of large aggregates of non-membrane bound amorphous granular electron dense material without viral-like particles.

When snakes demonstrating clinical signs are diagnosed with IBD the prognosis is grave, as the

disease is always fatal.<sup>11</sup> However, the long term survival of asymptomatic animals with IBD has not been evaluated. This case highlights the importance of evaluating blood films of susceptible snakes for IBD inclusions. Although there may be poor sensitivity, a blood film or buffy coat evaluation is a cheap, non-invasive test that can be used to detect this disease, especially if there is a lymphocytosis.

**\*\*Note:** Portions of this case have been published – Banajee KH, Chang L, Jacobson ER, Rich GA, Royal AB. What's your diagnosis? blood film from a boa constrictor. *Vet Clin Path.* 2012;41(1): 158-159.

**JPC Diagnosis:** Peripheral blood: marked lymphocytosis with numerous intralymphocytic and intraerythrocytic intracytoplasmic inclusions.

**Conference Comment:** The contributor provides an excellent review of IBD. Participants discussed recent findings that suggest IBD may be caused by novel viruses that appear to be related to both arenaviruses and filoviruses.<sup>10</sup> In this study, viral nucleoprotein from three viruses with a typical but divergent arenavirus genome was localized within large cytoplasmic inclusions in infected cells. Interestingly, envelope glycoproteins from the viruses are more similar to those of filoviruses than to other arenaviruses. The presence of these viral proteins in 6/8 confirmed IBD cases and 0/10 controls indicates that these newly discovered viruses are possible etiologic agents of IBD.<sup>10</sup>

Additionally, participants discussed characteristics of reptilian blood cells and reptilian hemoparasites that can be found on blood films. In reptiles, mature erythrocytes are larger than their mammalian or avian counterparts, and, as in birds, reptile erythrocytes are ellipsoidal with centrally-placed, oval to round nuclei with dense purple chromatin and irregular margins.<sup>2</sup> Often, small (0.5  $\mu$  m to 2.0  $\mu$  m) round to irregular, basophilic intracytoplasmic inclusions are found in the cytoplasm of reptile erythrocytes and are suspected to be degenerate organelles, likely due to slide preparation artifact. Likewise, the basophilic stippling associated with a regenerative response or lead poisoning should not be confused with pathogen associated intracytoplasmic inclusions. Reptilian lymphocytes are similar in appearance and function to mammalian and avian

lymphocytes. Circulating monocytes (azurophils) are usually found in small numbers; however, increased numbers are seen with granulomatous inflammation. Granulocytes in reptiles are classified as acidophils (heterophils and eosinophils) and basophils. Heterophils have bright orange fusiform cytoplasmic granule, and function similarly to avian heterophils (i.e. they rely heavily on oxygen-independent mechanisms to destroy phagocytized microbes.) Eosinophils contain eosinophilic spherical granules, and function much like their mammalian counterparts. Basophils appear and function similarly to those found in mammals and birds; however, their relative number can be higher and in some species comprise up to 40% of the leukocyte differential. Reptilian thrombocytes are elliptical to fusiform with a centrally-positioned nucleus, dense chromatin and colorless cytoplasm with few azurophilic granules.<sup>2</sup>

Hemoparasites and microfilaria are common in reptiles and are often encountered on blood films. Although usually considered incidental, some can cause disease (i.e. hemolytic anemia).<sup>2</sup> Common hemoprotozoa in reptiles include hemogregarines (*Hemogregarina*, *Hepatozoon*, *Karyolysus*), trypanosomes and *Plasmodium* spp. Less common hemoprotozoans of reptiles include *Leishmania*, *Saurocytozoon*, *Haemoproteus*, *Schellackia*, and the *piroplasmids*. Hemogregarines are identified in erythrocytes by the presence of intracytoplasmic gametocytes that lack refractile pigment granules. Trypanosomes are large extracellular flagellate protozoa. *Plasmodium* trophozoites are small, signet-ring structures found in erythrocyte cytoplasm and have gametocytes with refractile granules; whereas *Sauroleishmania* appear as round to oval 2-4  $\mu$  m blue organisms with an oval, red nucleus in the cytoplasm of thrombocytes or mononuclear leukocytes. *Saurocytozoon* can be identified by their round gametocytes that lack pigment granules in the cytoplasm of lizard leukocytes. *Haemoproteus* gametocytes have refractile pigment granules and, similar to *Haemoproteus* in birds, can cause dehemoglobinization of the infected erythrocyte.<sup>2</sup> *Lainsonia* and *Schellackia* are coccidian parasites whose sporozoites appear as intracytoplasmic inclusions in erythrocytes and lymphocytes of lizards and snakes. The sporozoites are round to oval, pale staining, nonpigmented inclusions that deform the host cell nucleus into a crescent shape. Piroplasmids such

as *Babesia*, *Aegyptianella*, *Sauroplasma* or *Serpentoplasma* appear as small nonpigmented, round to piriform and signet ring-like inclusions in erythrocyte cytoplasm.

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