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Specimen: Blood smear

Signalment: Adult, FS American Pit Bull Terrier

History and Clinical Signs: An adult female spayed American Pit Bull Terrier presented to the University of Georgia's oncology service as a referral for suspected lymphoma due to firm, enlarged peripheral lymph nodes of one month duration. Fine needle aspiration of a peripheral lymph node confirmed the diagnosis and flow cytometry allowed for further classification as intermediate to predominantly large-sized B cells that labeled CD21+ MHCII+ and CD45+, consistent with B cell lymphoma. Additionally, on flow cytometry, there were also very low percentages of small to intermediate T cells within the lymph node. At that time, a CBC, chemistry panel, and urinalysis were performed and revealed no significant abnormalities, with the exception of a mild thrombocytopenia (141 x $10^3/\mu l$) and no evidence of circulating neoplastic cells. Chemotherapy was initiated with vincristine.

The patient returned in one week for chemotherapy and another CBC was submitted prior to treatment. A pathology review was performed on the CBC, which revealed lymphocytes morphologically concerning for an emerging circulating neoplastic population. These lymphocytes were predominantly small to intermediate in size with a mildly expanded, lightly basophilic cytoplasm that occasionally contained a few magenta granules and rarely contained vacuoles. Nuclei of these atypical lymphocytes were round to ovoid, and contained lightly clumped to smooth chromatin with no overt nucleoli. At this time, Adriamycin was administered due to concern for disease progression. Approximately two weeks later, the following CBC was received for pathology review.

Hematology Data:

TEST	UNITS	RESULT	REFERENCE INTERVAL
RBC	x $10^6/\mu L$	4.39 **L**	5.90 – 8.66
HGB	g/dL	10.7 **L**	13.7 – 20.7
HCT	%	32.4 **L**	42.2 – 59.8
MCV	fL	73.8	63.9 – 75.4
MCHC	g/dL	33.0	32.4 – 35.2
RDW	%	13.4 **H**	11.2 – 13.2
Platelets	x $10^3/\mu L$	57 **L**	226 – 424
MPV	fL	15.6 **H**	8.9 – 14.4
PDW	%	65.2	39.9 – 67.2
PCT	%	0.09 **L**	0.15 – 0.45
MPC	g/dL	23.0	17.2 – 23.6

WBC **4.0 L x $10^3/\mu L$** **4.2 – 12.9**

TEST	UNITS	RESULT	REFERENCE INTERVAL
Segmented Neutrophils	x $10^3/\mu L$	2.000 **L**	2.700 – 8.500
Lymphocytes	x $10^3/\mu L$	1.680	0.500 – 4.100
Monocytes	x $10^3/\mu L$	0.320	0.100 – 1.000

Eosinophils	x $10^3/\mu L$	0.000	0.000 – 1.200
Basophils	x $10^3/\mu L$	0.000	0.000 – 0.045
Others	x $10^3/\mu L$	0.000	0.000 – 0.000
Nucleated RBC	/100 WBC	1	0 - 5

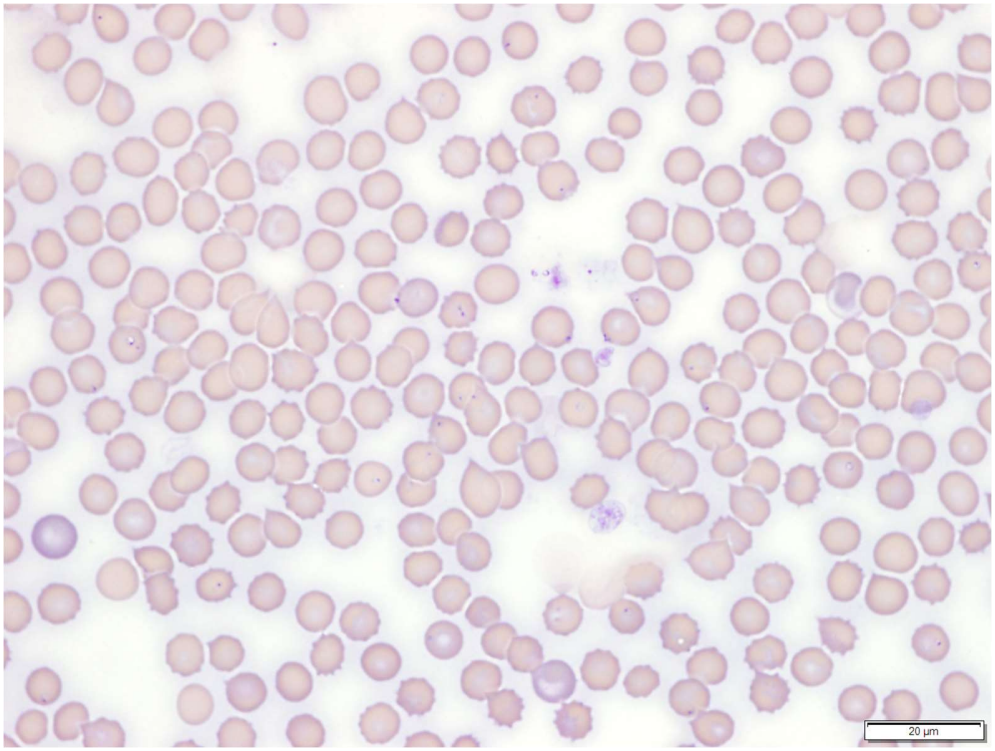

Figure 1. Blood smear from adult FS American Pit Bull Terrier. Wright-Giemsa stain. X100 objective.

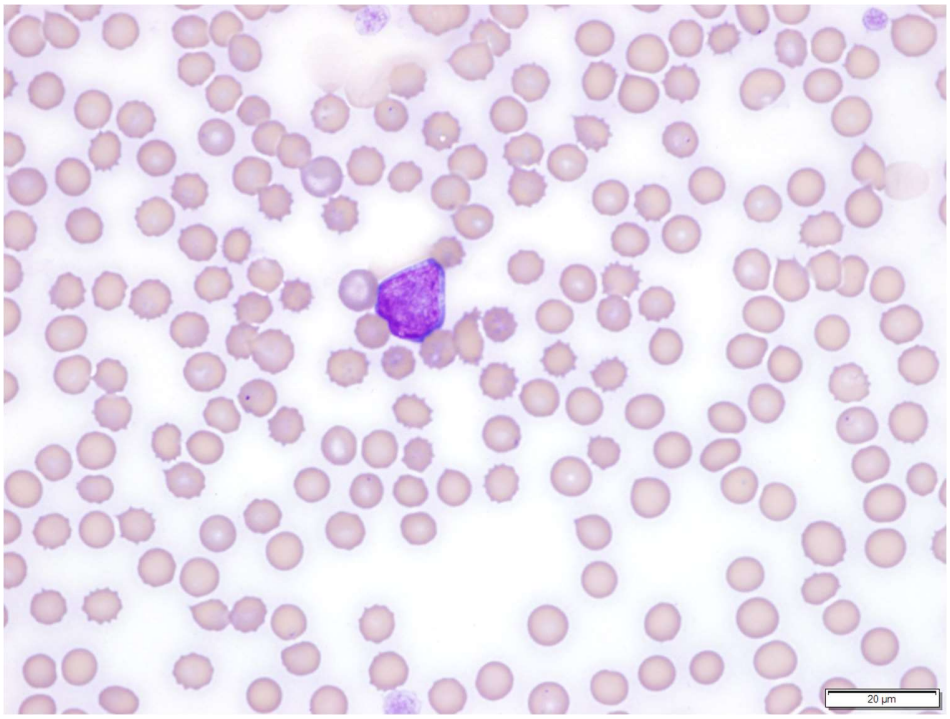

Figure 2. Blood Smear from adult FS American Pit Bull Terrier. Wright-Giemsa stain. X100 objective.

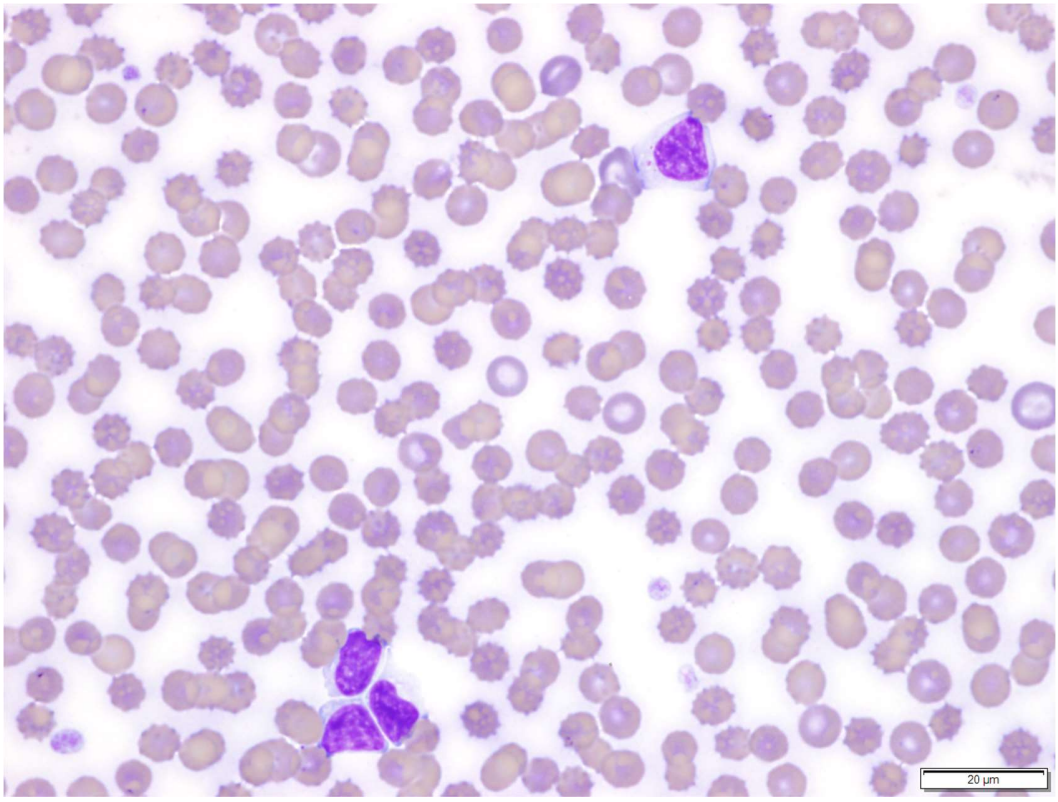

Figure 2. Blood Smear from adult FS American Pit Bull Terrier. Wright-Giemsa stain. X100 objective.

Questions:

1. **What are three differentials for the blood smear findings?**
2. **Given the history, what is the most likely diagnosis in this patient?**

Interpretation: Lymphoma, consistent with circulating phase of lymphoma with small form *Babesia spp.*

Clinical outcome: The blood smear evaluation confirmed the continued presence of atypical lymphocytes, similar to those seen in the blood smear from two weeks prior. The suspected neoplastic population was seen among erythrocytes that frequently contained round piroplasms (1-2 per cell), morphologically consistent with small form *Babesia spp*. At that time, the patient was started on a combination of anti-protozoal and antibiotic medications (atovaquone, azithromycin) for treatment of *Babesia* infection as well as l-asparaginase, due to the concern for circulating neoplastic lymphocytes. At subsequent visits, the patient remained clinically stable with progressive lymphadenopathy and no significant response to multiple chemotherapy treatment protocols. Over the next few weeks, the owners reported declining quality of life and observed the patient's inability to remain comfortable, and euthanasia was elected. A necropsy was performed and revealed disseminated lymphoma within the lymph nodes, liver, spleen, kidneys, and bone marrow. No discussion of babesiosis was mentioned in the necropsy report. PCR on peripheral blood confirmed infection with *B. gibsoni*.

Answers to Questions:

1. Although genetically and clinically distinct, three small piroplasms that cause disease in dogs include *Babesia gibsoni*, *Babesia conradae* and *Babesia vulpes* (formally known as *B. microti-like Theileria* annae and *Baesia* "Spanish isolate'). All three have been reported in the United States. *B. conradae* is

considered to be more pathogenic than *B. gibsoni* and results in higher parasitemia with more severe anemia.[1,5,9]

2. In our case, the most likely diagnosis would be *B. gibsoni*. The signalment (Female spayed American Pit Bull Terrier), unknown history prior to adoption and small form babesiosis identified in the blood smear are findings most consistent with a diagnosis of *B. gibsoni*, especially in the southeastern United States, due to the potential for direct dog-to-dog transmission. [5,9]

Discussion: Canine babesiosis is an important hemoprotozoal, vector-borne disease that results in a variety of clinical manifestations, ranging from subclinical to more severe presentations which classically include thrombocytopenia, hemolytic anemia, and multi-organ dysfunction in infected dogs.[1] Currently, there are over 100 *Babesia* spp. with worldwide distribution, making it the second most common vector-borne blood parasite behind trypanosomes.[2,3] Belonging to the order Piroplasmida, in the phylum Apicomplexa, *Babesia* spp. are intracellular protozoal parasites conventionally described by the morphology of their piroplasms (large >3µm vs. small, <3µm) that employ highly specific survival strategies within erythrocytes to complete their life cycle and secure transmission. [4]

In most *Babesia spp.* infections, animals become infected during the feeding of attached ticks. Tick vectors, (e.g., *Haemaphysalis longicornis* and *Haemaphysalis bispinosa*) spread the protozoal organism via the transmission of sporozoites into the subcutaneous tissue.[2,4] Sporozoites then make their way into the bloodstream where they invade, feed, and initiate reproduction via merogony within the erythrocytes through asexual reproduction, leading to the eventual release of merozoites that invade more erythrocytes. Merozoites then transform into gametocytes, a transient stage, and are uptaken by tick vectors to eventually complete their life cycle, allowing for transmission to susceptible hosts. Additionally, species within the *Babesia sensu stricto* linages ("true *Babesia* spp.," including *B. gibsoni*), utilize tranovarial transmission, ensuring dissemination of *Babesia* from a single infected tick to thousands of offspring.[4,5]

A notable difference to the established vector-borne transmission is demonstrated best by *B. gibsoni*. It has been suggested, with strong supporting evidence, that direct blood-to-blood transmission is possible during dog fights/bite wounds, as the parasite is directly transferred into the bites when blood from an infected dog enters the wound, resulting in mixing of capillary blood. [5,6] Additionally, *B. gibsoni* has become the most commonly diagnosed cause of canine babesiosis in the United States in the absence of a proven competent arthropod vector, with American Pit Bull Terriers and American Pit Bull Terrier mixed breeds being among those most commonly infected.[7] Interestingly, *B. gibsoni* DNA recently has been detected in *Rhipicephalus sanguineus* tick nymph stage, via nested-PCR, in client-owned dogs in Taiwan.[8] *R. sanguineus* (brown dog tick) has long been a proposed vector for *B.gibsoni* and is present in North America. However, further studies of vector competence and detection in North America are required for complete characterization.

Similar to *B. gibsoni*, *Babesia vulpes* may also be directly infectious via dog-to-dog transmission. These two species of *Babesia* have recently been associated with coinfections in similar breeds of dogs (American Pit Bull Terrier and American Pit Bull Terrier Mixes) in North America.[9] In our case, we cannot fully exclude the potential for dual infection in this patient, as the two species are not reliably distinguished via commonly utilized commercially available PCR assays. Screening and differentiation of canine *Babesia* infections through the use of quantitative real-time PCR assays are designed to amplify an 18S rRNA gene, a highly conserved region that amplifies most *Babesia* species via rDNA sequences. However, differences in 18S rRNA gene sequences of more distantly related clades, such as *Babesia senu lato* species which include *B. vulpes*, make design of primers a challenge.[9,10] However, *B. vulpes* DNA was identified and amplified in canine blood and tissue samples recently via a novel PCR assay. Of the 2.9% of dogs infected with *Babesia spp.*in this North American study, 1.7% were infected with *B. gibsoni*, 0.20% were infected with *B. vulpes*, and 0.31% were co-infected with both *B. gibsoni* and *B. vulpes*, demonstrating that *B. vulpes* is occasionally found with other

species and may demonstrate similar transmission strategies as seen with *B. gibsoni*.[9] The patient of this report was diagnosed by a combination of light microscopy and PCR, consistent with the typical diagnostic approach. Recently, a point of care, rapid PCR-based diagnostic test has been evaluated with 100% sensitivity and specificity for *B. gibsoni* detection based on real-time PCR confirmed positive/negative samples. This point-of-care alternative utilizes rapid DNA extraction in the QubeMDx system leading to a faster and more cost-effective diagnosis.[11]

In our case, the patient was an adopted adult Pit Bull Terrier with an unknown history prior to adoption who subsequently developed canine babesiosis parasitemia following initiation of immunosuppressive therapy for lymphoma. This sample was confirmed as *B. gibsoni* via PCR. Speciation was done for prognostic information, as the virulence, response to treatment and prognosis differ between *Babesia* species and subspecies.[12]

This case demonstrates an interesting presentation of a chronic, asymptomatic *B. gibsoni* infection in a commonly associated breed (American Pit Bull Terrier) that became apparent only after the initiation of chemotherapy for disseminated lymphoma. Due to the unknown history, the possibility of previous clinical signs and/or treatment is not known. However, the challenge of successful treatment of *B. gibsoni* is well-documented and has been associated with decreased parasitism without total clearance.[7,13]
Current recommendations for treatment of *B. gibsoni* are administration of azithromycin in combination with atovaquone for 10 days.[13] Recent studies have reported cytochrome b gene mutations associated with atovaquone resistance and treatment failure, although atovaquone combined with azithromycin is still considered the initial treatment of choice.[7] In our patient, the thrombocytopenia improved after combination therapy and the parasite load decreased to a low enough threshold that organisms were not seen on subsequent blood smears 8 and 22 days following treatment initiation. Clinical signs attributable to babesiosis depend largely on the virulence and strain of the parasite and factors that affect the host's ability to respond to an infection, such as age, immune status, and concurrent illness.[13] In this case, both concurrent illness and immunosuppression likely played a major role in developing parasitemia

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