**CASE REPORT**

**A novel form of macrothrombocytopenia in Akita dogs**

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**Key Words**
- Elongated platelets
- Large platelets
- Thrombocytopenia

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**Abstract:** Blood samples from 3 unrelated Akita dogs with a common history of persistent macrothrombocytopenia in the absence of clinical bleeding were sent to the Auburn University College of Veterinary Medicine (AUCVM) Clinical Pathology Laboratory for evaluation. Due to low platelet counts, one Akita dog had been treated with corticosteroids for presumed immune-mediated platelet destruction, and one Akita dog was treated with doxycycline for one month for presumed infection by a tick-borne agent. In spite of treatment, platelet counts remained low in both dogs. Given the absence of abnormal bleeding in all 3 dogs and lack of response to treatment in 2, congenital macrothrombocytopenia was suspected. Interestingly, platelets from all 3 dogs exhibited a consistent elongated platelet morphology. There were no morphologic abnormalities observed in other cell lines. While there have been anecdotal reports of a possible inherited macrothrombocytopenia in Akita dogs, scientific studies have not been done to verify these reports. This manuscript represents the first case report describing what is likely a congenital macrothrombocytopenia in Akita dogs based on persistently low platelet counts in the absence of clinical signs, and characterized by a unique platelet morphology.

**Case Presentation**

This case report includes 3 unrelated healthy Akita dogs. Based on pedigrees, 2 dogs were Japanese Akitas, one Akita (dog 1) living in the US and the other (dog 2) in Japan. Pedigree information was not available for the third Akita dog (dog 3), living in the US. The one Japanese Akita (dog 1) was a 10-month-old spayed female that had a persistent thrombocytopenia (PLT counts ranged from approximately 30,000–80,000/μL, no information available for reference interval [RI] or instrument). Buccal mucosal bleeding time and coagulation screening assays including APTT and PT were within normal limits in dog 1. The Akita living in Japan (dog 2) was a 2.5-year-old neutered male. It had a history of persistent thrombocytopenia ranging from 25,000 to 109,000/μL (provided RI: 175,000–500,000/μL, instrument not specified). The dog was treated with doxycycline twice a day (dose unknown) for one month because of suspicion of tick-borne disease, but the thrombocytopenia persisted.

Blood samples were collected from all 3 Akita dogs into EDTA, and multiple blood smears were prepared immediately by the referring veterinarians. Blood samples and smears were sent to the Auburn University College of Veterinary Medicine (AUCVM). Complete blood cell data were also provided when available. A CBC was performed immediately upon arrival of the sample from dog 1 at the AUCVM using an Advia 120 hematology analyzer in the Clinical Pathology Laboratory (Siemens, Tarrytown, NY, USA). However, no CBC from the other 2 Akita dogs could be determined due to the prolonged shipping time of the blood. Multiple blood smears were prepared from all 3 samples upon arrival at the laboratory.

The PLT count for dog 1 (Table 1), obtained on the Advia 120 hematology analyzer, was 81,000/μL (RI: 164,000–510,000/μL), and the plateletcrit was 0.19%. Platelet counts from CBC data provided by the
referring veterinarians were 81,000/uL (provided RI 175,000–500,000/μL, instrument not specified) for dog 2 (Table 1) and 47,000/μL (provided RI 200,000–500,000/μL) for dog 3 (Table 1). Submitted blood smears were used for evaluation of platelets. Platelet counts were estimated by multiplying an average of platelet numbers in 10 different fields using a ×100 objective by 20,000. Platelet density on blood smears was mildly to moderately decreased, ranging from 80,000 to 140,000/μL in all blood smears evaluated. There were many large elongated platelets (Figure 1) mixed with variable numbers of normal-sized platelets and large round platelets. Rarely, there were long linear platelets (40–70 μm) with a morphology similar to proplatelets (Figure 1). No morphologic abnormalities were observed in other cell lines. No inclusions were seen in leukocytes.

Based on the findings of persistent thrombocytopenia in these Akita dogs in the absence of clinical signs or response to treatment, a congenital macrothrombocytopenia was considered likely. This hypothesis was supported by the consistent observation of large and elongated platelets on blood smears. To screen for the presence of the 2 known mutations in the gene encoding β1 tubulin documented to cause congenital macrothrombocytopenia in Cavalier King Charles Spaniels (CKCS)1–4,7 and Norfolk and Cairn Terriers5, genomic DNA was extracted from EDTA blood samples collected from all 3 Akita dogs using a commercially available kit (QuickGene DNA Whole blood kit 5; Kurabo Industries Ltd., Osaka, Japan). Primers were designed based on the available canine genome sequence in GenBank for amplification of the coding region of the β1-tubulin gene in an overlapping fashion, including splice sites. Polymerase chain reaction (PCR) products were separated by electrophoresis on agarose gels, extracted (QIAtquick Gel Extraction Kit; Qiagen, Valencia, CA, USA), and submitted to the Genomics and Sequencing Laboratory at Auburn University, AL for sequence determination. The determined genomic sequences of the β1-tubulin gene in all dogs matched the GenBank dog genome sequence, and no substitutions, deletions, or other mutations were identified.

Table 1. Hematologic results from Akita dogs 1, 2 and 3.

<table>
<thead>
<tr>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC*</td>
<td>CBC**</td>
<td>CBC**</td>
</tr>
<tr>
<td>RBC (x 10⁶/μL)</td>
<td>7.22</td>
<td>33.5</td>
</tr>
<tr>
<td>HCT [%]</td>
<td>48.8</td>
<td>11.8</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>14.4</td>
<td>7.3</td>
</tr>
<tr>
<td>WBC (x 10⁶/μL)</td>
<td>7.99</td>
<td>6.17</td>
</tr>
<tr>
<td>PLT (x 10⁳/μL)</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>23.7</td>
<td>164-510</td>
</tr>
<tr>
<td>*Advia 120 hematology analyzer.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>**Analyzer unknown, Reference Interval provided by referring veterinarian.</td>
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Figure 1. (A–C) Peripheral blood smears from 3 Akita dogs with macrothrombocytopenia. Wright–Giemsa, × 100 objective. (A) Large elongated platelet and a normal-sized platelet (arrow). (B) Long linear platelet that is folded in the middle. (C) Long linear platelet and a normal-sized platelet (arrow).
Discussion

Akita dogs originate from the Akita prefecture in the northern part of the Island of Honshu in Japan. Akita dogs can be divided into Japanese Akitas and American Akitas, which are different from each other in terms of body size, shape, and coat color. In some countries or breed associations such as the Australian National Kennel Council (ANKC), Fédération Cynologique Internationale (FCI), Japanese Kennel Club (JKC), Kennel Club (KC), and New Zealand Kennel Club, the Japanese Akitas and American Akitas are considered 2 distinct breeds, while the American Kennel Club (AKC) and the Canadian Kennel Club (CKC) do not separate them.

Our 3 cases, including 2 Japanese Akitas and one Akita dog of unknown origin exhibited thrombocytopenia and abnormal platelet morphology. Because one Akita dog did not have a pedigree we cannot determine whether our findings are restricted to Japanese Akitas. Importantly, anecdotal reports from various clinicians in the US and Japan as well as from breeders and owners of Akita dogs have described persistently low platelet counts in some dogs that were not associated with clinical signs. The prevalence of the disorder in Akita dogs, however, is not known.

So far, congenital macrothrombocytopenia has not been documented in Akita dogs. The best documented asymptomatic congenital canine macrothrombocytopenia is the one reported in CKCS. That highly prevalent congenital macrothrombocytopenia has been related to a mutation in the gene encoding β1 tubulin. However, in our studies we could not confirm mutations in the β1 tubulin gene in these 3 Akita dogs. May–Hegglin anomaly, another type of macrothrombocytopenia, has been documented in a dog. However, this seemed an unlikely anomaly in the 3 Akita dogs considering the lack of cytoplasmic inclusions within leukocytes, and the distinct elongated platelet morphology.

Different causes for the unusual platelet morphology and low platelet counts must be considered. Platelet formation is dependent on interactions of various proteins including microtubules and accessory proteins. Potential candidate genes are presently being investigated in an effort to determine the genetic basis for this disorder.

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References