WHAT IS YOUR DIAGNOSIS?

# What is your diagnosis? Abnormal platelets dot plot from a dog

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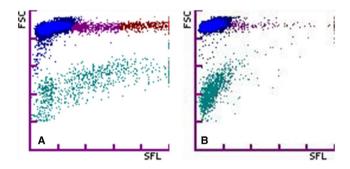
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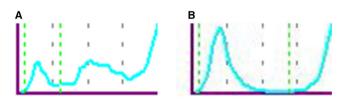
## 1 | CASE PRESENTATION

A 1.5-year-old intact male Jack Russell terrier dog was presented for acute weakness and moderate diarrhea. The dog had been to Gabon, in Central Africa, with his owner for 6 months and was presented 15 days after returning to France for a clinical examination. Physical examination only showed pale mucous membranes.

A blood specimen was collected in an EDTA tube, and a complete CBC was performed with the Sysmex XT 2000 iV hematology analyzer (Sysmex, Kobe, Japan). The CBC revealed a marked macrocytic (MCV 80 fL; RI 60-71 fL), hypochromic (MCHC 28.3 g/dL; RI 34.4-38.1 g/dL), regenerative (reticulocytes  $142.7 \times 10^{9}$ /L; RI 19.4-150.1 × 10<sup>9</sup>/L) anemia (RBC:  $2.8 \times 10^{12}$ /L, RI 5.2-7.9 ×  $10^{12}$ /L; Hemoglobin: 6.4, RI 12.4-19.2 g/dL; hematocrit: 22.6%, RI 35%-52%), marked neutropenia ( $0.2 \times 10^{9}$ /L; RI 2.9-13.6 ×  $10^{9}$ /L), slight eosinopenia ( $0.1 \times 10^{9}$ /L; RI 0.1-13.6 ×  $10^{9}$ /L), and marked thrombocytopenia with the optical count (PLT-O:  $16 \times 10^{9}$ /L; RI 108-562 ×  $10^{9}$ /L) not confirmed by the impedance count (PLT-I: 73 ×  $10^{9}$ /L; RI 64-613 ×  $10^{9}$ /L).<sup>1</sup> The platelet flow cytometry dot plot and impedance histogram are shown in Figures 1 and 2, respectively.



**FIGURE 1** Sysmex XT-2000iV hematology analyzer optical platelet dot plot from a Jack Russell terrier dog with pale mucous membranes (A) and a healthy dog (B)



**FIGURE 2** Platele impedance histogram from a Jack Russell terrier dog with pale mucous membranes (A) and a healthy dog (B)

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**Cytologic interpretation**: Erroneous platelet counts with an abnormal platelet population on the scattergram and histogram due to organisms compatible with trypanosomes.

The normal banana-shaped platelet dot plot was more extended in this patient's blood compared with the blood from a healthy dog, with two diffuse but distinct populations. Similarly, the patient's impedance platelet histogram had two populations smaller than RBC, but the second population was included in the RBC population by the analyzer. A blood smear examination revealed numerous small (7-10  $\mu$ m of length), flagellated, extracellular organisms with undulating membranes identified as the trypanosome trypomastigote stage, and small round organisms (2-4  $\mu$ m in diameter) containing granular light blue cytoplasm and large magenta nuclei, identified as the trypanosome amastigote stage. Trypanosomes appear similar to platelets in size and internal complexity but not in color (Figure 3). Platelets were not observed in the blood smear.

## 2 | DISCUSSION

In humans, erroneously high platelet counts were reported with cell fragments from red blood cells or leukemic cells, microorganisms, lipids, cryoglobulins, air bubbles, or debris.<sup>2</sup> In veterinary medicine, erroneous platelet counts were reported in cases of immune-mediated hemolytic anemia in a dog and leek intoxication in a cat associated with RBC fragmentation and Heinz bodies, respectively.<sup>3,4</sup>

In our case, the abnormal platelet dot plot was suggestive of the presence of "particles" other than platelets, and platelets were not found during the blood smear examination. The blood smear examination revealed two forms of the extracellular organisms: a flagellated (trypomastigote) form and round form (amastigotes).

Three morphologic stages of trypanosomes (epimastigote, trypomastigote, and amastigote) occur during the life cycle of the parasite. The trypomastigote represents the infectious stage. Trypomastigotes are spindle-shaped, flagellated, 15-20  $\mu$ m long and found in the blood. After hematogenous spread, the trypomastigotes enter macrophages and myocytes where they transform into the amastigote stage.<sup>5,6</sup> This intracellular form is round to oval and measures approximately 1.5-4  $\mu$ m in diameter. This parasitic stage forms tissue pseudocysts and transforms into trypomastigotes before rupturing and releasing from infected cells. The trypomastigotes then enter the blood.<sup>6</sup>

In our case, two forms were observed in the blood smear and, as previously described in a case report, this could be due to timerelated morphologic changes in the trypomastigotes because of the delay between blood sampling and blood smear preparation (in vitro transformation of the trypomastigote stage to the amastigote stage).<sup>6</sup> In our case, more than 24 hours had elapsed between sampling and analysis.

We presumed that, as in babesiosis, the nucleic acid from the parasites could be identified by the analyzer.<sup>7,8</sup> Trypanosomes are extracellular protozoans and, depending on their size, can fluoresce similarly to that of platelets or giant platelets. The platelet scattergram was characterized by two separate "populations." We speculate that the round form represents the first "population," located on the left side, and the flagellate form represents the second "population."

The round, small-sized amastigotes probably represent the left smaller Gaussian peak on the PLT-histogram, and the elongated flagellate trypomastigotes represent the second peak on the right measured as small RBCs.

FIGURE 3 A photomicrograph of a blood smear from a Jack Russell terrier dog with pale mucous membranes. The blood smear revealed small, flagellated, extracellular organisms with undulating membranes suggestive of the trypanosome trypomastigote stage (black arrows) and round organisms with granular light blue cytoplasm and large magenta nuclei, suggestive of the trypanosome amastigote stage (red arrows)

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## DISCLOSURE

The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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## KEYWORDS

CBC, dog, flow cytometry, impedance, platelet, scattergram, spurious platelet count, trypanosomiasis

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