

Macrothrombocytopenia in a Cavalier King Charles Spaniel

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A 6-year-old neutered Cavalier King Charles spaniel was presented with a 2-week history of difficulty climbing stairs and jumping into the car.

History

The Cavalier King Charles spaniel (CKCS) had a previous diagnosis of a grade III/VI left systolic heart murmur.

Physical Examination

The heart murmur as previously described was detected. Instability in the left stifle was also noted. The remainder of the examination was unremarkable.

Laboratory Evaluation

CBC was unremarkable with the exception of an automated platelet count of 2350/ μL (reference interval, $181\text{--}525 \times 10^3/\mu\text{L}$; Cell-Dyn 3500 hematology analyzer [abbott.com] using impedance technology for platelet count). A blood smear was evaluated.

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Ask Yourself



1. What should you take into consideration when evaluating platelet counts in CKCSs?
2. What is a potential pitfall with automated platelet counts?
3. What test could you complete in your clinic to verify the automated platelet count?

Diagnosis

Inherited Macrothrombocytopenia

Platelets that are the same size as or larger than an erythrocyte are called *macroplatelets* or *macrothrombocytes*.¹ Macroplatelets were identified in this patient (Figures 1 and 2). While occasional macroplatelets can be present in normal dogs, increased numbers have been associated with certain breeds.¹ CKCSs have a high prevalence of an inherited genetic mutation, which affects early platelet formation and results in an increased percentage of macroplatelets and thrombocytopenia; this clinical condition is termed *inherited macrothrombocytopenia*.² The prevalence of inherited macrothrombocytopenia in the US population of CKCSs is reported to be 30% to 50%.

It is important to note that these dogs may mistakenly be considered to have an underlying disease that results in thrombocytopenia, such as immune-mediated thrombocytopenia, tick-borne infection, or a bone marrow disorder. Unlike other dogs with platelet counts of 30,000/ μ L or lower, CKCSs with the mutation do not have signs of spontaneous mucocutaneous hemorrhage because their overall platelet function is normal.^{2,3} Platelet function is believed to depend more on total platelet mass (number \times volume) than platelet number alone. The platelet mass of CKCSs with inherited macrothrombocytopenia is reportedly similar to CKCSs without the mutation (normal platelet count and no large platelets).^{4,5}

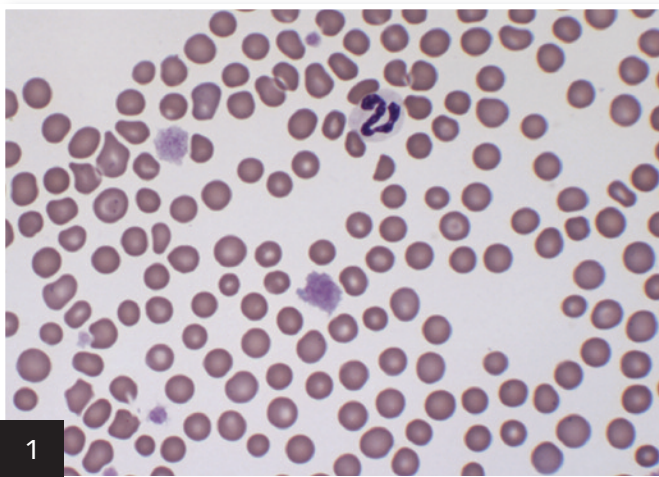
Detecting Genetic Mutations

An identical inherited genetic mutation has been documented in other breeds, including Chihuahua, Labrador retriever, poodle, English toy spaniel, labradoodle, shih tzu, Maltese, Jack Russell terrier; the mutation has been suspected in a group of related Norfolk terriers.⁷ The Department of Pathobiology at Auburn University (vetmed.auburn.edu/diagnostics) offers a DNA assay to determine the presence or absence of the mutation correlated with inherited macrothrombocytopenia.

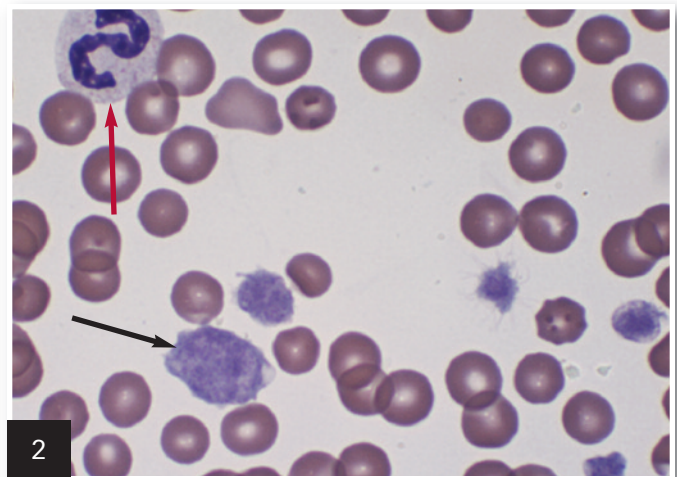
The presence of macroplatelets can also reflect increased platelet production, with marrow release of large, immature platelets in response to ongoing or resolving thrombocytopenia. In addition, feline platelets are more variable in size than other domestic species, and healthy cats often have macroplatelets.¹

Systems & Techniques

A potential problem with automated hematology systems (impedance and flow cytometry technology) is the reporting of falsely low platelet counts because the analyzer does not recognize, and therefore count, large platelets or individual platelets that comprise a clump. Some commercial hematology analyzers used at reference laboratories have reported better success at identifying large platelets, which would provide a more accurate platelet count and a better estimation of platelet mass.⁵



1 Patient blood smear. (Wright-Giemsa stain; 500 \times original magnification)



2 Note variation in platelet size. One macroplatelet is approximately 2 \times the size of an erythrocyte (**black arrow**). A segmented neutrophil is present in the top left corner (**red arrow**). (Wright-Giemsa stain; 1000 \times original magnification)

CKCS = Cavalier King Charles spaniel

In addition, a manual platelet count may be performed at some reference laboratories, using a hemocytometer. If platelet clumps are identified in the hemocytometer counting chamber, the technician can roughly estimate how many platelets are comprised within the clump as well as identify macroplatelets.

Unfortunately, the technique of performing a manual platelet count requires practice, which may not be a practical option for most clinics. For this case, a manual count was performed and reported as 66,000/ μL , markedly increased from the impedance automated analyzer platelet count of 2350/ μL but still consistent with thrombocytopenia. Using manual platelet counts or hematology instruments that utilize flow cytometry, reported platelet counts in healthy CKCSs with the mutation (without evidence of bleeding) have been reported to range from 30,000 to 200,000/ μL .^{4,2}

In practice, the best way to verify an automated platelet count is to perform a blood smear platelet estimate. Platelet estimation should be performed on a blood smear made from a freshly collected blood sample, ideally obtained by nontraumatic venipuncture, as platelets can become activated and clump as the blood is flowing from the vessel into the syringe or collection tube.

Estimation & Identification

Platelet estimation should begin by evaluating the feathered edge of the smear for platelet clumps using the 10 \times objective

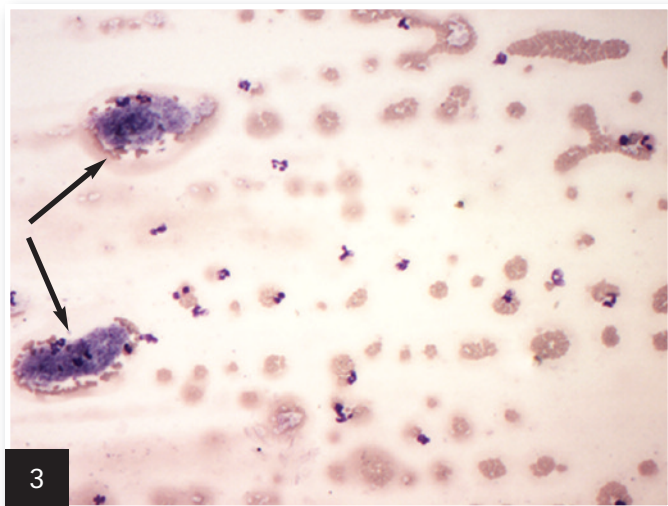
(Figure 3), which can lead to an underestimation of the platelet count. Next, the blood smear monolayer should be identified with the 10 \times objective. The monolayer of the blood smear is the area directly behind the feathered edge, where red cells touch occasionally but do not pile on top of one another (Figure 4). Once the monolayer has been identified, a drop of immersion oil is placed on the slide and the 100 \times oil objective is used to screen 10 separate viewing fields. A new viewing field should only require a subtle directional movement of the slide, but each viewing field should represent an entirely new area of the slide that is still within the monolayer.

Platelets are usually 25% to 50% the size of an erythrocyte and have light blue to gray cytoplasm with irregular edges, or sometimes spiderlike cytoplasm extensions.⁶ Platelets will often have bright magenta granules but some platelets may appear degranulated, which likely reflects recent activation.¹

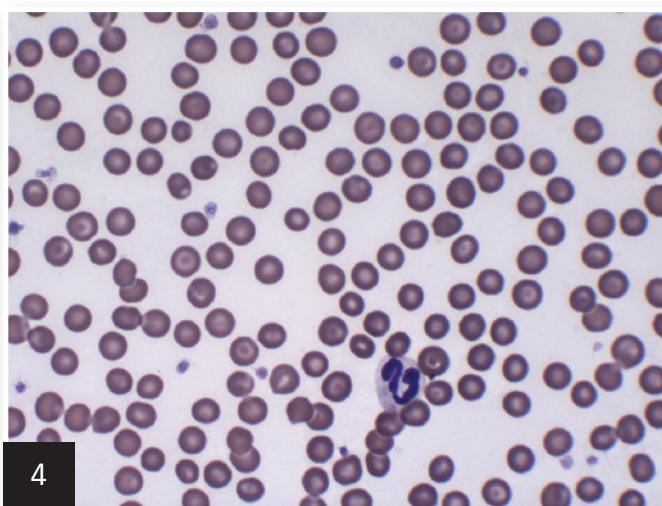
The average number of platelets per 10 fields is multiplied by 20,000 to give a final estimate of the number of platelets per

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Feline blood smear; note large platelet clumps along the feathered edge of the smear (arrows). (Wright-Giemsa stain; 100 \times original magnification)



Blood smear from a dog without macrothrombocytopenia and a normal hematocrit and platelet count. This image represents the blood smear monolayer, and platelets are significantly smaller than surrounding erythrocytes. (Wright-Giemsa stain; 500 \times original magnification)

microliter. The normal platelet count for dogs and cats is approximately 200,000 to 500,000 platelets/ μ L. If 200,000/ μ L is divided by the conversion factor of 20,000, the result is 10 platelets per field using the 100 \times oil objective.


Follow-up

As the dog did not have signs to suggest an underlying bleeding disorder or a hypercoagulative state, thrombocytopenia and macroplatelets were presumed to be a result of inherited macrothrombocytopenia. The dog was also diagnosed with a cranial cruciate tear of the left stifle.

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Thrombocytopenic or Not?

- The patient should have a minimum of 10 platelets per 10 fields of view when scanning the monolayer with 100 \times oil objective.
- If the platelet number is lower than 10 and platelet clumps were not appreciated, it should be suspected that the patient is truly thrombocytopenic.
- If platelet clumps were appreciated and the platelet estimate is only mildly decreased, it is likely that the patient has a normal platelet count.



See Aids & Resources, back page, for references & suggested reading.

CKCS = Cavalier King Charles spaniel

Did You Answer



1. Approximately 30% to 50% of the US CKCS population has inherited macrothrombocytopenia.
2. Automated hematology analyzers may not recognize and count large platelets and platelet clumps, resulting in a falsely decreased platelet count.
3. A platelet estimate could be completed from a blood smear to verify automated platelet counts.

TRIFEXIS™

(spinosad + milbemycin oxime)

Chewable Tablets

Before using TRIFEXIS chewable tablets, please consult the product insert, a summary of which follows:

Caution: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Indications:

TRIFEXIS is indicated for the prevention of heartworm disease (*Dirofilaria immitis*), TRIFEXIS kills fleas and is indicated for the prevention and treatment of flea infestations (*Ctenocephalides felis*), and the treatment and control of adult hookworm (*Ancylostoma caninum*), adult roundworm (*Toxocara canis* and *Toxascaris leonina*) and adult whipworm (*Trichuris vulpis*) infections in dogs and puppies 8 weeks of age or older and 5 pounds of body weight or greater.

Contraindications:

There are no known contraindications to the use of TRIFEXIS Chewable Tablets.

Warnings:

Not for human use. Keep this and all drugs out of the reach of children.

Serious adverse reactions have been reported following concomitant extra-label use of ivermectin with spinosad alone, one of the components of TRIFEXIS Chewable Tablets (see **ADVERSE REACTIONS**).

Precautions:

Treatment with fewer than 3 monthly doses after the last exposure to mosquitoes may not provide complete heartworm prevention (see **EFFECTIVENESS**).

Prior to administration of TRIFEXIS, dogs should be tested for existing heartworm infection. At the discretion of the veterinarian, infected dogs should be treated with an adulticide to remove adult heartworms. TRIFEXIS is not effective against adult *D. immitis*. While the number of circulating microfilariae may decrease following treatment, TRIFEXIS is not indicated for microfilariae clearance. Mild, transient hypersensitivity reactions manifested as labored respiration, vomiting, salivation and lethargy, have been noted in some dogs treated with milbemycin oxime carrying a high number of circulating microfilariae. These reactions are presumably caused by release of protein from dead or dying microfilariae.

Use with caution in breeding females. The safe use of TRIFEXIS in breeding males has not been evaluated. Use with caution in dogs with pre-existing epilepsy. Puppies less than 14 weeks of age may experience a higher rate of vomiting.

Adverse Reactions:

In a well-controlled US field study, which included a total of 352 dogs (176 treated with TRIFEXIS chewable tablets and 176 treated with an active control), no serious adverse reactions were attributed to administration of TRIFEXIS chewable tablets. All reactions were regarded as mild.

Reactions that occurred at an incidence >2% (average monthly rate) within any of the 6 months of observation are presented in the following table:

Average Monthly Rate (%) of Dogs With Adverse Reactions

Adverse Reaction	TRIFEXIS Chewable Tablets*	Active Control Tablets*
Vomiting	6.13	3.08
Pruritus	4.00	4.91
Lethargy	2.63	1.54
Diarrhea	2.25	1.54

*n=176 dogs

In the US field study, one dog administered TRIFEXIS experienced a single mild seizure 2½ hours after receiving the second monthly dose. The dog remained enrolled and received four additional monthly doses after the event and completed the study without further incident.

Following concomitant extra-label use of ivermectin with spinosad alone, a component of TRIFEXIS, some dogs have experienced the following clinical signs: *trembling/twitching, salivation/cooling, seizures, ataxia, mydriasis, blindness and disorientation*. Spinosad alone has been shown to be safe when administered concurrently with heartworm preventatives at label directions.

In US and European field studies, no dogs experienced seizures when dosed with spinosad alone at the therapeutic dose range of 13.5-27.3 mg/lb (30-60 mg/kg), including 4 dogs with pre-existing epilepsy. Four epileptic dogs that received higher than the maximum recommended dose of 27.3 mg/lb (60 mg/kg) experienced at least one seizure within the week following the second dose of spinosad, but no seizures following the first and third doses. The cause of the seizures observed in the field studies could not be determined.

For technical assistance or to report an adverse drug reaction, call 1-888-545-5973. Additional information can be found at www.TRIFEXIS.com.

Effectiveness:

Heartworm Prevention:

In a well-controlled laboratory study, TRIFEXIS was 100% effective against induced heartworm infections when administered for 3 consecutive monthly doses. Two consecutive monthly doses did not provide 100% effectiveness against heartworm infection. In another well-controlled laboratory study, a single dose of TRIFEXIS was 100% effective against induced heartworm infections. In a well-controlled six-month US field study conducted with TRIFEXIS, no dogs were positive for heartworm infection as determined by heartworm antigen testing performed at the end of the study and again three months later.

Flea Treatment and Prevention:

In a well-controlled laboratory study, TRIFEXIS demonstrated 100% effectiveness on the first day following treatment and 100% effectiveness on Day 30. In a well-controlled laboratory study, spinosad, a component of TRIFEXIS, began to kill fleas 30 minutes after administration and demonstrated 100% effectiveness within 4 hours. In field studies conducted in households with existing flea infestations of varying severity, flea reductions of 98.0% to 99.8% were observed over the course of 3 monthly treatments with spinosad alone. Dogs with signs of flea allergy dermatitis showed improvement in erythema, papules, scaling, alopecia, dermatitis/pyodermitis and pruritus as a direct result of eliminating the fleas.

Treatment and Control of Intestinal Nematode Infections:

In well-controlled laboratory studies, TRIFEXIS was \geq 90% effective in removing naturally and experimentally induced adult roundworm, whipworm and hookworm infections.

NADA #141-321, Approved by the FDA

Manufactured for Elanco Animal Health

A Division of Eli Lilly & Co.

Lilly Corporate Center, Indianapolis, IN 46285

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