

# NATIONAL VETERINARY LABORATORY

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## NEWSLETTER

### What's On Your Plate---lets? *Bartonella*?<sup>©</sup>

Evelyn E. Zuckerman, Editor

Winter 2009

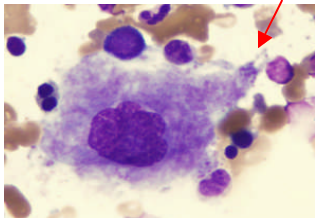
Vol. 8, Number 1

#### In This Issue:

In the winter 2009 issue of the NVL Newsletter we will discuss *Bartonella* effects on platelets of cats, dogs, and humans. Yes, what's on your platelets may be important for the health of your patients or you. Platelets are not merely passive plugs floating in the blood stream waiting for a break in a blood vessel to assist in clotting. They are very important in the every day maintenance of blood vessel integrity. From skin bruises or purpura, bloody noses (epistaxis), hematochezia (bloody stool), and melena, platelets are central to a healthy vascular system.

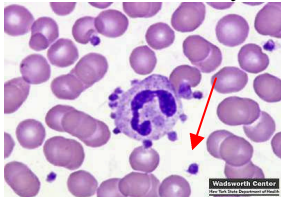
#### Platelets:

Platelets are irregular disk-shaped cytoplasmic fragments of megakaryocytes which are shed in the bone marrow and eventually end up in the peripheral blood where they function in clotting.



Megakaryocyte releasing platelets (arrow). A megakaryocyte can produce 1,000 platelets.

Platelets do not possess a nucleus, have no hemoglobin and are about one-fifth to one-third the size of a red blood cell. They have granules



Platelets in the peripheral blood.

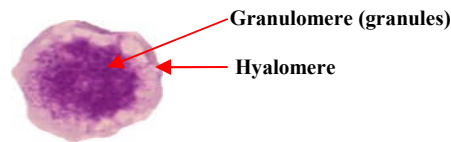
in the central region (granulomere) and a clear periphery (hyalomere). Platelets are the main elements that help to maintain blood circulation by controlling hemorrhage after injury to blood vessel endothelial cells. Membrane-associated events during clotting of subendothelial adhesion of platelets, platelet granule release, cohesion, aggregation, and plug formation is provided by ligand-receptor interactions.<sup>1</sup>

We will not cover clotting here. Instead, we will concentrate on the interaction between platelets and endothelial cells of blood vessels.

Understanding this mechanism will allow us to understand the pathogenesis of *Bartonella* infection which alters the balance between these cells leading to hemorrhages in various tissues. The bone marrow appears to be an early site for *Bartonella* replication in humans.<sup>2</sup>

#### Endothelial Cell Megakaryocyte Platelet Axis:

Endothelial cells and megakaryocytes are the only cells in the bone marrow that synthesize von Willebrand factor, a glycoprotein whose primary function is binding to other proteins, particularly Factor VIII, which acts like glue to help the platelets stick together and form a blood clot.<sup>3</sup> In the bone marrow, endothelial cells and megakaryocytes define a specific vascular niche where hematopoietic precursors and sinusoidal endothelial cells interact directly.<sup>4</sup> Platelets are shed from megakaryocytes that have direct contact with sinusoidal endothelial cells.<sup>4</sup> The endothelial cells in the bone marrow produce trophogens (factors) that influence megakaryocytes to produce platelets. In turn, megakaryocytes and platelets produce different trophogens (factors) that help to maintain the structure and stability of endothelial cells which maintain the integrity of blood vessels.



**Platelet: Granulomere is the central zone containing trophogen granules.**

In the peripheral blood, the most important interaction of platelets and the endothelial cells lining blood vessels is the maintenance of vascular integrity by the constitutive release of proangiogenic cytokines and growth factors (called trophogens) from platelets. These molecules are stored in the platelet granules and bind to specific receptors on the surface of endothelial cells which act to stabilize their intercellular junctions and maintain integrity of the blood vessels.<sup>5</sup>

#### Clinical Implications of Platelets:

##### Disorders of Platelet Numbers:

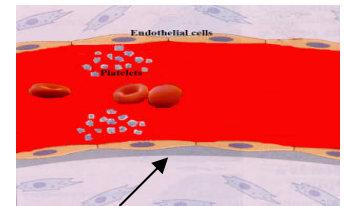
1. Thrombocytosis: A rare condition where the bone marrow produces too many platelets. This may cause an increased risk of clotting.
2. Thrombocytopenia: Too few platelets can be caused by the failure of the bone marrow to produce normal numbers of platelets or the increased

destruction of platelets after they are released into the circulating blood. Causes:

**Decreased platelet production-**1) Drugs such as Clarithromycin, Ganciclovir, Fluconazole, Amphotericin B and others, 2) Deficiencies- folate and vitamin B12, 3) Infection- *Bartonella*, HIV, Parvovirus B19, and *Mycobacterium tuberculosis*.

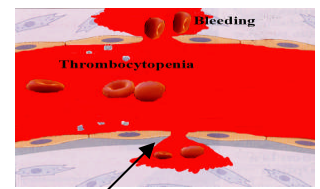
**Increased platelet destruction-** immune thrombocytopenia purpura, and thrombotic thrombocytopenic purpura and hypersplenism.

The complex platelet-endothelial cell interactions are maintained continuously under healthy conditions and the blood vessels do not leak blood into tissues.



**Vascular homeostasis- normal number of platelets produce trophogens to maintain intact endothelial intercellular junctions (arrow).**

Interruption of the interactions can occur by marked thrombocytopenia or impaired platelet granule (trophogen) release caused by aspirin. A severe reduction in platelets numbers causes a reduction in the platelet derived trophogen which leads to failure of the intercellular zippers holding endothelial cells of blood vessels together. The resulting loss of vessel integrity causes bleeding into the tissues.



**Thrombocytopenia: Due to their lower numbers, platelets cannot produce enough trophogens to maintain endothelial intercellular junctions- thus bleeding into tissues occurs (arrow).**

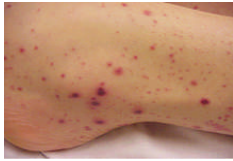
#### *Bartonella*- Platelet Disorders:

##### Humans:

Bleeding and platelet disorders, caused by *Bartonella*, were first described in humans with Oroya Fever in 1886 and in cat scratch disease in 1952.<sup>6,7,8</sup> Since then there have been many reports of similar observations in *Bartonella* infected people.<sup>9,10</sup> Low platelet numbers may lead to bleeding, petechiae, purpura and Henoch-Schönlein purpura (HSP).<sup>9</sup>

## Clinical Signs of Thrombocytopenia:

- Epistaxis
- Multiple small bruises
- Purpura
- Gingival bleeding
- Hematuria
- Excess vaginal bleeding
- GI bleeding- melena
- Mucosal spontaneous bleeding
- Increased cutaneous bleeding
- Prolonged bleeding after tooth extractions



**Purpura- human ankle.**

HSP is a rare disease of small blood vessels in the skin and kidneys that leak because of systemic inflammation characterized by deposition of immune complexes containing IgA antibody. It occurs mainly in young children, tends to last 4 weeks and resolves spontaneously. The purpura typically appears on the legs and buttocks, but may also be seen on the arms, face and trunk. Although several infectious agents are associated with HSP, the pathogenesis of *Bartonella*-associated HSP is unknown.<sup>9,10</sup> Platelet disorders occur in <5% of *Bartonella* infected people.

## Dogs:

Platelet disorders occur far more often in *Bartonella* infected dogs than in people or cats (see following Tables). Epistaxis, excess bleeding from surgical wounds, bleeding gums, and skin petechiae are the result of the *Bartonella*-associated thrombocytopenia.<sup>11</sup>

### Occurrence of Thrombocytopenia (TP) in *Bartonella* Infected Dogs and Cats

Species	# Infected	# TP	%
Dog	656	32	5%
Cat	84,081	40	0.05%

### Occurrence of Epistaxis in *Bartonella* Infected Dogs and Cats

Species	# Infected	# Epistaxis	%
Dog	656	3	0.4%
Cat	84,081	18	0.02%

### Dogs and Cats with Thrombocytopenia (TP) who were Infected with *Bartonella*

Species	# TP	# Infected	%
Dog	260	32	12%
Cat	80	40	50%

### Dogs and Cats with Epistaxis who were Infected with *Bartonella*

Species	# Epistaxis	# Infected	%
Dog	24	3	12%
Cat	30	18	60%

### Multiple Sites of Bleeding, Anemia, and Thrombocytopenia in 2 Dogs.

**Dr. Steven Petcher**

**Citronelle Veterinary Clinic, Citronelle, AL**

**Case 1:** A 2 year-old female Boxer-cross presented with heavy flea infestation, severe weakness, vaginal bleeding, petechiae and ecchymosis of the groin (pictured).



***Bartonella* infected dog from the south with thrombocytopenia and severe abdominal bruising-ecchymosis. Courtesy Dr. Steven Petcher**

The dog was anemic, had a moderate leukocytosis, and a profound thrombocytopenia (<10,000 platelets/ $\mu$ L). Serology for *Anaplasma*, Lyme, *Babesia* and *Ehrlichia* were negative but the dog had a positive titer of 1:64 for RMSF and the *Bartonella* FeBart<sup>®</sup> western blot was a strong +4 positive. The dog was treated for 6 weeks with Doxycycline and made a rapid and complete recovery. The hemogram returned to normal (422,000 platelets/ $\mu$ L) by day 18 of therapy.

**Case 2:** A 10 year-old male Labrador Retriever presented comatose with petechiae and ecchymoses of the groin, and gums. Laboratory findings were anemia, thrombocytopenia (15,000 platelets/ $\mu$ L), elevated liver enzymes and elevated BUN and creatinine. Serology for *Anaplasma*, Lyme, and *Ehrlichia* were negative but the dog had a positive titer of 1:256 for RMSF and the *Bartonella* FeBart<sup>®</sup> western blot was a strong +4 positive. The dog was treated for 6 weeks with Doxycycline and made a complete recovery. The post therapy comparative titer test showed an 8 fold titer deduction (1:512,000 to 1:64,000) indicating eradication of the *Bartonella*.

Ten of 16 (63%) dogs with thrombocytopenia and bleeding disorders were seropositive for *Bartonella* from Dr. Petcher's practice in Alabama.

## Cats:

As discussed previously, cats appear to have fewer *Bartonella* associated platelet disorders compared to people and dogs. This seems unusual since the cat's bone marrow is more sensitive to injury as cat erythrocytes have a 25% shorter life span. In addition, more cats are infected carriers of *Bartonella* than dogs or humans.

### Multiple Sites of Inflammation, Thrombocytopenia, and Purpura in a Cat.

**Dr. Becky Richardson**  
**Cascade Animal Medical Center**  
**Rochester, Minnesota**

A 6 month-old male kitten, adopted from a local shelter, was presented with lethargy, fever of 104.4°F, and petechiae on both ears. Clinical examination revealed gingivitis, anemia, chorioretinitis and uveitis. Laboratory findings were: FeLV and FIV negative, *Toxoplasma* titer negative, a normal hemogram except for a mild thrombocytopenia (130,000 platelets/ $\mu$ L) and a positive +3 *Bartonella* FeBart<sup>®</sup> test. The kitten was treated with Azithromycin for 21 days. There was rapid clinical improvement, the platelet count returned to normal (>300,000/ $\mu$ L) by day 5 of therapy. However, 4 months later there was recurrence of the thrombocytopenia and ocular disease. Upon the second round of therapy, consisting of Azithromycin for 40 days and Doxycycline for 21 days, the thrombocytopenia and all oral, ocular and skin inflammatory lesions

resolved completely. The post therapy titration test revealed eradication of the *Bartonella* infection (8 fold titer reduction- 1:128,000 to 1:16,000) 8 months after therapy. This case demonstrates the occurrence of severe multi-organ *Bartonella* disease, in a cat from Minnesota, a low *Bartonella* prevalence area.

## Pathogenesis of *Bartonella* Induced Platelet and Endothelial Disorders:

Although the mechanism(s) by which *Bartonella* cause platelet and vascular endothelial disorders in humans or animals are unknown, several possibilities exist. *Bartonella sp.* infect many cells types including, erythrocytes, neutrophils, macrophages, and endothelial cells. It is certainly possible that *Bartonella* may infect megakaryocytes in the bone marrow causing them to produce fewer platelets or causing the platelets to be released containing *Bartonella*. Most infected animals mount a vigorous antibody response to *Bartonella* proteins and these antibodies may attach to exposed *Bartonella* proteins, or homologous cellular proteins, on megakaryocyte, platelet and endothelial cell surfaces causing their removal by macrophages in the spleen or other tissues. Alternatively, intracellular *Bartonella* may directly kill their host cells by replication in those cells. More work must be done to elucidate the pathogenic mechanisms by which *Bartonella* disrupts the platelet-endothelial cell homeostasis.



**Home Plate:** "Let" it be said that Yogi Berra was one of the best behind the "plate." Yogi said "when you come to the fork in the road- take it!"

## References:

1. Davi, G, and Patrono, C. Platelet activation and atherothrombosis. *N Engl J Med* 357: 2482-94, 2007.
2. Jacomo, V, Kelly, PJ and Raoult, D. Natural history of *Bartonella* infections (an exception to Koch's postulate). *Cl Diagn Lab Immunol.* 9:8-18, 2002.
3. Jaffe, EA, Hoyer, LW, and Nachman, RL. Synthesis of von Willebrand factor by cultured human endothelial cells. *Proc Natl Acad Sci USA.* 71: 1906-9, 1974.
4. Aveilla, ST, Hattori, K, Heissig, B, *et al.* Chemokine-mediated interactions of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nat Med* 10:64-71, 2004.
5. Nachman, RL and Rafii, S. Platelets, petechiae, and preservation of the vascular wall. *N Engl J Med* 359: 1261-70, 2008.
6. Medina, C, Mestanza, E, Arce, J, Alcedan, M and Miranda, R. La verruga peruana y Daniel A. Carrion. Lima 1886. (*An Fac Med Extraordinary number* 1925).
7. Ricketts, WE. *Bartonella bacilliformis* anemia (Oroya fever) a study of thirty cases. *Blood* 3:1025-49, 1948.
8. Daniels, S and MacMurray, FG. Cat-scratch disease; nonbacterial regional lymphadenitis: a report of 60 cases. *Ann Intern Med* 37: 697-713, 1952.
9. Robinson, JL., *et al.* *Bartonella* seropositivity in children with Henoch-Schonlein purpura. *BMC Infect Dis* 5:21-25, 2005.
10. Golden, SE. Hepatosplenic cat-scratch disease associated with elevated anti-*Rochalimaea* antibody titers. *Pediatr Infect Dis* 12: 868-71, 1993.
11. Breitschwerdt, EB *et al.* *Bartonella* species as a potential cause of epistaxis in dogs. *J Clin Microbiol* 43: 2529-33, 2005.