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CASE REPORT

Cryoglobulinemia concurrent with *Leishmania infantum* infection in a dog and its interference with two automated hematology analyzers

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Abstract

A 6-year-old, intact male English Bulldog presented for evaluation of weakness. Physical examination revealed mildly pale mucous membranes and ophthalmologic alterations. A complete blood cell count was performed on the Urit Smart V5 and the Sysmex XN-1000V, and showed several hematologic discrepancies between the analyzers. The Urit reported marked leukocytosis and thrombocytosis, and the Sysmex reported marked thrombocytosis on the impedance and optical RET channel but mild thrombocytopenia on the optical PLT-F channel. On the blood smear examination, there was an abundant proteinaceous material compatible with cryoglobulins. The red blood cell exhibited a notable degree of poikilocytosis. The number of leukocytes was found to be correlated with the Sysmex total white blood cell count, while the platelet count demonstrated a better correlation with the optical PLT-F channel from the Sysmex. Centrifugation of the blood in a capillary tube (at room temperature) identified a cryoglobulin precipitate. Additionally, the blood was reanalyzed soon after warming it to 37°C, which partially corrected most of the interferences. The serum protein electrophoresis demonstrated a marked hyperproteinemia with mild hypoalbuminemia and a marked hypergammaglobulinemia. On diagnostic imaging, a mild splenomegaly was noted. Cytology of the spleen showed *Leishmania infantum* infection, plasma cell hyperplasia, and extramedullary hematopoiesis. This is the first description of a dog infected with *Leishmania*. *infantum* with concurrent monoclonal hypergammaglobulinemia and cryoglobulinemia. We present a detailed description of the interference of cryoglobulin with the Urit Smart V5 and the Sysmex XN-1000V, along with the usefulness of Sysmex PLT-F in this condition.

KEYWORDS
cryoglobulins, hematological interferences, leishmaniosis, monoclonal gammopathy, pseudoleukocytosis, pseudothrombocytosis

1 | CASE PRESENTATION

A 6-year-old, intact male English Bulldog was brought to the veterinary center for evaluation after a period of generalized weakness and sporadic vomiting. The dog was up to date on vaccinations and duly dewormed. A general physical examination of the animal revealed mildly pale mucous membranes. On ophthalmologic examination, bilateral corneal edema, normal intraocular pressure, decreased Schirmer's test (<15 mm/minute in both eyes), and a single corneal ulcer (positive fluorescein test) on the right eye were found.

A complete blood cell count (CBC) was performed at the veterinary clinic on a point-of-care test system, the Urit Smart V5 (URIT Medical Electronic Group, Guilin, Guangxi China), and repeated 24 h later on a Sysmex XN-1000V (Sysmex Corporation, Norderstedt, Germany) with peripheral blood smear examination. The CBC results of both analyzers are shown in Table 1, and representative histograms and scattergrams of both analyzers are presented in Figures 1 and 2. Several discrepancies were observed between the analyzers. The total WBC count reported by the Urit Smart V5 (95.36×10^9 cells/L) was significantly higher than the total WBC count reported by the Sysmex XN-1000V (5.26×10^9 cells/L). The automated WBC differential also differed between the analyzers. The Urit Smart V5 WBC scattergram was found to be morphologically unreliable, and the Sysmex XN-1000V WDF scattergram was manually regated to exclude the eosinophil region, which appeared to include numerous non-eosinophil events. The platelet count, calculated by impedance on the Urit Smart V5 (1379×10^9 cells/L), was not significantly different from the impedance (945×10^9 cells/L) and the optical RET channel (1619×10^9 cells/L) on the Sysmex XN-1000V. However, it was significantly different from the optical PLT-F channel (126×10^9 cells/L) on the Sysmex XN-1000V. No significant discrepancies were found for the erythron.

The blood viscosity was judged to be increased during the preparation of the blood smear. Microphotographs of the blood smear are shown in Figure 3. There was a very abundant proteinaceous material, homogeneously distributed throughout the background, bluish to grayish, amorphous to globular, compatible with protein precipitates/aggregates. This material was interpreted as cryoglobulins. The RBC showed a marked poikilocytosis, mainly due to marked acanthocytosis and schistocytosis. In the context of this case, this was interpreted as probable in vitro RBC fragmentation. The number of leukocytes appeared to be slightly decreased, correlating with the Sysmex total WBC count. Most leukocytes appeared to be poorly preserved. There were activated macrophages, and both neutrophils and macrophages contained phagocytized proteinaceous bluish material. The manual differential WBC is shown in Table 1. There were frequent platelet aggregates on the feathered edge, which were morphologically distinguishable from protein precipitates. The platelet concentration in the blood smear appeared to correlate better with the optical PLT-F channel determination of the Sysmex XN-1000V compared to the optical RET channel or the impedance determination of either analyzer.

The hematologic interpretation was cryoglobulinemia, moderate, normocytic, normochromic, mildly regenerative anemia, with appropriate rubricytosis, probable in vitro RBC fragmentation, mild leukopenia, leukocyte phagocytosis of proteinaceous material, and aggregated platelets (possible mild thrombocytopenia or normal count).

To demonstrate the presence of cryoglobulins, two methods may be employed. The first is the centrifugation of the blood in a capillary tube (at room temperature) to identify the cryoglobulin precipitate; as illustrated in Figure S1A, a significant precipitate fraction was observed and interpreted as cryoglobulin precipitate (45% of all capillary volume, 60% of the plasma fraction).

The second method is the reanalysis of the whole blood soon after warming it to 37°C. This was done on the Sysmex XN-1000V to ascertain whether the erroneously altered values had been corrected. The CBC results and graphical histograms and scattergrams are presented in Table 1 and Figure 2, respectively. The interference caused by cryoglobulin precipitates in the platelet determination by impedance and optically in the RET channel has been corrected and improved, respectively. The optical platelet determination based on the PLT-F channel was not affected by cryoglobulins at either 37°C or at room temperature. In the WDF channel, the interference on the eosinophil region was successfully resolved.

A new blood smear was prepared soon after warming blood to 37°C (Figure 4). The viscosity of the blood during smear preparation decreased significantly. Large cryoglobulin precipitates almost completely disappeared on this new blood smear. The background was markedly proteinaceous, with granular to amorphous bluish to grayish material, homogeneously distributed throughout the slide. The RBC poikilocytosis remained unchanged. The majority of leukocytes appeared to be poorly preserved, with many exhibiting prominent blue-proteinaceous material phagocytoses. Other blood smear findings remained unaltered.

Heparin plasma clinical chemistry was performed on a VetScan VS2 (Zoetis, Parsippany, NJ, USA); relevant abnormalities included: elevated total protein (11.8 g/dL [reference interval {RI}: 5.4–8.2 g/dL]), globulins (9.6 g/dL [RI: 2.3–5.2 g/dL]), and decreased albumin (2.2 g/dL [RI: 2.5–4.4 g/dL]). A rapid immunochromatographic test (Uranotest Quattro, Uranovet, Spain) was performed to screen for vector-borne infections (for the detection of *Erlichia canis*, *Anaplasma* spp. [*A. platys* and *A. phagocytophilum*] and *Leishmania infantum* antibodies, and *Dirofilaria immitis* antigens), which gave an uncertain/suspicious positive result for *L. infantum*. Serum protein electrophoresis (SPE) and serology (ELISA) for *Leishmania* spp. were sent to an external laboratory for analysis. The SPE was performed on a Capillarys 3 Tera (Sebia, Lisses, France), and the results are presented in Table 2 and Figure 5. The ELISA for *Leishmania* spp. was negative. The clinical chemistry interpretation was marked hyperproteinemia with mild hypoalbuminemia and monoclonal marked hypergammaglobulinemia.

A radiographic investigation of the thorax and abdomen revealed no significant findings. Mild splenomegaly was noted on abdominal ultrasound, and fine-needle aspirate biopsies of the spleen

TABLE 1 Complete blood cell counts performed on the Urit Smart V5 (blood analyzed at room temperature) and the Sysmex XN-1000V (blood analyzed at room temperature and at 37°C).

Parameter (units)	Result of Urit Smart V5	Reference interval (Urit)	Result of Sysmex XN-1000V	Result of Sysmex XN-1000V	Reference interval (Sysmex)	Manual data
Temperature of blood at analysis	At room temperature	–	At room temperature	After warming to 37°C	–	–
Red blood cell count (10^{12} cells/L)	**3.45**	5.1–8.5	**3.41**	**3.05**	5.5–8.5	–
Hematocrit (%)	**24.3**	36–56	**25.3**	**23.6**	37–55	PCV: **25%**
Hemoglobin (g/dL)	**10.2**	11–21	**10.1**	**8.6**	12–18	–
Mean cell volume (fL)	70.7	62–78	74.2	**77.4**	62–77	–
Mean cell hemoglobin (pg)	**29.5**	21–28	**29.6**	**28.2**	21.5–26.5	–
Mean cell hemoglobin concentration (g/dL)	**41.9**	30–38	**39.9**	36.1	33–37	–
Reticulocyte count (10^9 cells/L)	–	–	**110**	59.2	0–60	–
Reticulocyte-Hemoglobin (RET-He; pg)	–	–	28.6	26.1	>20.9	–
Nucleated red blood cell count (10^9 cells/L; cells/100 white blood cells)	–	–	0.86; 16.3	0.69; 15.6	–; ≤1	0.89; 17
White blood cell count (10^9 cells/L)	**95.36**	6–17	**5.26**	**4.41**	6–17	[5.26]
Neutrophils (10^9 cells/L)	**11.68**	3.6–11.3	**2.87**	**1.62**	3–11.5	3.3 [63%]
Lymphocytes (10^9 cells/L)	**66.51**	0.8–4.7	1.77	2	1–4.8	1.3 [25%]
Monocytes (10^9 cells/L)	**15.87**	0.14–1.97	0.58	0.73	0.15–1.35	0.6 [12%]
Eosinophils (10^9 cells/L)	0.97	0.04–1.56	–	0.03	0.1–1.5	0 [0%]
Basophils (10^9 cells/L)	**0.32**	0–0.12	0.04	0.03	0–0.2	0 [0%]
Platelet count [impedance] (10^9 cells/L)	**1379**	117–460	**945**	**134**	200–500	–
Platelet count [optical – RET channel] (10^9 cells/L)	–	–	**1619**	**530**	200–500	–
Platelet count [optical – PLT-F channel] (10^9 cells/L)	–	–	**126**	**101**	200–500	–
Plateletcrit (%)	1.2	0–2.9	**0.9**	**0.11**	0.14–0.61	–
Mean platelet volume (fL)	9	5–15	9.6	**8.5**	9–12.7	–
Immature platelets count (10^9 cells/L)	–	–	23	20.3	1.7–25.6	–
Immature platelet fraction (%)	–	–	**18**	**20.1**	1.2–10.4	–

Note: Right column: Manual packed cell volume (PCV), nucleated red blood cells and white blood cell differential based on peripheral blood smear examination. Bolded values are outside the reference interval.

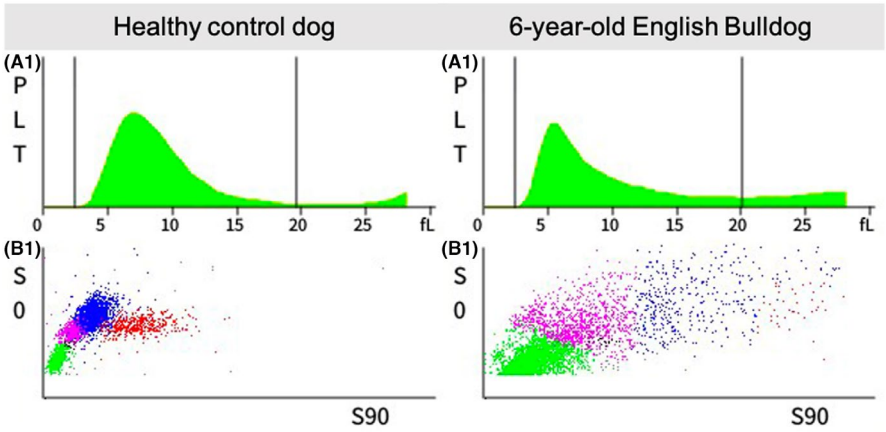


FIGURE 1 Graph representations from the Urit Smart V5. The left column shows an example of a healthy dog, and the right column shows this 6-year-old English Bulldog. (A) platelet impedance histogram, and (B) white blood cell light scattergram. FL, femtoliter; S0, forward scatter; S90, side scatter; PLT, platelets. Particle representation in light scattergram: Green dots (lymphocytes), pink dots (monocytes), blue dots (neutrophils), and red dots (eosinophils).

(Figure S2) and liver were performed. On splenic cytology, heterogeneous lymphoid tissue was present, with a significant increase in well-differentiated plasma cells. A low to moderate number of megakaryocytes, erythroid, and myeloid/granulocytic precursors were present. There was a low number of macrophages. Frequent *Leishmania infantum* amastigotes were seen in the background and phagocyted by macrophages. Cytologic interpretation of the spleen was *L. infantum* infection, plasma cell hyperplasia, and extramedullary hematopoiesis. Liver cytology revealed mild lymphocytic inflammation and extramedullary hematopoiesis (erythroid and myeloid). No *L. infantum* amastigotes were observed at this location. The global interpretation was visceral leishmaniosis with monoclonal hypergammaglobulinemia and cryoglobulinemia.

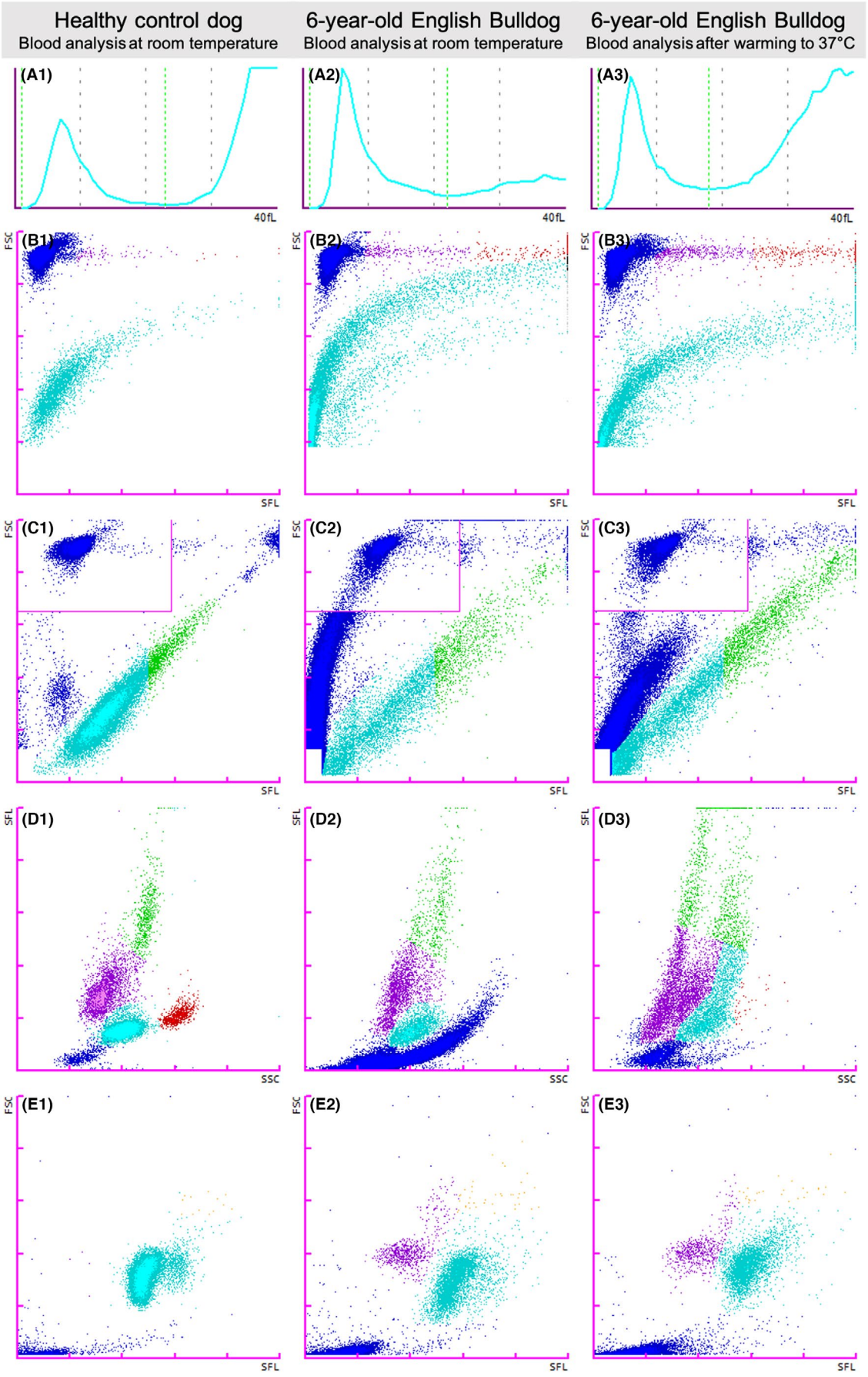
Ocular treatment was administered at time of the first visit: polymyxin B sulfate, neomycin sulfate, gramicidin (Oftalmowell, Teofarma Srl, Pavia, Italy), diclofenac sodium (Voltaren, Laboratoires THEA, Clermont-Ferrand France), and lubricant gel (Lubrithal Gel, Dechra Veterinary Products, Barcelona, Spain). The therapeutic plan was to treat keratoconjunctivitis sicca after the corneal ulcer had been resolved. Additionally, allopurinol 10 mg/kg twice a day (Zyloric, Faes Farma, Leioa, Spain), meglumine antimoniate 100 mg/kg once a day (Glucantime, Boehringer Ingelheim Animal Health España, Sant Cugat del Vallès, Spain), and prednisolone 0.7 mg/kg once a day (Prednisolona Fatro, Ozzano dell'Emilia, Italy) were administered for the treatment of canine leishmaniosis. At the time of this manuscript submission, no follow-up information is available.

2 | DISCUSSION

Cryoglobulinemia is the presence of cryoglobulins in the blood, which are abnormal immunoglobulins (Ig) that precipitate at low temperatures (less than 37°C) and dissolve again after rewarming.[1] The biochemical basis of cold insolubility is not completely understood. Protein solubility can depend on various factors, including primary structure and steric conformation, which are influenced by temperature, pH, and ionic strength.[2]

The simplest way to confirm cryoglobulins is to show that there is protein precipitation or gelation at a temperature below 37°C (i.e., centrifugation in a capillary tube to identify visible cryoglobulin precipitate) and that this material dissolves after reheating to 37°C (i.e., reanalysis of the warmed sample should correct the false alterations on automated hematology).[3] This work has not completely followed the current recommendations for the identification of cryoglobulins made by Napodano et al. (2021).[4] Due to logistical constraints related to the distance between the sample collection site and our institution, the sample was not maintained at 37°C (conventional refrigerated shipment was utilized). Secondly, the centrifugation of the whole blood for the detection of cryoglobulin precipitates was conducted at room temperature after 24-h incubation at 4°C rather than after a one-week incubation period at 4°C. As a result, the precipitate fraction of cryoglobulin described above (45% of all capillary volume, 60% of the plasma fraction) may not be completely accurate.

FIGURE 2 Graph representations from the Sysmex XN-1000V. The left column shows an example of a healthy dog, the middle and right columns show blood from this case analyzed at room temperature and after warming to 37°C, respectively. (A) platelet impedance histogram (PLT-I), (B) platelet scattergram from RET channel (PLT-O), (C) platelet scattergram from PLT-F channel (PLT-F), (D) white blood cell scattergram from WDF channel, and (E) nucleated cell scattergram from WNR channel. FL, femtoliter; FSC, forward scatter; SFL, side fluorescence light; SSC, side scatter. Particle representation: In RET channel, clear blue dots (platelets); in PLT-F channel, clear blue dots (mature platelets), green dots (immature platelets); in WDF channel, clear blue dots (neutrophils), purple dots (lymphocytes), green dots (monocytes), and red dots (eosinophils); in WNR channel, clear blue dots (white blood cells [except basophils]), purple dots (nucleated red blood cells), yellow dots (basophils).



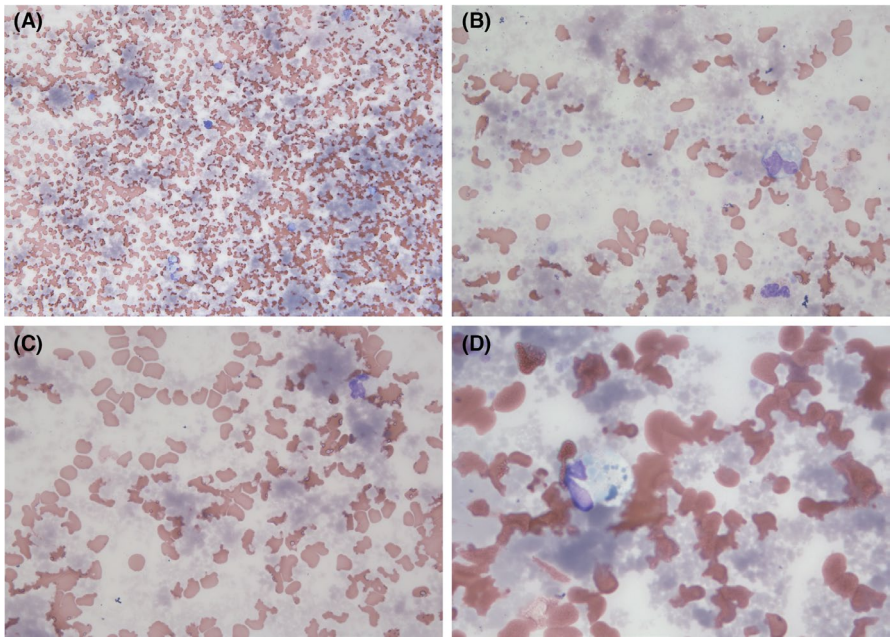


FIGURE 3 Peripheral blood smear micrographs. Modified Wright stain, original magnification ×20, ×63, ×63, and ×100 objectives; (A–D), respectively.

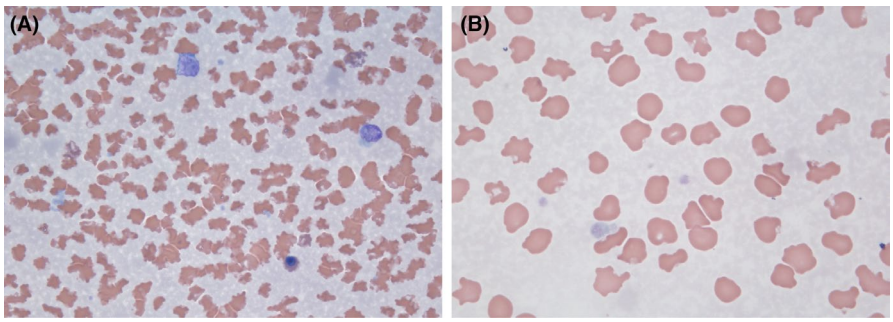


FIGURE 4 Peripheral blood smear micrographs. Smear prepared after warming the blood sample to 37°C. Modified Wright stain, original magnification ×63, and ×100 objectives; (A, B), respectively.

TABLE 2 Serum protein electrophoresis: Absolute concentrations.

Parameter (units)	Result	Reference interval
Total protein (g/L)	**138**	47–68
Albumin (g/L)	**24.3**	25–40
Total globulin (g/L)	**113.8**	15–35
Alfa-1–globulin (g/L)	4	3–7
Alfa-2–globulin (g/L)	10.9	3–11
Beta–globulin (g/L)	**17.3**	8–14
Gamma–globulin (g/L)	**81.6**	7–16
Albumin: globulin ratio	**0.21**	0.5–1.1

Note: Bolded values are outside the reference interval.

Cryoglobulinemia is infrequently reported in veterinary medicine. To the authors' knowledge, peer-reviewed reports in dogs are limited to four cases with multiple myeloma[5–8] and two cases with Waldenström's macroglobulinemia.[5,9]

In human medicine, Brouet's classification is used for distinguishing different types of cryoglobulinemia. Type I cryoglobulinemia is composed of a single monoclonal Ig, usually an IgM or IgG (simple type cryoglobulinemia). Type II cryoglobulinemia is characterized by immune complexes formed by polyclonal Ig and one or more monoclonal Ig, typically monoclonal IgM and polyclonal IgG (mixed monoclonal cryoglobulinemia). Type III cryoglobulinemia involves only polyclonal Ig (mixed cryoglobulinemia). The most common cause of type I cryoglobulinemia is B-cell neoplasia (i.e., multiple myeloma, Waldenström's macroglobulinemia). Mixed cryoglobulinemia (types II and III) is usually attributable to an infectious cause, primarily hepatitis C virus infection (70% to 90% of the cases). Many other causes can cause type II and III cryoglobulinemia, including other infections, B-cell lymphoid malignancies, and autoimmune diseases.[1]

In this case, the SPE revealed a monoclonal gammopathy involving the gamma–globulin region. However, the nature of the monoclonal gammopathy of this dog has not been studied further. Immunofixation could be of value to further classify this anomaly. Considering the *L. infantum* infection in this dog, type II

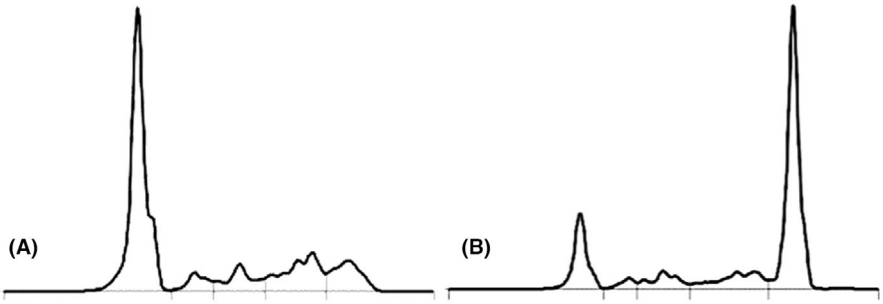


FIGURE 5 Serum protein electrophoresis: Densitometer tracing. (A) Normal SPE from a healthy control dog, and (B) SPE from this 6-year-old English Bulldog.

cryoglobulinemia is suspected. However, a type I cryoglobulinemia should also be considered, especially considering that a plasma cell tumor involving the spleen cannot be completely ruled out by cytology alone due to the increased plasma cell population in this location, nor can Waldenström's macroglobulinemia or lymphoplasmacytic lymphoma. An additional splenic cytology following treatment for leishmaniosis may assist in the demonstration of a possible concurrent plasma cell tumor.

There are scant human reports of cryoglobulinemia (type III) secondary to *L. infantum* infection.[10] It has been hypothesized that some circulating immune complexes in cases of canine leishmaniosis may include cryoglobulins, and this may contribute to some clinical signs.[11] However, to the best of our knowledge, this is the first report of *L. infantum* infection with concurrent cryoglobulinemia in dogs. While it is likely that the cryoglobulinemia was induced by or reacting to the *L. infantum* infection, there is currently insufficient evidence to support a clear association.

Although most cases of monoclonal gammopathies are associated with neoplastic conditions (B-cell neoplasia), a significant proportion of dogs with this laboratory abnormality are caused by an infectious etiology. In particular, leishmaniosis (3/18 cases) and ehrlichiosis (2/18) are the most common infectious causes.[5] Additionally, it is also described that a small number of dogs (3/31 cases) infected with *L. infantum* may exhibit clonal rearrangement of PCR for antigen receptor rearrangement (PARR) test.[12] Of the three cases, two were monoclonal for Ig heavy chain gene rearrangement, and one was biclonal for TCR gamma chain gene rearrangement. The study did not identify the presence of monoclonal gammopathy in any of the cases.[12] However, the use of SPE alone has poor sensitivity and specificity for detecting monoclonal gammopathy, which may be missed in cases with a low concentration of monoclonal proteins and overcalled in cases of oligoclonal or restricted polyclonal gammopathy.

The negative result of the ELISA against *Leishmania* spp. was attributed to the cryoglobulin precipitation since it has been described that cryoprecipitates may disrupt the measurement of specific Ig through techniques such as immunofluorescence and ELISA.[13] A cryoglobulin-induced false negative could have been tested by re-running with a warmed sample. A false negative may occur because the precipitated cryoglobulins were reactive with *Leishmania* spp. antigen or because the cryoprecipitate interfered with the binding or identification of non-cryoglobulin Ig. In human medicine, the most common ocular manifestation of cryoglobulinemic vasculitis is keratoconjunctivitis sicca, which occurs in 43% of the cases with ocular involvement. This is somewhat expected, given that most of these cases were hepatitis C virus-positive, and it is well known that between 10% and 67% of hepatitis C virus-infected patients suffer from dry eye disease, with or without cryoglobulinemia.[14] On the other hand, the prevalence of keratoconjunctivitis sicca in dogs suffering from leishmaniosis with ocular signs ranges from 2.8% to 26.8%.[15,16] The proposed pathogenic mechanisms of dry eye syndrome in dogs with leishmaniosis involve a granulomatous infiltrate around the ducts of lacrimal glands, leading to retrograde dilation and subsequent accumulation of the secretions.[15]

It is well established that cryoglobulinemia can give rise to a number of interferences in automated hematology analysis. In this case, both pseudoleukocytosis and pseudothrombocytosis were present.

The Urit Smart V5 employs two distinct methodologies for calculating the total WBC count: impedance and flow cytometry; the latter was the default configuration in our equipment. Both counts (Table 3) were erroneously elevated and interfered with by cryoglobulins, with the impedance-based count being more affected. In contrast, the Sysmex XN-1000V employs optical technology to calculate the total WBC count: the WNR and the WDF channels. The WNR channel-based count is used for reporting purposes, as it allows for the subtraction of the nucleated RBC. No evidence of cryoglobulin interference was observed in either of the Sysmex XN-1000V WBC counts (Table 3). With regard to the WBC differential, both analyzers employ flow cytometry. Notable interference that invalidates all WBC differential calculations was observed only in the Urit Smart V5, which utilizes solely light scatter properties without fluorescence. In contrast, the interference observed in the Sysmex XN-1000V, which employs both light scatter properties and fluorescence in the WDF channel, was limited to interference with eosinophil calculation. The aberrant morphology of the WDF channel following the warming of the sample has been attributed to the poorly preserved WBC of the sample, which on the blood smear showed prominent blue-proteinaceous material phagocytoses (this could have increased the fluorescence intensity on the scattergram). This phenomenon of cryoglobulin

TABLE 3 WBC counts of the two analyzers. WBC counts of the Urit Smart V5 based on impedance determination and optical determination with light scatter properties. WBC counts of the Sysmex XN-1000V based on optical determination on the WNR and WDF channels.

Analyzer	Temperature of blood at analysis	White blood cell count [impedance] (10^9 cells/L)	White blood cell count [optical—light scatter] (10^9 cells/L)	White blood cell count [optical—WNR channel] (10^9 cells/L)	White blood cell count [optical—WDF channel] (10^9 cells/L)
Result of Urit Smart V5	At room temperature	259.46	95.36	–	–
Result of Sysmex XN-1000V	At room temperature	–	–	5.26	5.01
Result of Sysmex XN-1000V	After warming to 37°C	–	–	4.41	7.86

phagocytosis by WBC has already been described in the literature.[17] The mild leukopenia observed in this case, in the absence of a decrease in any of the specific WBC types, was considered to have limited clinical significance.

The RBC parameters appear to be unaffected by the cryoglobulins in both analyzers. Regarding RBC morphology, some reports indicate that RBC poikilocytosis in cryoglobulinemic cases corrects after warming the blood to 37°C.[18] However, this did not occur in our case, potentially due to the presence of residual cryoprecipitates (visible on the blood smear) and/or due to the analysis of warm blood being conducted more than 24 h after collection.

The two main differential diagnoses for moderate regenerative anemia were blood loss (which is not clinically evident) or hemolysis. Although the poikilocytosis (acanthocytosis, schistocytosis) is suggestive of RBC fragmentation, it was considered a most likely in vitro artifact. However, it is possible that some degree of clinical RBC fragmentation may be occurring in this dog, and therefore, evaluation of a directly extracted blood sample with a warmed syringe should be conducted to definitively determine the presence of poikilocytosis. Although cold agglutinin activity was not excluded with a Coombs test in our case, it seems unlikely, given the absence of microscopic RBC agglutination on the blood smear.

Pseudothrombocytosis represents one of the most prominent interferences of cryoglobulinemia. Other causes of falsely increased platelet count include small, fragmented, or hemolyzed erythrocytes, leukocyte fragments (from normal or leukemic cells), or particulate cellular debris, including bacteria, protozoa, fungi, or lipids.[19]

The platelet count calculated by impedance on both analyzers is aberrantly high and corrects after warming the sample to 37°C. Spurious impedance count with a surplus of small particles (left side) is suggestive of cryoprecipitates,[3,20] see platelet histograms above (Figures 1 and 2). Our fluorescent optical platelet determination on the RET channel exhibited a spuriously increased count, contrary to other reports in which the Sysmex XE-2100 and Sysmex XN-9000 were not affected on the same channel.[17,18] Other authors have described interferences similar to ours (in impedance and optical platelet counts) but not when using an immunoplatelet count with CD61 labeling on a CELL-DYN CD4000 system (Abbott Laboratories).[21] The Sysmex PLT-F channel uses a fluorescent oxazine dye that binds preferentially to platelet organelles that are rich in nucleic acids, such as ribosomes and mitochondria.[22] These staining properties allow one to clearly differentiate between platelets and small particles like RBC fragments.[22,23] The PLT-F channel differentiation between platelets and WBC fragments is still controversial, with some studies claiming no interference[22] and others claiming interference.[24,25] In accordance with other reports, the PLT-F channel is useful for platelet determination in cases of cryoglobulinemia and does not suffer from interference,[3] similar to our case.

A definitive assessment of the platelet count in this case was not possible due to the presence of platelet aggregation on the blood smear. However, the count of PLT-F, together with the moderate number of medium to large platelet clumps on the blood smear, makes it suspect from a normal count, although mild thrombocytopenia cannot be definitively excluded. Examination of a new sample would be beneficial.

In this case, the cryoglobulin deposits were clearly visible on the blood smear. They were very abundant, proteinaceous, homogeneously distributed, bluish to grayish, and amorphous to globular. Several cryoglobulin deposit morphologies have been described, including needle-shaped crystals, as well as amorphous and roundish deposits.[17]

In conclusion, we describe the first case of a dog infected with *L. infantum* with concurrent monoclonal hypergammaglobulinemia and cryoglobulinemia. It is important to be aware of the several false alterations induced by cryoglobulins on automated hematology analysis and to be able to identify them. A detailed description of the interference of cryoglobulin on the Urit Smart V5 and the Sysmex XN-1000V, along with the usefulness of Sysmex PLT-F in this condition, is provided herein.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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