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CASE REPORT

Unusual Electrophoretic Pattern in a Dog Infected With *Angiostrongylus vasorum*

Alice Kéfer[1,2], Hélène Machiels[2], Gaëlle Vincken[2], Clare Pitchford[3], Élodie Roels[2]

[1]*Department of Internal Medicine, CHV OnlyVet, Saint- Priest, France*

[2]*Department of Small Animal Clinical Sciences, Faculty of Veterinary Medicine, University of Liege, Liege, Belgium*

[3]*Veterinary Pathology Group, Exeter, UK*

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ABSTRACT

A 7- year- old female intact pug was presented for a six- month history of cough, dyspnea, and exercise intolerance. Treatment with fenbendazole was initiated 3 days before referral based on a positive blood quantitative PCR for *Angiostrongylus vasorum* (A. vasorum) without improvement of the clinical signs. The dog lived in Belgium with a traveling history to Corsica and Canada. On physical examination, the dog was tachypneic with expiratory dyspnea. Mild non- regenerative anemia, monocytosis, eosinophilia, and basophilia were identified on hematology. Biochemistry revealed hyperproteinemia due to hyperglobulinemia. The protein capillary zone electrophoresis (CZE) identified a restricted polyclonal (or oligoclonal) peak in the beta- globulin region and a tall and narrow peak in the gamma- globulin region, suggestive of either restricted polyclonal or monoclonal gammopathy. Further diagnostic investigations included thoracic radiographs, echocardiography, abdominal ultrasound, urinalysis, snap test 4Dx, *Leishmania* spp. serology, and *Leishmania* spp. PCR on bone marrow aspirates. Severe proteinuria was noted with a urine protein to creatinine ratio (UPCR) of 7.54. No infectious agents other than A. *vasorum* were identified. Treatment with fenbendazole was pursued for 7 days, followed by a spot-on application of imidacloprid/moxidectin combined with tapering the anti- inflammatory dose of steroids, with complete resolution of the

respiratory signs. At 1-month recheck, hyperglobulinemia and proteinuria completely resolved, and the thoracic radiographic images improved. To the authors' knowledge, this case is the first to describe such a gammopathy associated with chronic angiostrongylosis.

1. Case Presentation

A 7- year- old 8 kg entire female pug was presented for a six- month history of cough, dyspnea, and exercise intolerance. The dog lived in Belgium and had a travel history to Corsica 3 years before and Canada 1 year before (Montreal, Quebec, and Toronto). Five months before presentation, an empirical therapy with antibiotics and corticosteroids (unknown duration and dosage) resulted in a mild improvement without further diagnostic investigations. As clinical signs persisted, thoracic radiographs were performed at the referring veterinarian 1 month prior to presentation. The lung pattern was consistent with lungworm infection. A positive quantitative *A. vasorum* PCR on EDTA blood (Cycle threshold—Ct 30.8; RI maximum 45) was detected at the same time, suggesting a moderate to low *A. vasorum* DNA load in the tested sample. An antiparasitic treatment with a single oral administration of milbemycin oxime was given by the referring veterinarian 3 weeks prior to presentation, without improvement of the clinical signs. Once- daily oral fenbendazole therapy was started 3 days prior to presentation. The dog was up to date with core vaccinations. A non- core vaccine against leishmaniasis was administered at the age of 1 year, with no booster thereafter.

Physical examination at presentation revealed tachypnea at 65 breaths per minute associated with restrictive expiratory rest of the physical examination.

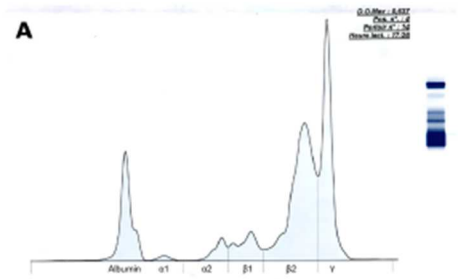
Initial diagnostic investigations included hematology, biochemistry (including electrolytes), thoracic radiographs, echocardiography, abdominal ultrasound, urine analysis, snap test 4Dx, and leishmania serology (IFAT; Indirect Fluorescent Antibody Technique). Hematology revealed a mild non- regenerative anemia (34.7%; RI 37.3%–61.7%) with a reticulocyte count of 66 700/µL (RI 10 000–110 000/µL), monocytosis (1520/µL; RI 160–1120/µL), eosinophilia (1610/µL; RI 60–1230/µL), and basophilia (730/µL; RI 0–100/µL). Platelets were within normal limits. A marked hyperproteinemia (109 g/L; RI 52–82 g/L) due to hyperglobulinemia (81 g/L; RI 25–45 g/L) was identified on biochemistry; the remaining biochemical parameters were within reference intervals. Protein CZE (Capillarys 2 Sebia, Antech) revealed a restricted polyclonal (or oligoclonal) peak in the beta-globulin region and a tall and narrow peak in the gamma- globulin region, which initially raised

concern for a monoclonal gammopathy suggestive of neoplasia (Figure 1A). Thoracic radiograph findings were compatible with chronic parasitic pneumonia (Figure 2). Urine specific gravity was 1.028 (RI > 1.030) with 3+ protein on dip- stick evaluation. Urine sediment analysis was unremarkable. Urine protein to creatinine ratio (UPCR) was increased at 7.54 (RI < 0.5). Echocardiography was within normal limits, without any signs of pulmonary hypertension. The snap test 4D× was negative. Leishmania serology was borderline positive with a titer of 1:50 (RI < 1:50), possibly due to leishmaniasis vaccination at a young age. Abdominal ultrasonography revealed a well- defined hypoechoic and heterogeneous splenic target nodule, measuring 7 mm in diameter, and localized in the head of the spleen. No other notable abnormalities were seen in abdominal ultrasonography.

Respiratory symptoms were attributed to angiostrongylosis according to compatible thoracic radiographic findings and the positive quantitative PCR for *A. vasorum* on EDTA blood. Fenbendazole treatment at 50 mg/kg/day was continued for a total treatment duration of 10 days. A topical antiparasitic agent composed of imidaclopride and moxidectin (Advocate Spot- on) was applied at the end of fenbendazole therapy, and then 1 month later after the end of fenbendazole therapy. Corticosteroids at a tapering anti-i nflammatory dose (prednisolone 0.5 mg/kg/ day for 5 days, then 0.5 mg/kg every other day for 5 days) were prescribed.

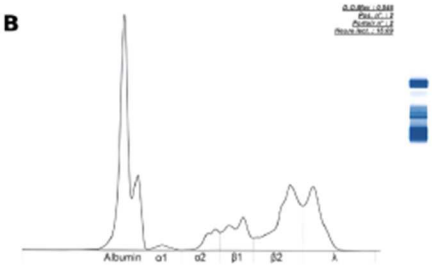
The dog returned 2 weeks after initial presentation for further investigations into the origin of the hyperglobulinemia. A marked improvement in physical activity and appetite, as well as in respiratory symptoms, was reported by the owners. Physical examination was within normal limits with normalization of the respiratory rate at 24 breaths per minute. Hematology results normalized: Hematocrit (43.8%; RI 37.3%–41.7%), leucocytes 8400/µL; RI 5050–16 760/µL. A bone marrow sample was aspirated from the sternal manubrium under sedation (butorphanol 0.3 mg/kg IV) and local anesthesia (subcutaneous lidocaine). No major abnormalities were detected on bone marrow cytology, and no infectious agents or neoplastic cells were seen. Leishmania PCR on bone marrow was negative.

FIGURE 1 *- (A) Initial and (B) post- treatment protein capillary zone electrophoresis of a dog with marked hyperglobulinemia due to Angiostrongylus vasorum. (C) An example of an electrophoretic tracing from a healthy dog is provided for comparison. (A) Initial electrophoretic tracing, revealing a restricted polyclonal peak in the beta- globulin region and a tall and narrow peak in the gamma- globulin region consistent with either a monoclonal or a restricted polyclonal gammopathy (B) Post- treatment electrophoretic tracing is within normal limits.*



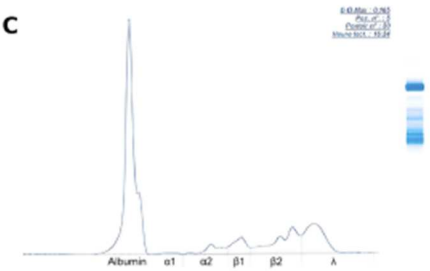
Electrophorèse Capillaire des Protéines Sériques

Fractions	%	Ref. %	Ref. g/dl
Albumine	**16,5**	55,8 - 66,1	40,20 - 47,60
Alpha 1	**3,7**	2,9 - 4,9	2,10 - 3,50
Alpha 2	**7,5**	7,1 - 11,8	5,10 - 8,50
Beta	**40,9**	7,9 - 13,7	6,00 - 9,40
Gamma	**31,4**	11,1 - 18,8	8,00 - 13,50



Electrophorèse Capillaire des Protéines Sériques

Fractions	%	Ref. %	Ref. g/dl
Albumine	**43,1**	55,8 - 66,1	40,20 - 47,60
Alpha 1	**4,9**	2,9 - 4,9	2,10 - 3,50
Alpha 2	**10,6**	7,1 - 11,8	5,10 - 8,50
Beta	**24,1**	7,9 - 13,7	6,00 - 9,40
Gamma	**17,3**	11,1 - 18,8	8,00 - 13,50



Electrophorèse Capillaire des Protéines Sériques

Fractions	%	Ref. %	Ref. g/dl
Albumine	**59,4**	55,8 - 66,1	40,20 - 47,60
Alpha 1	**3,8**	2,9 - 4,9	2,10 - 3,50
Alpha 2	**5,5**	7,1 - 11,8	5,10 - 8,50
Beta	**15,0**	7,9 - 13,7	6,00 - 9,40
Gamma	**16,3**	11,1 - 18,8	8,00 - 13,50

At the third recheck, 1 month after initial presentation, the dog was in good general condition. Only a slight exercise intolerance persisted. Total proteins (65 g/L; RI 52–82 g/L) and globulins normalized (40 g/L; RI 25–45 g/L) and proteinuria resolved (UPCR 0.16; RI < 0.5). The electrophoretic tracing was within normal limits (Figure 1B). Thoracic radiographs showed improvement, without normalization of the pulmonary images (Figure 3), which were considered compatible with sequelae of angiostrongylosis. A follow-up abdominal ultrasound showed a stable splenic target nodule of 6.5–7 mm in diameter in the head of the spleen. Continued monthly administration of internal antiparasitic agent composed of imidaclopride and moxidectin (Advocate Spot- on) was advised to prevent lungworm reinfection as per manufacturer instructions.

FIGURE 2 *- (A) Ventro- dorsal, (B) right lateral, and (C) left lateral radiographs of a 7- year- old female intact pug, showing the typical lesion of angiostrongylosis. Mixed interstitial to alveolar patterns are seen in the periphery of the lung, more marked in the caudo- dorsal region. Note the scalloped, retracted lung ventrally in lateral views (B and C), signs of chronicity (arrowhead).*

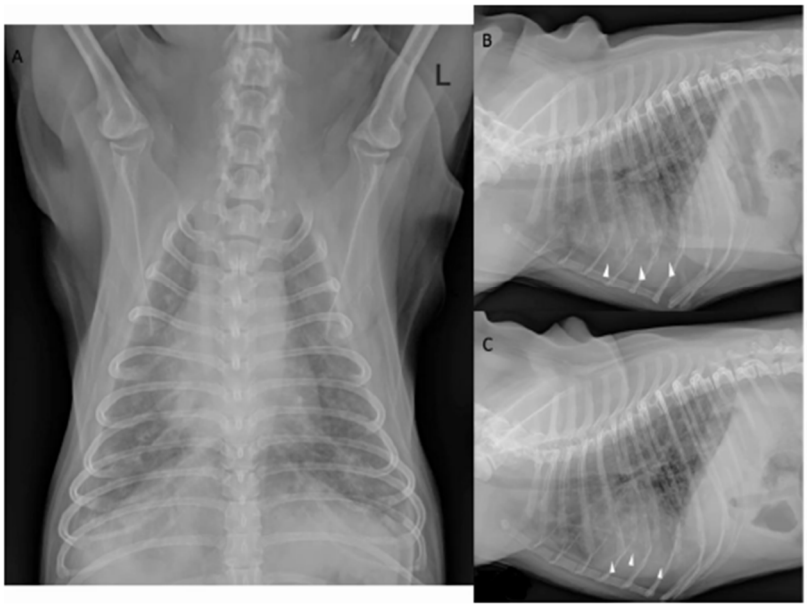
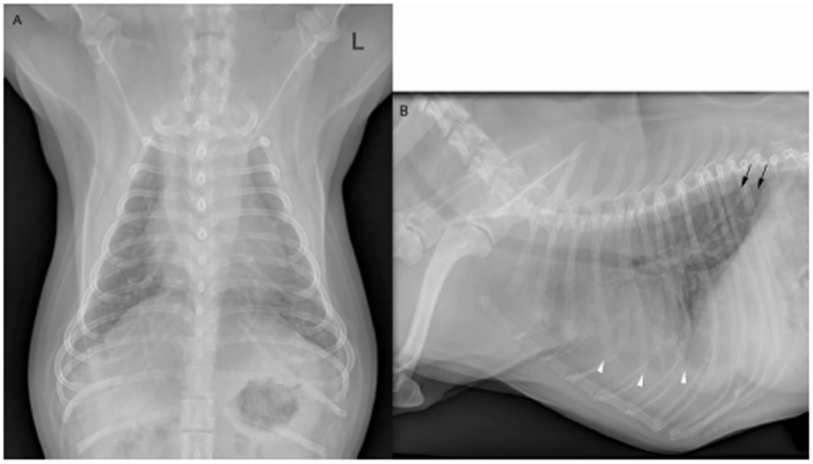


FIGURE 3 *- (A) Ventro- dorsal and (B) right lateral radiographs of a 7- year- old female intact pug 1 month after initiation of the treatment showing improvement of pulmonary lesions of angiostrongylosis. Some interstitial to alveolar lesions are still noted in the left caudo- dorsal lung lobe (black arrow), with persistence of scalloped, retracted lung ventrally (white arrowhead).*



Five months after initial presentation, the dog came back for a follow-u p of the splenic nodule detected during the first and second abdominal ultrasounds. The dog was in very good general condition, with no respiratory symptoms apart from the mild exercise intolerance previously reported without significant impact on the dog's quality of life. Total proteins (65 g/L; RI 52–82 g/L) and globulins (40 g/L; RI 25–45 g/L) remained within normal limits. The previously described splenic nodule was not identified on abdominal ultrasound, suggesting that the structure observed at presentation was likely a regenerative nodule.

Two years after the initial presentation, the dog returned for two episodes of epileptiform seizures versus syncope. No other symptoms were reported apart from a more marked fatigability over the past 2 –3 weeks, warranting a complete hemato-biochemical blood test 10 days prior to presentation, which showed no abnormalities. Repeated thoracic radiographs showed stable lesions compared with the previous pulmonary images, consistent with old lesions of parasitic pneumopathy. Equivocal right cardiomegaly was also present. Echocardiography, however, showed no significant abnormality. Brain MRI revealed post- ictal lesions, but no other cause to explain the symptoms. Cerebrospinal fluid analysis was within normal limits. No anticonvulsant treatment was initiated to determine the exact interval between two seizures. No more seizures were observed over the following 12 months.

2. Discussion

This case report describes for the first time the presence of a tall and narrow peak in gamma-globulins on protein CZE, suggestive of either restricted polyclonal or monoclonal gammopathy in a dog affected with angiostrongylosis.

A. vasorum is a metastrongyloid nematode whose adult worms inhabit the right cardiac ventricle and the pulmonary arteries of dogs and other wild carnivores [1, 2]. The parasite has an indirect life cycle in which slugs and snails act as intermediate hosts [1, 2]. Canine angiostrongylosis is a disease known to be endemic in many countries in Europe, Africa, South America, and Atlantic provinces of Canada (Newfoundland and Labrador) [2–6]. It has been endemic in Belgium since 2016 [6]. In line with the symptoms reported in this dog, the most common clinical signs associated with angiostrongylosis are respiratory and/or cardiovascular symptoms such as exercise intolerance, dyspnea, tachypnea, and sometimes cough. Bleeding disorders have also been described [2, 3].

Dogs naturally infected with *A. vasorum* usually have nonspecific hematology and biochemistry profiles [1]. Occasionally, eosinophilia and hyperglobulinemia (up to 23.4% of cases) can be seen [7, 8]. Electrophoretic profiles of *A. vasorum*- infected dogs are variable [7]. Serum protein electrophoresis is a useful laboratory method for identifying neoplastic processes as well as other pathological changes associated with acute or chronic inflammation of infectious or non- infectious origins [7, 9]. Alpha- 1, alpha- 2, and beta fractions can show alterations during the acute phase of the disease [1, 9]. In chronic diseases, the gamma fraction is usually increased, resulting in a polyclonal hypergammaglobulinemia [7]. Mostly, a broad and symmetrical beta-2,peak and occasionally polyclonal hypergammaglobulinemia have been previously reported in a total of 22 dogs naturally infected with *A. vasorum*. Restricted polyclonal or monoclonal gammopathy was not reported in that previous study [7]. The dog in the present case report had a chronic cough for at least 6 months duration, suggestive of a very long- standing form of angiostrongylosis. The electrophoretic profile shows a restricted oligoclonal beta peak, which aligns with previous findings [7], and restricted polyclonal or monoclonal gammopathy, a finding that has never been described in *A. vasorum*- infected dogs and may be linked to the prolonged duration of the disease in the present case.

Monoclonal gammopathy, as identified through electrophoresis, refers to an electrophoretic profile indicative of a clonal immunoglobulin, which is typically characterized by a single peak that is either of equal height or taller than the albumin peak, and has a width that is similar to or narrower than the albumin peak, often resulting in a peak with a height- to- width ratio of 4:1 on protein electrophoresis [9]. Monoclonal gammopathy is rare in dogs and is usually associated with multiple myeloma, the primary cause of this condition. Other causes identified are: chronic lymphocytic leukemia, plasma cell leukemia, B- cell lymphoma, amyloidosis, and Waldenström macroglobulinemia [9–13]. Monoclonal gammopathy has also been described in some chronic infectious diseases, including mainly ehrlichiosis and leishmaniasis [10–13]. Cases of plasmacytic gastroenterocolitis, chronic pyoderma, and *Dirofilaria immitis* infection associated with monoclonal gammopathy have also been described [14–16]. Restricted polyclonal gammopathy results from the expansion of a small number of plasma cell clones, unrelated to neoplastic processes, potentially leading to the formation of one or more restricted immunoglobulin bands that can be wider than or closely resemble those seen in monoclonal gammopathies [9]. Such gammopathies may be secondary to any reactive or inflammatory process [9]. Accordingly, extensive investigations were performed to screen for malignancies or co- infection in the present case, which did not reveal significant findings. The rapid resolution of hyperglobulinemia after angiostrongylosis therapy supports the association between this parasitic disease and the observation made on the electrophoregram.

Initially, the severe proteinuria (UPCR > 2) raised the possibility of a renal cause, such as glomerulopathy. However, the complete resolution of proteinuria following the treatment of angiostrongylosis, along with the normalization of hyperglobulinemia, without any specific treatment for proteinuria, suggests that hyperglobulinemia alone was responsible for the proteinuria. In contrast, if glomerulopathy had been the cause, we would have expected the proteinuria to persist despite treatment.

The differentiation between monoclonal and restricted polyclonal gammopathy is challenging and often necessitates complementary testing such as immunofixation [17–19]. A study revealed that combining protein electrophoresis with immunofixation enhances both sensitivity and specificity for detecting M- proteins [17], and that restricted polyclonal patterns on serum protein electrophoresis alone often lead to incorrect diagnoses of monoclonal gammopathy or neoplasia [18]. Another recent study demonstrated that only a minority of suspected monoclonal

gammopathies linked to vector- borne diseases were confirmed when both protein electrophoresis and immunofixation were employed [19]. In the present case of angiostrongylosis, the absence of immunofixation constitutes a main limitation, as it would have enabled a more accurate interpretation of the electrophoretic profile, though this technique is not routinely available in clinical practice. Nevertheless, based on the observed pattern, further investigations to rule out underlying neoplastic or infectious etiologies were still justified. Given the lack of immunofixation, a definitive distinction between a monoclonal and a restricted polyclonal gammopathy cannot be established in this case. However, the infectious nature of the disease favors the latter interpretation, suggesting that the observed hyperglobulinemia more likely reflects a restricted polyclonal gammopathy rather than a monoclonal gammopathy [18].

Chronic antigenic stimulation caused by infectious diseases can lead to immune dysregulation, promoting the expansion of a single or small number of B- lymphocyte clones and resulting in non-myelomatous monoclonal or restricted polyclonal gammopathies, respectively [7, 20]. Infections such as leishmaniasis or ehrlichiosis are believed to trigger such chronic stimulation, which can rarely result in the production of paraproteins and the subsequent occurrence of monoclonal or restricted polyclonal gammopathies [12, 13, 20]. In the present case of chronic angiostrongylosis, the electrophoretic changes, particularly the narrow and tall peak, suggest an expanded B- cell or plasma cell population responding more intensely to a specific antigen compared to other populations.

Diagnosis in this case was based on a positive quantitative PCR identification of *A. vasorum* on EDTA blood, along with compatible clinical signs and thoracic radiographic findings. Several diagnostic tests are available to confirm angiostrongylosis, such as the Baermann method in fecal samples; antigen snap test or PCR in blood, lung tissues, bronchoalveolar lavage fluid, or feces [2, 6, 21–24]. Quantitative PCR on blood could potentially be more sensitive than the antigen snap- test [22].

The dog described in this case report was initially treated with milbemycin oxime without improvement but responded to a course of 10 days of oral fenbendazole followed by two spot-on applications (1 month apart) of imidacloprid 10%/moxidectin 2.5% (Advocate). The treatment protocol was chosen according to the recent literature recommendations [2, 23]. The lack of initial response to milbemycin may be attributed to it not being administered weekly for 4 weeks, as recommended in the literature [2, 23]. While the imidacloprid 10%/moxidectin 2.5% Spot- on

formulation showed no significant difference in efficacy compared to fenbendazole, it was chosen in this case as a preventive measure against reinfection with canine angiostrongylosis [2, 25]. Corticosteroids have also been used to prevent anaphylactic reactions post- parasiticide treatment [2, 23].

In conclusion, this case report describes an *A. vasorum*- infected dog with a protein CZE revealing a tall and narrow peak in the gamma- globulin region, which was consistent with either a monoclonal or a restricted polyclonal gammopathy. Such an electrophoretic pattern had never been described in a dog with chronic angiostrongylosis, but had been previously described in dogs with other non-myelomatous gammopathies, such as ehrlichiosis and leishmaniasis. Further studies are needed to determine whether this is an isolated case or whether several cases of chronic angiostrongylosis may be associated with monoclonal or restricted polyclonal gammopathies. In the future, chronic angiostrongylosis should be considered in the differential diagnosis of hypergammaglobulinemia with monoclonal or restricted polyclonal gammopathies, leading to appropriate therapeutic management and prognosis.

Conflicts of Interest

The authors declare no conflicts of interest.

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