# Intra-abdominal bleeding in a horse: not always of traumatic origin

*Intra-abdominale bloeding bij een paard: niet altijd het gevolg van trauma*

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## ABSTRACT

A nine-year-old warmblood mare was presented with clinical signs of mild colic and fever. On percutaneous ultrasound of the abdomen, a mass was identified on the left side of the abdomen between the spleen and the stomach. During examination the following day, intra-abdominal blood was observed. On rectal examination, a mass was palpated in the pelvis. The presumptive diagnosis of hematoma with intra-abdominal bleeding was made. On consecutive ultrasounds and radiographs, the mass evolved in shape and other masses were identified in the liver and the lungs. No change was noticed in the pelvic mass. Cytology and histology of a tru-cut liver biopsy revealed abnormal, most likely neoplastic cells, whereas cytology of the abdominal and thoracic fluid did not reveal any neoplastic cells. Due to the presence of several rapidly growing masses, a neoplastic process was most likely. Because of the malignant character of the disease and the persistence of the clinical signs, euthanasia was suggested but refused by the owner. Supportive treatment was instituted. Initially, the general condition remained stable, after which the horse suddenly collapsed and died. Post-mortem examination revealed a primary neoplasm located in the pelvic cavity, as well as multiple disseminated masses within several tissues. The mass found in the liver had ruptured with loss of probably 50 liters hemorrhagic fluid within the abdominal cavity. Based on gross pathology, cytological and histological findings, a hemangiosarcoma was suspected. This diagnosis was confirmed using immunohistochemistry for von Willebrand factor. In this case report, the importance of differentiating hematoma from hemangiosarcoma in the horse is highlighted.

## SAMENVATTING

Een negen jaar oude merrie werd aangeboden met klachten van milde koliek en koorts. Met behulp van echografie werd een mixed hypo- en hyperechogene massa gediagnosticeerd tussen de maag en de milt. Op het rectale onderzoek werd eveneens een massa waargenomen in de bekkenholte. Bij herhaling van deze onderzoeken een dag later werd intra-abdominaal bloed waargenomen. Er werd gedacht aan een hematoom met intra-abdominale bloeding van traumatische oorsprong. Gedurende de volgende dagen werden echografische en radiografische veranderingen waargenomen in de longen en de lever. De massa ter hoogte van het bekken bleef stabiel. Abnormale, vermoedelijk neoplastische cellen werden waargenomen op cytologisch en histologisch onderzoek van een biopt genomen ter hoogte van de massa aanwezig in de lever. Dergelijke cellen werden niet waargenomen in het abdominale en thoracale vocht. Door de aanwezigheid van een snel groeiende massa werd de diagnose van maligne neoplasie het meest waarschijnlijk. Euthanasie werd voorgesteld maar geweigerd door de eigenaar. Ondersteunende behandeling werd ingesteld, waardoor het paard stabiel bleef. Enkele dagen later is het paard plotseling gestorven. Tijdens het post-mortemonderzoek werd in het bekken een primaire neoplasie waargenomen met metastasen in de longen en de lever met lekkage van 50 liter hemorragisch vocht in het abdomen. Gebaseerd op de macroscopische bevindingen, cytologische en histologische kenmerken werd een hemangiosarcoma als meest waarschijnlijke diagnose vooropgesteld. Dit werd bevestigd via een immunohistochemische kleuring voor von Willebrand-factor. Deze casuïstiek toont aan dat differentiatie tussen hematoom en hemangiosarcoma belangrijk is.

## INTRODUCTION

Hemangiosarcoma, also known as angiosarcoma or malignant hemangioendothelioma, is a malignant neoplasm originating from the vascular endothelium (Pulley et al., 1990). It affects middle-aged horses and no apparent sex predilection has been reported (Southwood et al., 2000). Locally invasive or disseminated forms have been reported (Southwood et al., 2000; Johns et al., 2005; Ferrucci et al., 2012; Taintor et al., 2014). The respiratory and musculoskeletal systems are mostly affected (Johns et al., 2005). Cutaneous, ocular and cervical vertebral forms have also been described (Scherrer et al., 2017; Taintor et al., 2014). In horses, disseminated hemangiosarcoma is more commonly found in the lungs and pleura, skeletal muscle, spleen, heart, kidney and brain (Taintor et al,. 2014). Kuipel et al. (2000) described an intrapelvic hemangiosarcoma involving the pelvis and hind limb musculature causing periodic episodes of colic and lameness. Due to the involvement of multiple organ systems, different clinical signs have been reported depending on the body systems involved. The main clinical signs include lethargy, depression, anorexia, weight loss and anemia. In addition, epistaxis, dyspnea, lameness caused by hematoma formation within the skeletal muscle and signs of abdominal pain associated with hemoabdomen may be observed (Southwood et al., 2000; Taintor et al., 2014). The duration of the clinical signs varies from one day to several years (Southwood et al., 2000). Anemia, neutrophilic leucocytosis and thrombocytopenia have been described as the most common hematologic abnormalities in horses with disseminated hemangiosarcoma (Southwood et al., 2000). Ultrasonographic examination or radiography of thorax and/or abdomen often reveals masses (Johns et al., 2005). Biopsy of the tissue and histological examination are useful for diagnosing neoplasia. However, immunohistochemistry for endothelial markers, e.g. von Willebrand factor, may be necessary for diagnosis (Bertazzolo et al., 2005). Due to the malignant character of the disease, euthanasia is often suggested.

## CASE REPORT

A nine-year-old warmblood mare weighting 497 kg was presented at the Department of Large Animal Internal Medicine of Ghent University with signs of mild colic and pyrexia (39.0°C). Two days prior to admission, the mare had been in Morocco for three weeks for a show jumping tournament; she was transported back to Belgium by truck. She had no previous history of illness.

Upon presentation, the horse showed clinical signs of abdominal discomfort but was alert. No abnormalities were found during heart and lung auscultation. The heart rate (40 beats/min) and respiratory rate (24 breaths/min) were within normal ranges. The mucous membranes were pale and the capillary refill time was two seconds. Rectal temperature was 38.3°C and gut sounds were reduced. No abnormalities were found on ultrasonography of the thorax. Abdominal ultrasound however showed a large heterogeneous mass situated between the stomach and the spleen with a diameter of 10 cm. A small amount of hypoechogenic free fluid was present in the abdominal cavity. On rectal palpation, a 15 cm-diameter mass was found in the pelvic cavity. A heterogeneous, moderately echogenic mass with slightly hypoechoic cavities was found on rectal ultrasound.

Blood analysis showed a decreased packed cell volume (PCV) (24%, ref: 35-45%) and a white blood cell count of $13.0x10^9$ cells/l (ref: $3.5-9.0x10^9$ cells/l), with 87% of neutrophils. The total serum protein concentration was within normal limits (59 g/l, ref: 56-72 g/l), but the albumin concentration was decreased (24 g/l, ref: 35-55 g/l). The total calcium concentration was low (2.40mmol/l, ref: 2.60-3.22 mmol/l). Blood lactate was normal.

A presumptive diagnosis of hematoma with intra-abdominal bleeding as a result of trauma was made. Initial treatment consisted of transexamic acid infusion (Exacyl® Sanofi, Belgium, 10 mg/kg bwt, in 1L NaCl 0.9% IV two times a day). Flunixine meglumine (Emdofluxin®Ecuphar, the Netherlands, 1.1 mg/kg bwt IV) one time a day and broad-spectrum anti-biotics, sodium penicillin (Penicilline®, Kela, Belgium, 10M IU IV) three times a day) and gentamycin (GentaEquine, Dechra®, Ireland, 6.6 mg/kg bwt IV one time a day) were instituted. No signs of colic of pyrexia were seen over the next days. The horse was kept on broad-spectrum antibiotics IV for seven days. After seven days, oral antibiotics (Trimethoprimum Sulfadiazinum, Emdotrim, Ecuphar®, Belgium, 30 mg/kg bwt, two times a day) were administered for ten days.

The day after admission, thoracic and abdominal ultrasound along with rectal palpation were repeated. In addition to the previous findings, the abdominal cavity was now filled with cloudy free fluid (Figure 1A). The infusion of transexamic acid was continued (Exacyl® Sanofi, Belgium, 10 mg/kg bwt, in 1L NaCl 0.9% IV two times a day, for two consecutive days). The PCV remained 20-28% over the next days. Because the horse showed symptoms of intestinal obstruction due to the intrapelvic mass, laxative food was administered.

The general condition remained stable although the appetite remained poor. Ultrasound was repeated during the following twelve days after initial presentation, and ultrasonographic changes were noticed. The initial mass on the left side was still present but cloudy fluid gradually disappeared. On the right side of the horse, a mass could be identified within the liver (Figure 1B). In the right cranio-ventral thoracic cavity, hypoechoic free fluid was noticed with atelectasis of the ventral lung tip (Figure 1C). Multiple masses were identified at the lung surface (Figure 1D). Thoracic radiographs revealed the presence of multiple
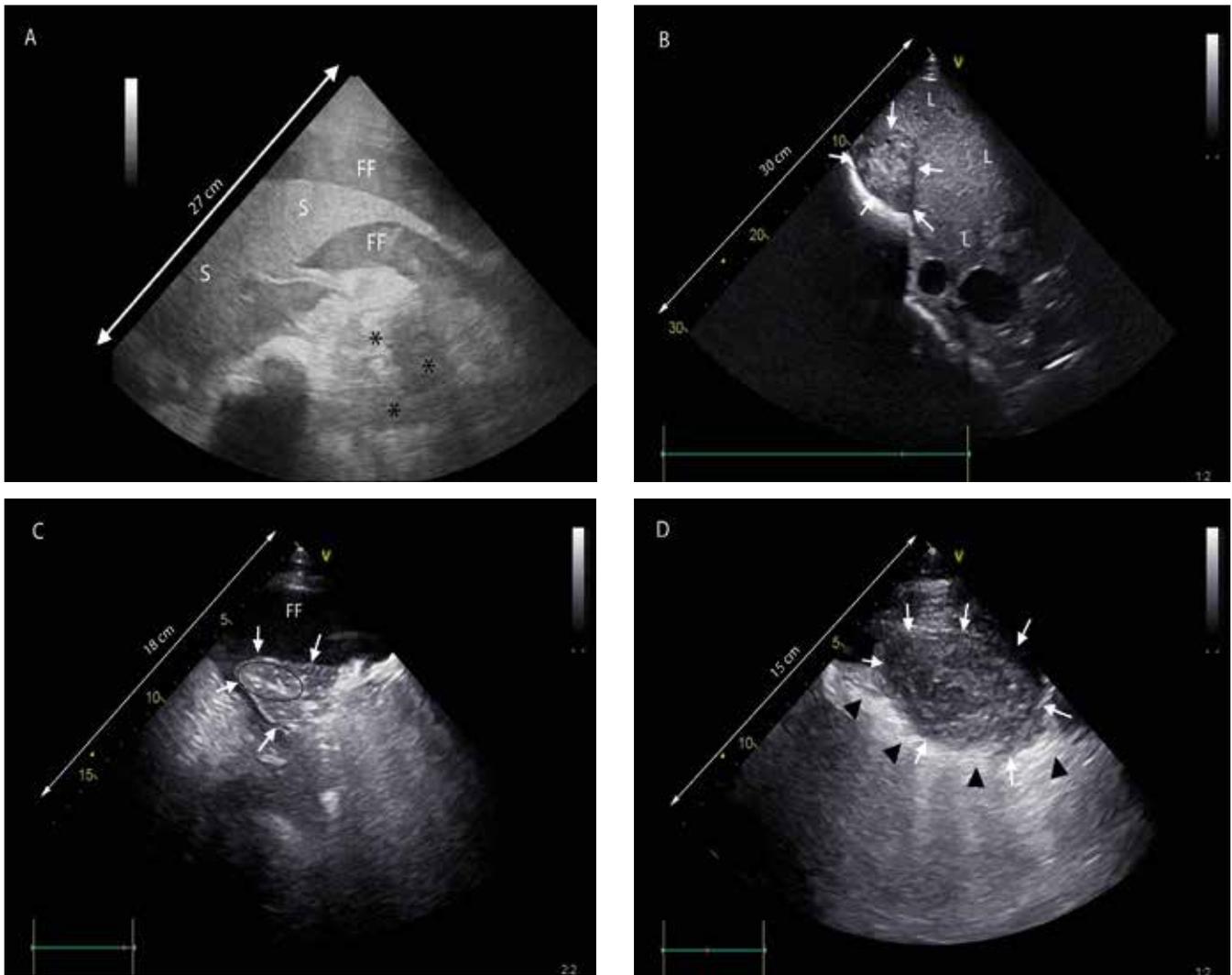
**Figure 1. Ultrasonographic images taken from A. the left and B., C., D. right side of the horse using a phased array transducer (Esaote Mylab30gold and GE Vivid IQ) (dorsal is right on the screen). A. US image taken on the second day. B., C., D. US images taken during the follow-up examinations. A. Cloudy fluid is present lateral and medial to the spleen (FF). Medial to the spleen (S), a mass (asterix) is seen. B. A tissue structure (arrows) was identified within the liver (L) C. Atelectasis of the lungtip (arrows); an air-bronchogram (black circle) and free fluid (FF) are visible. D. An irregular mass (white arrows) is visualized at the lung surface.**

nodular masses within the lungs. A rapid spreading and growing neoplastic process was suspected.

A transcutaneous ultrasound-guided (3.5 MHz convex probe) biopsy of the mass present adjacent to the liver was taken in the standing, sedated (Detomidine, Domosedan®, Dechra, the Netherlands, 0.1 mg/kg bwt IV and Butorfanol, Dolorex®, MSD, Germany, 0.1mg/kg bwt IV) horse. Care was taken to avoid puncturing large hepatic blood vessels within the mass. After aseptical preparation, local anesthetic (1.5 ml Procaine, Procainii Chloridum®, Kela, Belgium) was injected subcutaneously and in the intercostal muscles. A 5-mm incision was made with a No. 24 scalpel blade. A 16-gauge tru-cut biopsy needle (Quick-core, cook®) was inserted. An impression smear was made and the biopsy was fixed in 10%-neutral buffered formalin for 24 hours. The biopsy was paraffin-embedded, routinely processed

and 10 μm sections were made. Both impression and biopsy sample were stained with hematoxylin and eosin (H&E) for routine light microscopy.

Abdominocentesis and thoracocentesis were performed with a venoject 20G needle. The fluid obtained by thoracocentesis was serohemorragic fluid. The lactate concentration was below 2 mmol/l. The protein level was increased (total protein: 35g/l, ref <25 g/l) and a leukocyte count of $6.37 \times 10^9$ cells/l (ref: $<8 \times 10^9$ cells/l with neutrophilia: 76% neutrophils) was present. Cytologic examination revealed no neoplastic cells. Similar results were observed from the abdominal fluid with an even higher protein concentration (total protein: 45g/l, ref<25 g/l), a leucocyte cell count of $9.34 \times 10^9$ cells/l (ref<7.5$\times 10^9$cells/l) and a low lactate (< 2 mmol/l). No neoplastic cells were identified on cytological examination.

On microscopic evaluation of the impression

smear, large atypical cells were present with multiple nucleoli, a small amount of cytoplasm and marked anisokaryosis. This raised the suspicion of a neoplastic process.

Histopathologic examination of the biopsy revealed dense masses of moderate to big polygonal to spindle-shaped cells with clearly delineated pale cytoplasm. The nuclei were big, round to oval and had a small amount finely stippled chromatin. The nucleus/cytoplasm ratio was increased. Mitotic figures ranged from 1 to 3 per high power field. Moderate cellular atypia was present. There were some blood filled cavities present. The diagnosis of a malignant neoplasia was made.

Considering all above mentioned findings, a grave prognosis was given, euthanasia was proposed but the owner declined. The horse's condition remained stable for three more days, after which the horse suddenly collapsed and died. On necropsy, a large, friable but invasive retroperitoneal mass measuring 25 x 30 x 20 cm was found, strongly adherent to the ventral aspect of the pelvic bone, uterus and rectum. On cross section, cavernous cavities filled with blood and white fibrotic to soft necrotic spots were present. A huge amount of blood was present in the abdominal cavity. The lungs were pale and edematous, similar masses ranging from 1 to 10 cm in size were identified. One liter of serohemorrhagic fluid was present in the thoracic cavity. A soft tissue mass of 50 x 23 x 30 cm and multiple small masses were present in the liver, hemoabdomen was caused due to rupture of this mass. The pelvic mass was suspected to be primary while the others were probably metastatic.

Histopathological examination of the masses revealed an invasively growing, densely cellular and non-encapsulated proliferation of a monomorphous cell population. The cells were organized in thick sheets with a scant amount of stroma and sporadic organization into small blood-filled cavities. The cells were spindle-shaped to polyhedral with a small amount of eosinophilic cytoplasm and distinct cell borders (Figure 2A). The nuclei were round to oval with fine granular chromatin. Mitoses were frequent, ranging from 5 to 10 per high power field, often with atypical mitotic figures. There was pronounced anisocytosis and anisokaryosis. Edema was present and multifocal areas of necrosis and an influx of neutrophils were observed. Immunohistochemistry for von Willebrand factor was performed on multiple slides to confirm the endothelial origin of the neoplastic cells. Most slides from the pelvic mass showed large areas with positive immunoreaction, sometimes highlighting the small cavernous blood-filled spaces (Figure 2B). Other slides, like from the liver and lung metastases, revealed very faintly or no specific immunostaining.

These findings confirmed the diagnosis of a malignant neoplasia, more specifically a disseminated hemangiosarcoma.

## DISCUSSION

Hemangiosarcoma is relatively uncommon in horses. Affected patients often present a major diagnostic challenge. Kuipel et al. (2000) described an intrapelvic hemangiosarcoma involving the pelvis and hind limb musculature causing periodic episodes of colic and hind limb lameness. The infiltrative, multilobulated, dark red and yellow, mottled soft mass identified in that case report originated from the pelvis, dorsally to the pubic and ischiatic bones. The mass covered the whole floor of the pelvis, extended ventrally through the obturator foramina and invaded the pubic and ischiatic bones along the symphysis, causing severe bone lysis. The mass extended bilater-
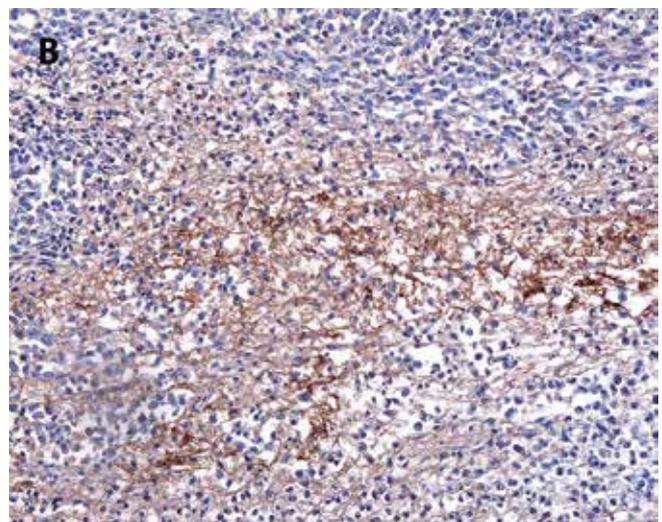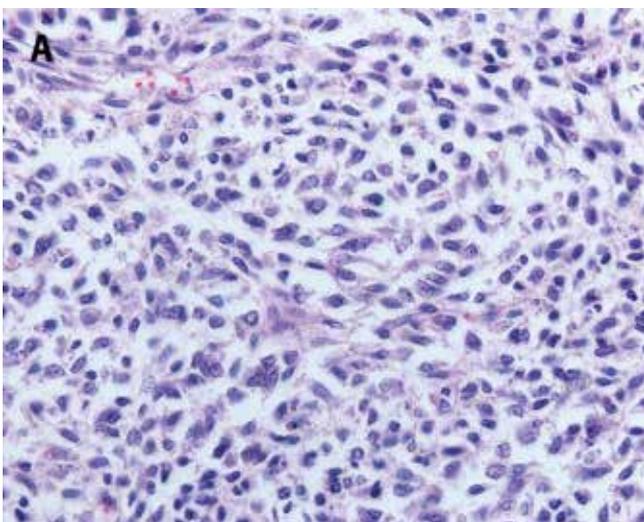


**Figure 2. A. Histopathological slide of the soft tissue mass showing spindle-shaped cells with ovoid nuclei (40x). B. Positive immunohistochemical staining of neoplastic cells for von Willebrand factor (brown pigment) confirms the diagnosis of hemangiosarcoma in this horse (20x).**

ally into the semimembranous, adductor femoris, and medial quadriceps muscles causing diffuse hemorrhages and focally necrotic zones. In the present case, the suspected primary mass was found retroperitoneal, invading the ventral pubic bones. It was soft and cavernous areas filled with blood and white necrotic spots were present. Smaller metastatic masses were found in the lungs and liver.

In this case, hemoabdomen due to bleeding as result of trauma was first suspected. The horse did not show any chronic signs of systemic illness and was able to participate to show jumping until a few days before referral to the clinic. Due to the suspicion of hematoma formation after trauma, transexaminic acid was administered. The treatment of acute abdominal hemorrhage depends on the severity of the clinical signs. In cases of acute hemorrhage and subsequent hypovolemic shock, rapid expansion of blood volume with isotonic crystalloids, hypertonic saline or colloids is appropriate (Magdesian, 2008). Indications for administration of whole blood include clinical and laboratory findings of hemorrhagic shock that persist after adequate restoration of hydration with crystalloid fluids. These signs include persistent hypotension, mucous membrane pallor, lethargy, cool extremities and tachycardia (Magdesian, 2008). The need for whole blood transfusion may be based on clinicopathological data, including packed cell volume less than 12%, acute blood volume loss of 30-40%, hyperlactatemia, an oxygen extraction ratio greater than 50% and decreased venous oxygen tension (Magdesian, 2008). Aminocaproic acid and transexaminic acid, both inhibitors of fibrinolysis, are used to treat hemorrhage (Wong et al., 2009).

No neoplastic cells were found on the abdominocentesis and thoracocentesis. On the impression smear, neoplastic-like cells could be identified. Histopathologic examination of the biopsy also revealed neoplastic cells. During examination of fine needle aspirates of a hemangiosarcoma, neoplastic cells are rarely found (Taintor et al., 2014) and often, only red blood cells are identified (Bertazzolo et al., 2005). Cytology is usually more consistent with hemorrhage and not particularly useful for diagnosing hemangiosarcoma (Southwood et al., 2000; Johns et al., 2005). The neoplasms can be well or poorly differentiated and a variable number of mitotic figures might be seen (Bertazzolo et al., 2005). Spindle-shaped to enlarged ovoid, hyperchromatic nuclei located in blood filled vascular channels may be present in well-differentiated hemangiosarcomas (Bertazzolo et al., 2005). Poorly differentiated neoplastic cells can appear as solid sheets of spindle-shaped cells or pleomorphic cells, of which the vascular channels may not be obvious (Bertazzolo et al., 2005). In the present case, similar histopathological findings were present and immunohistochemical staining was necessary for diagnosis (Bertazzolo et al., 2005). Von Willebrand factor (factor VIII-related antigen) is expressed by normal and neoplastic cells of both vascular and lymphatic origin, with the largest expression by the vascular endothelium (Bertazzolo et al., 2005; Jennings et al., 2012). The absence of (strongly) positive immunoreaction of the metastatic masses can be explained by loss of the expression of the von Willebrand factor antigen in very anaplastic and dedifferentiated cells.

The hemangiosarcoma in this case was not amenable to resection. Supportive treatment is often unrewarding (Southwood et al., 2000). In young horses (younger than three years old) however, the progression of the disease may be different. Euthanasia was proposed, but the owner declined. The horse collapsed probably due to the rupture of the well-vascularized mass present in the liver. In rare cases, early resection of the affected tissue may be successful (Johns et al., 2005).

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