Severe hindlimb lameness and pathological femur fracture in a horse secondary to haemangiosarcoma

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Summary

An 18-year-old Warmblood stallion was presented for an acute onset nonweightbearing right hindlimb lameness following a mild lameness of 2 weeks' duration. Severe swelling was present in the right femoral area. Despite extensive diagnostic procedures, no definitive diagnosis could be made. Packed red cell volume was persistently low. The horse improved with supportive treatment and was discharged at the owner's request. The horse presented 4 days later for bleeding from a previously made incision on the lateral femoral area. Due to deterioration of his condition, the stallion was subjected to euthanasia. Diagnosis of disseminated haemangiosarcoma, affecting primarily the hindlimb musculature and leading to pathological fracture of the femur was made at necropsy and subsequent histopathology.

Keywords: horse; haemangiosarcoma; femur; lameness; fracture; histopathology

Introduction

Haemangiosarcoma is a malignant tumour of vascular endothelial origin (Cottle *et al*. 2008) that rarely affects horses: it occurred in 0.01 % (Braun 2007) and 0.05 % (Johns *et al*. 2005) of patients admitted to equine hospitals in two large retrospective studies. Two different age-related manifestations of haemangiosarcoma have been reported in horses (Johns *et al*. 2005). Younger horses often present with cutaneous masses, leg swelling or joint effusion and have a variable prognosis for survival (Johns *et al*. 2005), whereas middle aged and older horses typically have multiple organ involvement and show rapid clinical deterioration. In the latter disseminated manifestation the respiratory and musculoskeletal systems are most commonly

affected (Southwood *et al*. 2000). This disseminated form poses a diagnostic challenge to the clinician and a diagnosis is often only reached after a detailed *post mortem* examination.

Lameness and muscular or subcutaneous swelling accounted for up to 36% of the primary complaints in 35 cases with disseminated haemangiosarcoma (Southwood *et al*. 2000). Other isolated cases of haemangiosarcoma leading to lameness have also been described (Pelt, Langham and Gill 1972, Waugh *et al*. 1977, Kiupel *et al*. 2000, Knottenbelt and Clegg 2004, Pille *et al*. 2004, Cottle *et al*. 2008).

This report describes the clinical, diagnostic and *post mortem* findings of a primary periosteal or skeletal muscular haemangiosarcoma causing severe hind limb lameness and leading to pathological femur fracture in a horse. To the authors' knowledge this is the first equine report of this kind.

Case details

History:

An 18-year-old European Warmblood breeding stallion was referred to Onderstepoort Veterinary Academic Hospital (OVAH) for evaluation of severe right hind limb lameness. The stallion had a history of mild right hind limb lameness of two weeks' duration which acutely progressed to non-weight bearing. The horse had been active as a breeding stallion for two months prior to the onset of lameness. The referring veterinarian had performed physical and radiographic examinations of the distal limb up to the tarsus with reported absence of significant findings and the horse was referred for a suspected pelvic fracture. The horse received phenylbutazone (8.8 mg/kg bwt IV) before referral.

***Clinical findings*:**

The stallion was in good overall body condition. There was atrophy of the right gluteal muscles and severe right hind limb swelling extending from the proximal femoral area to the distal aspect of the tarsus **(Fig 1)**. The stallion had a dull demeanour, rectal temperature of 37.5 ^{o}C, tachycardia (80 beats per minute), tachypnoea (40 breaths per minute), pale-pink mucous membranes and showed sweating. Grade 5/5 lameness (Anon 1991) was present in the right hind limb. The right metatarso-phalangeal joint was held in flexion resulting in weight bearing

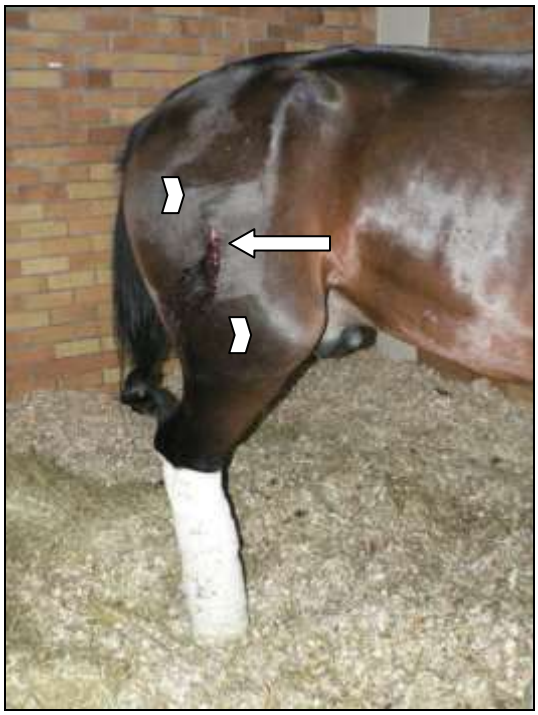

Fig 1: Severe right hind limb swelling extending from the proximal femoral area to the distal aspects of the tarsus (arrow heads). A stab incision, packed with sterile gauze (arrow), is present on the lateral femoral area.

on the dorsum of the digit. No abnormalities were detected on rectal examination. Evaluation of a peripheral blood smear was unremarkable with blood packed cell volume (PCV) and total serum protein (TSP) 34% (reference range 24-44%) and 64 g/l (reference range 66-83g/l), respectively.

Diagnostic imaging:

The horse was sedated with romifidine (Sedivet)[1] (0.04mg/kg bwt i.v.) and ultrasonographic examination of the right hemi-pelvis, right femur and surrounding soft tissues was performed. This revealed severe soft tissue swelling and multiple irregular, poorly marginated hyper- to hypoechoic areas at the caudo-lateral aspect of the mid-femur.

To obtain proximal femur and pelvis radiographs the horse was sedated with butorphanol (Torbugesic)[3] (0.02mg/kg bwt i.v.) and romifidine (0.08 mg/kg bwt i.v.) and induced into general anaesthesia with ketamine (Ketamine-Fresenius)[4] (2mg/kg bwt i.v.). Anaesthesia was maintained with halothane (Halothane M and B)[5], oxygen inhalation and a constant rate infusion

of ketamine (2 mg/kg bwt per hour i.v.) and metdetomidine (Domitor)[6] (1µg/kg bwt per hour i.v.). Ventro-dorsal views of the right hemi-pelvis and medio-lateral views of the femur and stifle joint were obtained. No osseous abnormalities were evident in any of the radiographs. Ultrasonographic examination of the femoral area was repeated. A 7 x 12 cm round structure with an irregularly marginated capsule-like structure with a heterogeneous lumen filled with hypoechoic contents and some hyperechoic particles was found at the proximal lateral region of and adjacent to the femoral diaphysis (**Fig 2**). These findings were consistent with an organizing haematoma or an abscess. An ultrasound-guided fine needle aspirate of the mass yielded no material. Lancing of the structure with a stab incision from the lateral aspect of the femur was attempted and resulted in profuse haemorrhage. Sterile packing of the incision with a gauze roll was performed after which the horse recovered uneventfully from anaesthesia.

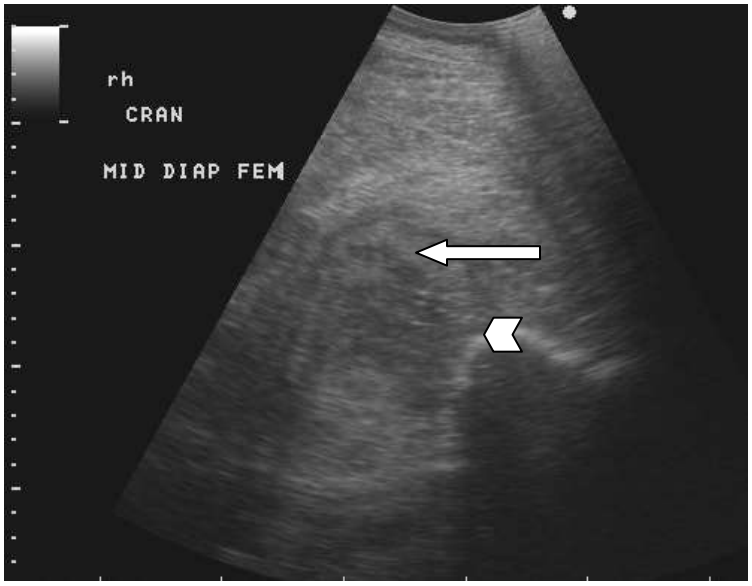

Fig 2: Ultrasonographic image of a well encapsulated mass (white arrow) in the region on the proximal cranio-lateral region of the femoral diaphysis (arrow head). Cranial is to the left.

Case management:

Haematological analysis was unremarkable with a haematocrit of 0.33L/L, red blood cell count of 7.11 $x10^9$/L (reference range of 5.5-9.5), white cell count of 6.14 $x10^9$/L (reference range 6-12.0), with 82% mature and 6% immature neutrophils. Plasma fibrinogen level was 4g/l

(reference range 1-4 g/l). The horse was administered penicillin (Benzyl Penicillin Fresenius)[2] (22 000 IU/kg bwt i.v. q 6 h), amikacin (Amikacin Fresenius)[4] (25mg/kg bwt i.v. q 24 h) and metronidazole (Bemetazole)[7] (15mg/kg bwt p.o. q 8 h). An epidural catheter (Epidural catheter set)[9] was placed in the first coccygeal space and epidural analgesia administered with morphine (0.04 mg/kg bwt), detomidine (Domosedan)[6] (0.002 mg/kg bwt) and lidocaine (Lignocaine 2%)[8] (0.04mg/kg bwt) every 6-8 hours.

A modified Robert Jones bandage which included the foot and a plantar splint was placed on the affected distal limb to maintain the digit in extension. A support bandage was placed on the contralateral limb. The horse received 12L intravenous crystalloid fluids during the night. During the first two days his level of comfort increased markedly.

On the 3rd day after admission, haematological analysis was repeated, revealing a low normal haematocrit of 25%. No other abnormalities were present. Plasma fibrinogen and serum creatinine were within normal limits. The gauze packing in the previously mentioned incision was replaced without further bleeding. A bandage was applied to the right tarsus to reduce swelling. The epidural catheter was no longer patent and was removed. Phenylbutazone (Phenylarthrite)[10] was added as an analgesic agent (2.2 mg/kg bwt i.v. q 12 h). Due to low faecal production, magnesium sulphate (1g/kg bwt) and 6L of water were administered via nasogastric intubation.

For the next five days the horse received hydrotherapy of the affected limb between daily bandage changes. The horse maintained a persistent tachycardia of 60-72 beats per minute and low PCV (the lowest being 18% on the 6th day, with TSP 68 g/l) but showed marked clinical improvement. The swelling of the affected limb decreased, he became bright and alert and was able to bear full weight on the splinted limb. Medication was discontinued except for phenylbutazone (Phenylbutazone)[11] (2,2 mg/kg bwt p.o. q 12 h) and the gauze packing was removed from the incision. Due to financial constraints the horse was discharged from hospital on phenylbutazone (2.2 mg/kg bwt p.o. q 24 h) with instructions to have the PCV monitored by the referring veterinarian. At this stage a torn muscle with peroneal nerve neuropathy possibly secondary to swelling or intramuscular haemorrhage (Katz *et al*. 1991, Kaymak *et al*. 2002) was considered the most likely diagnosis.

The owners reported profuse haemorrhage from the previous incision 4 days after discharge. The referring veterinarian packed and sutured the incision and referred the horse back to the OVAH. On presentation there was severe tachycardia (100 beats per minute), tachypnoea (40 breaths per minute), rectal temperature of 38.1 ºC, pale mucous membranes with a PCV of 21% and TSP of 70 g/l and grade 4/5 lameness of the right hind limb. The distal aspect of the affected limb was bandaged and marked swelling was present around the right stifle and femoral area. Ultrasonographic evaluation revealed similar but more extensive pathology than previously: the haematoma-like structure extended from the caudal proximal femur to the caudal aspect of the proximal tibia.

Treatment with ceftiofur (Excenel)[6] (2.2mg/kg bwt i.v. q 12 h), phenylbutazone (2.2 mg/kg bwt i.v. q 12 h) and intravenous crystalloid fluids (50ml/kg bwt per 24 hours) was initiated. Haematological analysis revealed leukocytosis of 14.09 $x10^9$ cells/L with 12.79 $x10^9$ mature neutrophils/L. Prothrombin time was 11,2 seconds and partial thromboplastin time 37,5 seconds, matching those of a healthy control horse. Serum urea and creatinine were within normal limits. Morphine (0.12 mg/kg bwt q 8 h) and xylazine (Chanazine 2%)[8] (0.012 mg/kg bwt q 8 h) were administered via an epidural catheter. To decrease the risk of gastrointestinal impaction, 4L of liquid paraffin with 4L of water were administered via nasogastric intubation.

During the following four days the PCV oscillated between 15 and 21%. The right limb was maintained in a distal limb bandage with a plantar splint, although the horse would not bear weight on this limb. A support bandage and frog support with high-density polystyrene wedges were applied to the left hind limb. The systemic antimicrobial coverage was extended by adding metronidazole (20mg/kg bwt per rectum q 8 h). By the 2nd day after re-admission the horse showed increased levels of discomfort. Ultrasonographic examination revealed similar findings to the previous scan. Purulent exudate was observed draining from the incision site: the packing was removed and profuse bleeding from the incision occurred. The incision was re-packed with sterile gauze. The epidural catheter was no longer patent and was removed. Pain medication consisting of a constant rate infusion of ketamine (0.6 mg/kg bwt/hr i.v.) was started. The following day the horse appeared more comfortable and would intermittently bear full weight on the affected limb. However, a day later the stallion became recumbent, showed severe signs of discomfort and was humanely destroyed.

Post Mortem Findings:

The right hind limb had a comminuted mid-shaft femur fracture with regional haemorrhage. Muscle surrounding the fracture area had numerous small (0,1 to 1,5cm) multifocal, red, poorly defined, slightly nodular lesions resembling haematomas (**Fig 3**); similar lesions were seen in the caudal internal abdominal wall (**Fig 4**), mesentery, on both diaphragmatic surfaces, in the endocardium and scattered in the myocardium. The multilobular right deep inguinal lymph node was enlarged (largest lobule approximately 6 cm in diameter), haemorrhagic and patchily necrotic on the cut surface (**Fig 5**). A large, elongated, clotted haematoma (70cm in length and from 10 to 15cm in irregular diameter) occupied the groove between two muscle bellies of the *biceps femoris* muscle and tapered off below the stifle and above the lateral aspect of the tarsus (**Fig 6**). The lungs, spleen and kidneys appeared macroscopically normal. Specimens of affected muscle from the right hind limb, as well as the affected lymph node and a mesenteric serosal nodule were preserved in 10% neutral buffered formalin for routine sectioning and staining with Haematoxylin and Eosin (HE). The disembodied femur was radiographed, showing moderate osteopaenic bone in the area of the severely comminuted fracture (**Fig 7**). Fracture-site bone was decalcified in formic acid before sectioning and staining. Immunohistochemical (IMH) staining using a standard avidin-biotin technique (Haines and Chelack 1991) for factor VIII-related antigen was applied to representative sections from muscle, mesentery and fracture-site bone and all sections examined by light microscopy.

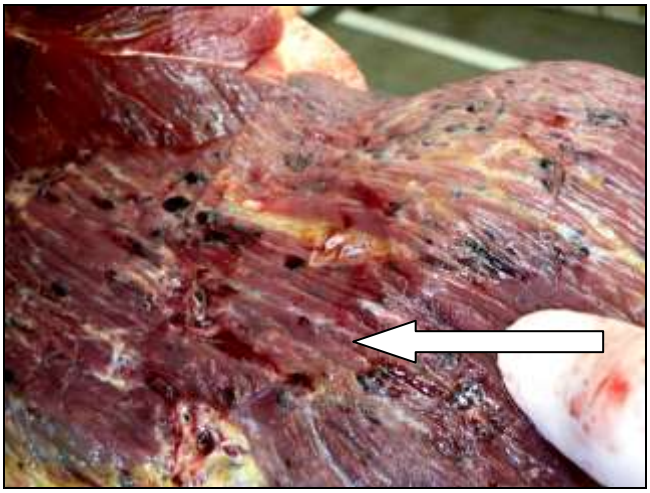

Fig 3: *Post mortem* examination of the right hind limb peri-femoral musculature. Red and yellow mottling is present due to multifocal small HASs (white arrow) often embedded in fibrous connective tissue.

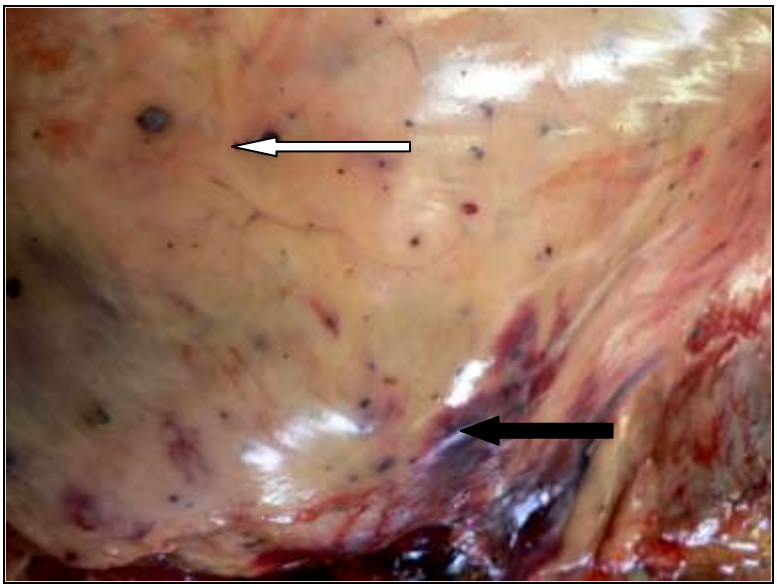

Fig 4: *Post mortem:* Caudal abdominal wall showing multifocal small nodular metastatic HASs (white arrow) and some petechiae and ecchymoses (black arrow).

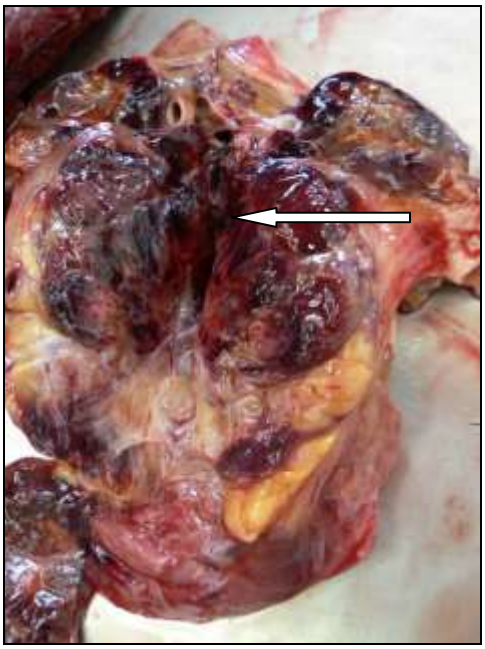

Fig 5: *Post mortem:* Cut surface (white arrow) of the enlarged, haemorrhagic, necrotic, HAS-invaded multi-lobular right deep inguinal lymph node.

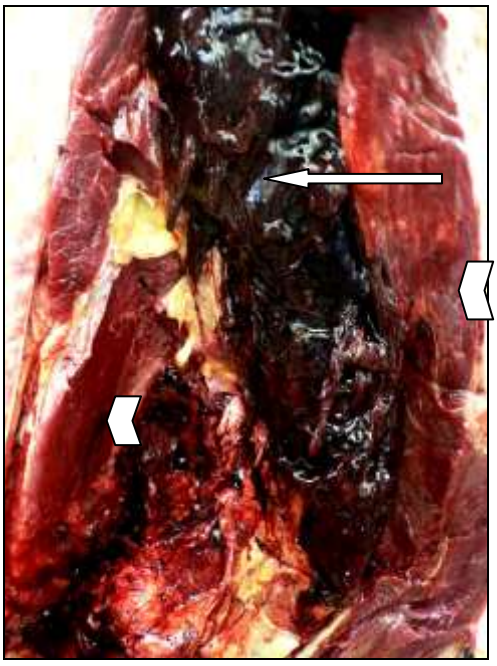

Fig 6: *Post mortem:* Right hind limb suspended from the hoof showing the massive lateral haematoma (white arrow) between muscle bellies of the *biceps femoris* muscle (arrow heads).

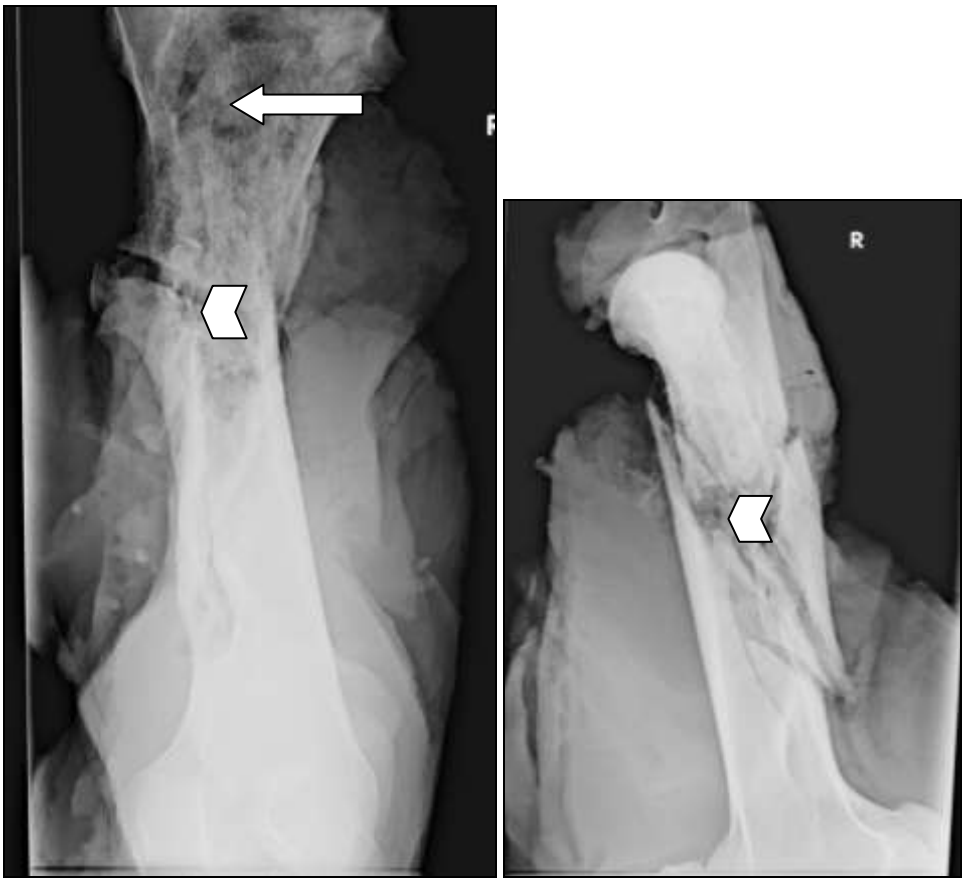

Fig 7: *Post mortem: C*raniocaudal (left) and lateromedial (right) radiographs of the disembodied right femur, with lateral and cranial to the left respectively, showing the moderate osteopaenia (white arrow) and comminuted fracture (arrow head).

Histopathological Findings

The right hind limb muscular and abdominal serosal nodules comprised variably-sized irregular blood-filled spaces, which were either encapsulated or infiltrating into muscle. The nodules were lined by either a single layer of small or pleomorphic, plump, neoplastic endothelial cells, or multilayered, spindle-cell dense, irregularly thickened walls (**Fig 8**), sometimes thrown into folds, and with robust supporting connective tissue. The deep inguinal lymph node parenchyma was mostly replaced by large areas of haemorrhage and necrosis with more peripheral tumour presence and compression of remaining lymphoid tissue to below the capsule. Factor VIII-related antigen IMH staining showed the better differentiated intramuscular tumours forming vascular channels to have strong endothelial cell cytoplasmic staining (**Fig 9**),

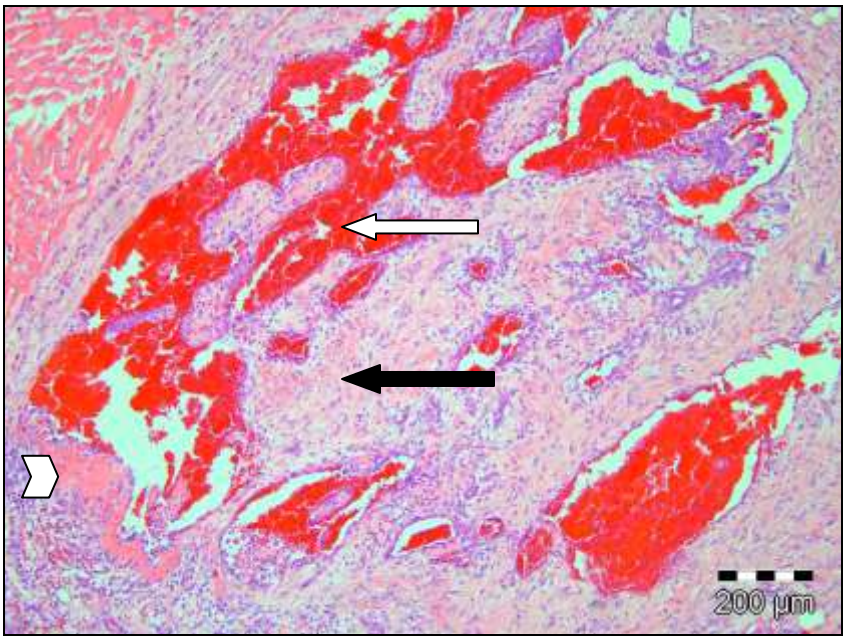

Fig 8: Intramuscular focal HAS showing vascular channel formation (white arrows) and robust extravascular connective tissue (black arrow) and occasional fibrin (arrow head).

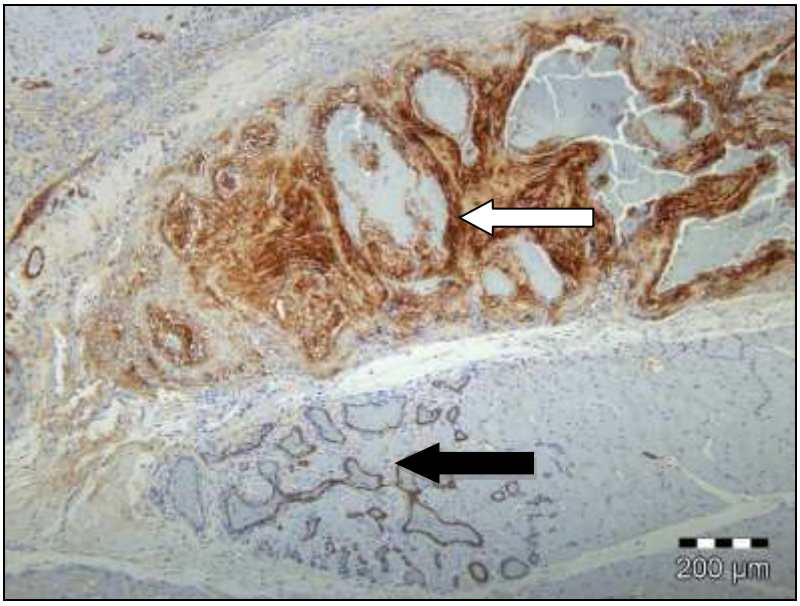

Fig 9: Factor VIII-related antigen immunohistochemical staining of focal HAS in right hind limb skeletal muscle showing marked expression in neoplastic proliferating endothelial cells (white arrow) and dilation of thin-walled vessels in an adjacent muscle bundle (black arrow).

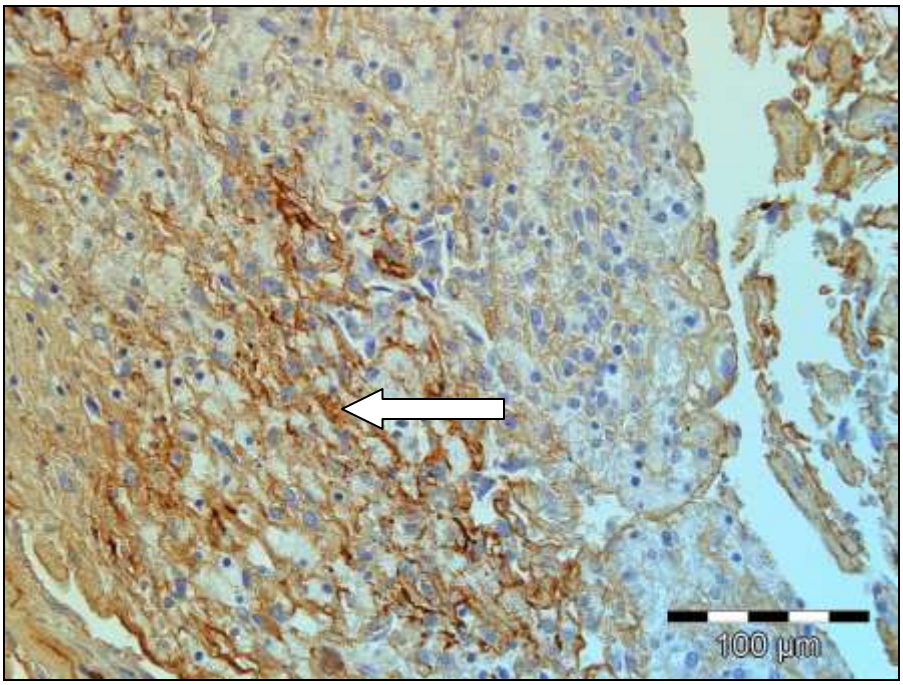

Fig 10: Immunohistochemical staining of neoplastic endothelial cells (white arrow) for factor VII-related antigen in the periosteum of the region of the right hind limb femur fracture.

whereas the solid tumours had scant staining. Although there was some loss of cellular detail and IMH staining caused by the decalcification, there was evidence of haemangiosarcoma involving the periosteum in the region of the fracture (**Fig 10**), with occasional round, cell-dense tumour emboli within the marrow cavity. The bone shards at the fracture site were necrotic on histopathological examination. The tumour was interpreted as having arisen periosteally or intramuscularly with muscle and bone invasion, rather than from the bone marrow.

Diagnosis*:*

A *post mortem* diagnosis of disseminated haemangiosarcoma, affecting the right-hind limb musculature, epicardium, myocardium, pleura, peritoneum, deep inguinal lymph node, mesentery and leading to a pathological femur fracture was made.

Discussion:

The presenting complaint for horses with disseminated haemangiosarcoma varies tremendously. In a review of 35 cases, 24% were presented for subcutaneous and muscular

swelling and 12% for lameness (Southwood *et al*. 2000). Lungs and pleura were most commonly affected, followed by skeletal muscle in up to 46% of cases (Southwood *et al*. 2000). Osseous involvement is rare and has been reported involving the equine vertebrae (Newton-Clarke *et al*. 1994), mandible (Southwood *et al*. 2000) and humerus (Cottle *et al*. 2008). To the authors' knowledge this is the first case report of haemangiosarcoma affecting the equine femur or of haemangiosarcoma leading to a pathological fracture in an equine long bone.

Radiography on the live patient failed to show osteopaenia seen on the *post mortem* radiographs of the femur. This could be explained by insufficient time elapsed for 30% demineralisation to occur in order to be evident radiographically (Butler *et al*. 2008). Also, on the *post mortem* radiographs, the femur was disembodied which allowed for removal of large amounts of soft tissue, thus avoiding soft tissue super-impositioning. During radiographic examination under general anaesthesia two orthogonal views of the femur might have revealed osseous abnormalities like a periosteal reaction; however this was unfortunately not done. Notwithstanding, the authors believe that the pathological fracture only occurred on the last day at the time when the animal became severely distressed and recumbent.

Alternatively, a scintigraphic examination could be performed without the risk of general anaesthesia in a horse with a suspected femur or pelvis fracture, (Pilsworth 2011) and is likely to have yielded increased radiopharmaceutical uptake, which has been described in haemangiosarcoma of the humerus of a Clydesdale gelding (Cottle *et al*. 2008). Scintigraphy was not preformed due to financial constraints.

A traumatic incident is often implicated in the history of horses presented for subcutaneous or muscular swelling or lameness caused by haemangiosarcoma; likewise, the *ante-mortem* diagnosis is often incorrectly made as a haematoma (Valentine *et al*. 1986, Pille *et al*. 2004, Johns *et al*. 2005). Ultrasonography and fine needle aspirates are commonly unrewarding (Southwood *et al*. 2000, Pille *et al*. 2004), whereas multiple biopsies and histopathological examination might yield the diagnosis (Southwood *et al*. 2000, Johns *et al*. 2005). Due to the low level of suspicion of haemangiosarcoma as cause of severe lameness in this case no biopsy was attempted during hospitalisation. Marked bleeding has been described following biopsy (Waugh *et al*. 1977, Cottle *et al*. 2008) or fine needle aspiration (Pille *et al*. 2004) of haemangiosarcoma as occurred in this case.

Anaemia, as was present in this case, is often the only consistent haematological abnormality in horses with haemangiosarcoma and is explained by blood loss and microfragmentation of the red blood cells (Waugh *et al*. 1977, Rebar *et al*. 1981, Valentine *et al*. 1986, Collins *et al*. 1994, Southwood *et al*. 2000, Johns *et al*. 2005). In the present case the anaemia was believed to be due to the continued intramuscular haemorrhage and haemorrhage resulting from the stab incision. Some intravascular haemolysis cannot be ruled out as serum bilirubin concentrations (Dhaliwal, Cornett and Tierney 2004) were not measured. The carcass however was not icteric at the time of necropsy. The case progressed to a neutrophilic leukocytosis which has also been reported (Waugh *et al*. 1977, Southwood *et al*. 2000).

Increased serum alkaline phosphate activity (ALP) is common in human cancer patients with bone metastasis (Garnero *et al*. 2000, Min *et al*. 2009). Likewise increased ALP is seen in dogs with osteosarcoma and has been correlated with the prognosis for survival (Garzotte *et al*. 2000). Although not measured, increased ALP in the present case was possible and could have aided in identifying the osseous involvement.

Treatment in the absence of a diagnosis is supportive and includes antimicrobial coverage, intravenous fluids, blood transfusion, corticosteroids and analgesic agents (Waugh *et al*. 1977, Valentine *et al*. 1986, Collins *et al*. 1994, Southwood *et al*. 2000, Johns *et al*. 2005, Reischauer *et al*. 2006). Isolated cases of successful surgical excision and even spontaneous resolution have been described (Pelt, Langham and Gill 1972, Johns *et al*. 2005, Lempe *et al*. 2008). Interstitial brachytherapy can be used in the management of these tumours (Burks *et al*. 2009); however in disseminated cases the outcome is invariably fatal (Waugh *et al*. 1977, Valentine *et al*. 1986, Collins *et al*. 1994, Southwood *et al*. 2000, Johns *et al*. 2005).

The use of distal limb bandages in a proximal limb fracture is usually contraindicated due to the added weight to the distal limb (Fürst 2011). In the present case a Modified Robert Jones splint bandage was needed to prevent the horse taking weight on the dorsum of the metatarso-phalangeal joint, since spontaneous extension of the metatarso-phalangeal joint during weight bearing was absent. The splint used was PVC and this bandage added very little weight to the distal limb.

A definitive diagnosis is often only reached with a thorough *post mortem* and histopathological examination (Collins *et al*. 1994, Southwood *et al*. 2000, Reischauer *et al*. 2006, Cottle *et al*. 2008). Staining of histopathological sections with Factor VIII, which is specific for endothelial cells, thrombocytes and megakaryocytes, is advised to obtain a definitive diagnosis (Collins *et al*. 1994, Southwood *et al*. 2000, Johns *et al*. 2005).

Although haemangiosarcoma most commonly affects the spleen in dogs (Brown, Patnaik and MacEwen 1985), osseous involvement (Gibbs, Denny and Lucke 1985) and more commonly intramuscular haemangiosarcoma leading to lameness (Shiu *et al*. 2011) have been described.

This case demonstrates the difficulty in reaching a clinical diagnosis of haemangiosarcoma as a cause of severe lameness in the horse. Neoplasia should be included in the list of differential diagnoses for horses with severe lameness and limb swelling, especially when routine diagnostic examinations fail to identify a cause and the affected horse shows anaemia or undifferentiated intramuscular masses (Knottenbelt and Clegg 2004, Cottle *et al*. 2008). To the authors' knowledge, this is the first case report describing haemangiosarcoma affecting the femur of a horse and leading to a pathological long bone fracture.

Manufacturers' addresses

1. Boehringer Ingelheim, Ingelheim Pharmaceuticals (Pty) Ltd., Randburg, South Africa
2. Adcock Ingram Critical care (PTY) Ltd., Johannesburg, South Africa
3. Fort Dodge Animal Health, Iowa, USA
4. Bodene (Pty) Ltd., Port Elizabeth, South Africa
5. Safeline Pharmaceuticals, Johannesburg, South Africa
6. Pfizer Laboratories (Pty) Ltd., Sandton, South Africa
7. Be-tabs Pharmaceuticals (Pty) Ltd., Roodepoort, South Africa
8. Bayer, Isando, South Africa
9. Arrow International, Inc., 2400 Bernvill Road, Reading, Pennsylvania US,
10. Schering-Plough Animal Health, Isando, South Africa
11. Kyron Laboratories (Pty) Ltd., Barney Road, Benrose, South Africa

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