

Paraneoplastic pruritus in a 7-year-old Slovak warmblood with malignant round cell tumour.

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Abstract

A seven year old Slovak warmblood gelding, used as a police horse, initially presented to the referral clinic when it was five years old, with an itching keloid on the distal limb, requiring surgical removal. After surgical removal of the keloid and successful closure of the skin, it was necessary to maintain bandaging the healed wound to prevent self mutilation as the healed surgical site remained pruritic post operatively. After two years, the horse presented to the clinic again with pruritic and alopetic regions of the head, neck, limbs and base of the tail. Attempts to find the cause of dermal irritation included dermatological, parasitological, microbiological and ultrasonographic examination. Treatment with corticosteroids, antiparasitics and antibiotics were not successful. Further investigation due to the horse's worsening general status led to the finding of marked pleural effusion. Cytology of the pleural fluid confirmed there was, as suspected, neoplastic changes in the thoracic cavity. Furthermore there were gross pathological changes of the lymphatic tissue of the cranial mediastinum, surroundings of the aorta in the thoracic cavity and lymph nodes of the abdominal cavity. Histology of the lymphatic nodes found poorly differentiated round cell tumours. Histopathological findings confirmed there was a very aggressive form of lymphoma. The pruritus, generalised moth-eaten alopecia and self-mutilation were the first symptoms of the terminal disease in this case.

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Summary

A seven year old Slovak warmblood gelding, used as a police horse, initially presented to the referral clinic when it was five years old, with an itching keloid on the distal limb, requiring surgical removal. After surgical removal of the keloid and successful closure of the skin, it was necessary to maintain bandaging the healed wound to prevent self mutilation as the healed surgical site remained pruritic post operatively. After two

years, the horse presented to the clinic again with pruritic and alopetic regions of the head, neck, limbs and base of the tail. Attempts to find the cause of dermal irritation included dermatological, parasitological, microbiological and ultrasonographic examination. Treatment with corticosteroids, antiparasitics and antibiotics were not successful. Further investigation due to the horse's worsening general status led to the finding of marked pleural effusion. Cytology of the pleural fluid confirmed there was, as suspected, neoplastic changes in the thoracic cavity. Furthermore there were gross pathological changes of the lymphatic tissue of the cranial mediastinum, surroundings of the aorta in the thoracic cavity and lymph nodes of the abdominal cavity. Histology of the lymphatic nodes found poorly differentiated round cell tumours. Histopathological findings confirmed there was a very aggressive form of lymphoma. The pruritus, generalised moth-eaten alopecia and self-mutilation were the first symptoms of the terminal disease in this case.

Key points

- The presentation of severe pruritus and self-mutilation with generalised moth-eaten alopecia could be the first clinical signs to suggest a diagnosis of neoplasia of the lymphatic system in the horse.
- The pattern of moth-eaten alopecia is important to notice and differentiate from other skin conditions that cause pruritus and alopecia.
- Moth-eaten alopecia pattern is not particularly similar to any other skin disease, so it could potentially be pathognomonic and help assist with ascertaining future and early correct diagnosis.

Introduction

The most common clinical signs of paraneoplastic syndromes in horses include hypercalcemia, hypoglycemia, cachexia, intermittent fever, anemia, thrombocytopenia, neutrophilia, protein losing enteropathy, vasculitis and pruritus (Axiak and Johnson 2011, Knottenbelt et al. 2015). Long before the tumour is clearly identified, paraneoplastic pruritus is often the main or only clinical sign observed, which can make early and accurate diagnosis of such pathologies very difficult. (Knottenbelt et al. 2015). Such pruritus can be accompanied by alopecia, which can be as a consequence of paraneoplastic changes or by self-mutilation (Knottenbelt et al. 2015). Skin biopsies and scrapings are often inconclusive or nonspecific and can be confused with the consequences of self-trauma (Knottenbelt et al. 2015). There is some evidence that pruritus as a consequence of neoplasia is caused by the destruction of T-cells arising from pathological cytokines produced by the tumour itself or the tumour's growth, nerve compression, bile duct compression with cholestasis or necrotic tumorous cells (Knottenbelt et al. 2015). Paraneoplastic pruritus has been described in horses with lymphoma (Finley et al. 1998), mast cell tumours (Combarros et al. 2020) and renal carcinomas (Curnow 2020).

Despite the fact that pruritus is reported as one of the relatively more common equine paraneoplastic clinical signs, there is a little clinical evidence to support it.

In this article, we would like to document a case whereby persistent pruritus was the only clinical sign in a horse suffering from paraneoplastic pruritus caused by malignant round cell tumours. Publications in equine oncology are few, and documentation of such cases such are necessary to aid and assist practitioners in achieving more expedited and correct diagnosis of such conditions

Case history

The gelding arrived at the Equine clinic UVLF for the first time in the summer of 2021, and presented with a keloid on the medial aspect of the fetlock on the left forelimb requiring surgical removal. The lesion was made worse due to repetitive trauma sustained when hitting the keloid scar with the contralateral limb. Intensive pruritus was localised only in this area and the compulsion to scratch the area did not subside even after surgical removal. Consequently the horse had to wear a firm rubber bandage to protect the limb to prevent self-mutilation, which was considered psychogenic and remained unchanged for the rest of the patient's life. Almost two years later, at 7 years of age, the horse once again developed pruritus and alopecia lesions in the head area (Fig 1 and 2). He was stabled with 30 other horses which did not suffer from a similar problem. Two weeks before the onset of the first clinical signs, the owner had begun adding nutritional supplements to the feed such as vit. C, biotine, and methylsulfonylmethane (MSM). After discovering the lesions, the owner

applied a topical spray with allantoin and later treated the alopecic areas with betadine solution. Within one week of the first appearance of the alopecic lesions, the pruritus became more aggressive and generalised, and with alopecic areas all over the body, including over the mane and tail (Fig 3; 4; 5; 6; 7). Soon after and upon deterioration of the condition and its clinical appearance the horse was taken to the referral Equine clinic at UVLF.

Clinical findings and treatment

On admission, a clinical assessment of the horse found that despite having poor body condition (BCS 4/9), the TPR values were within normal limits, the mucous membranes were normal, and the sub-mandibular lymph nodes were not enlarged. Hematological and biochemical parameters were also found to be completely normal. However the horse was extremely pruritic and presented with generalised “moth-eaten” alopecia, with scabs containing blood (Fig. 2) over some of the alopecic areas. The lesions were however superficial (Fig. 3 and 4). Additionally there was no skin efflorescence observable to indicate the pruritus was caused by a common skin disease. Skin smears and scrapings were performed and were positive for *Pantoea agglomerans*, *Staphylococcus epidermidis*, and aerobic spore-forming species. These pathogens were discounted as being the primary source of the pathology and interpreted as being a consequence of secondary infection. Additionally there was no evidence of ectoparasites or fungal infection present. After skin scraping samples were obtained, the horse was repeatedly bathed once a day with Vetericyn Foam Care medicated shampoo, combined with ketoconazole (Innovacyn Inc, California), but to no effect. The parasitologist who was asked to examine the horse strongly suspected hypersensitivity to the bite of *Culicoides* spp. 30 ml of Ancesol (Chlorphenamine maleate, 10 mg/ml, Richter Pharma AG, Austria) was administered IV for three days in a row, but also to no effect. Due to the tail rubbing and the suddenly slightly increased eosinophils ($0.84 \times 10^9/l$; $RI 0.00 -$

$0.80 \times 10^9/l$), a coprological examination was performed, which was highly positive for *Strongylus* spp. The horse was dewormed with a 5-day course of fenbendazole.

Because paraneoplastic pruritus was suspected, an ultrasonographic examination of the thorax and abdominal cavity was performed, but did not yield any pathological findings. Rectal examination was negative for a mass. Generally the horse continued to have an appetite, but on occasion was not interested in food especially during episodes of nervousness due to pruritus. On dental examination a few lesions around the incisors were observed as well as sharp edges on the premolars and molars, which were likely the cause of the erosions on the buccal mucosa. The lesions near the incisors were small and it is impossible to deduce if they could have been a clinical sign or if they arose as a consequence of self-mutilation and rubbing of the nose against the walls. After two weeks of hospitalization, the horse started to lose body condition to 3/9 BCS. During the third week, the hematological profile showed a decrease in lymphocytes ($0.91 \times 10^9/l$; $RI 1.50 -$

$5.10 \times 10^9/l$) and monocytes ($0.15 \times 10^9/l$; $RI 0.20 - 0.60 \times 10^9/l$) and at the same time a slight swelling in the preputial area was noticed. At the end of the third week of hospitalization the horse was euthanized.

Diagnosis

Poorly Differentiated Malignant Round Cell Tumour - Lymphoma

Pathological and histological examination and outcome

The results of a necropsy revealed, a large volume of free fluid in the thoracic cavity, with blood adhered to the diaphragm, and atelectasis of the edges of the lung lobes (Fig. 13). A large mass was found in the cranial mediastinum, approximately 15x15 cm in size, which surrounded the trachea and esophagus (Fig. 14), and continued up to the aorta. Enlarged lymph nodes were found in the stomach area. The stomach showed evidence of mucosal hemorrhages in the pyloric area. The intestines were without visual pathological changes except for the ileum, where small hemorrhages on the mucosa and enlarged mesenteric nodes were observed. The liver was intact, and the spleen was normal on first sight, but on palpation and later on dissection there were obvious changes in the area of the gastrosplenic vein. The vein was firm, with a thickened wall and the corresponding lymph nodes were markedly enlarged. While the kidney parenchyma remained intact, there were also changes in the lymphatic nodes around the kidneys.

Samples of the fluid on smear testing showed moderate cellularity. Cell preservation was fair. Cytologically, a small population of lymphocytes with round nuclei and reduced cytoplasm were observed (cross sections of the nuclei <1.5x the size of an erythrocyte). These were the predominant cells on the smears from the fluid and were the second most common nucleated cells in the pre-made smears. The cells showed mild anisocytosis and anisokaryosis. Mitotic figures were not found. Low numbers of neutrophils were found in the smears from the fluid samples and moderate numbers were found in the pre-made smears. Very low numbers of eosinophils and macrophages were found.

Microscopic examination of the spleen showed no lesions. The lymph node adjacent to the spleen had an intact capsule with some retention of normal cellularity and architecture. However, most of the medullary sinuses, some vascular spaces, and occasional foci of the lymph node parenchyma had a population of neoplastic cells. The normal cell population were instead replaced by monomorphic populations of large round cells. These cells had slightly ill-defined borders, moderate amounts of eosinophilic cytoplasm, and varying numbers of oval to convoluted nuclei with fine chromatin and some with particularly large nucleoli. Mitotic figures were moderately frequent and occasionally atypical. There was an increase in cortical small lymphocytes (perhaps reactive). Additionally the perinodal adipose tissue was infiltrated with tumorous round cells. The stomach and kidney lymph nodes showed the same findings as described in the lymph node above.

Findings in the mediastinum lymph node, thymus, and the surrounding adipose tissue were similar to those described in and around the splenic lymph node, above. In addition, rare large cells in the thymic sinuses showed erythrophagocytosis. These cells also resembled the aforementioned tumorous cells.

The ileum showed a moderate infiltration of plasma cells with eosinophils present in the mucosa. There were also a moderate number of eosinophils present in the submucosa, and surrounding glands. No neoplastic cells were found. These findings were indicative of eosinophilic to plasmacytic duodenal enteritis, ranging from a mild to moderate level of disease progression.

A histopathological examination was repeated to determine the type of the tumour, but the exact identity of the tumour cells remained unclear. The tissue showed large to huge blasts (Fig. 15) with a bizarre appearance, as they were surrounded by a rich mixture of small (probably T-cell) lymphocytes, which would support a diagnosis of an aggressive form of equine lymphoma.

Immunostaining was carried out for antibodies against CD3, CD79a. The residual lymphocytes within the follicles and paracortex show immunopositive staining for CD79a and CD3 respectively. The atypical neoplastic cells percolating throughout the sinuses failed to express CD79a and CD3.

Histopathological examination and immunostaining were not sufficient to determine the exact type of the tumour, although lymphoma or histiocytic sarcoma was suspected. Upon repeating the histological examination, the samples were found to have a greater population of poorly differentiated cells, and thus the results of which were more compelling towards supporting the diagnosis of an aggressive lymphoma.

Serological oncomarker testing for thymidine kinase was performed with the results obtained two weeks after euthanasia however with negative results 0.696 U/l (< 3).

Discussion

Despite the patient being repeatedly examined in order to determine the exact diagnosis, it took four weeks from the onset of the first clinical signs of generalised pruritus and alopecia until the patient started to develop additional clinical signs indicating the presence of a malignant tumour. Knottenbelt et al. (2015) reminds us that pruritus can be the only sign of the presence or progression of carcinoma.

Since the skin was altered, due to self-mutilation rather than the primary skin disease, a skin biopsy was not immediately performed. At the same time, the horse was on a high dose of corticosteroids, and consequently a skin biopsy was not performed until a few weeks after they were discontinued. As the use of corticoids resulted in a deterioration of the secondary skin infection, administration of antibiotic therapy was indicated.

As is also described in Knottenbelt et al. 2015, whereby horses with pruritis do not suffer from paraneoplastic syndrome, this horse also started to loose condition during hospitalization, despite maintaining to preserve an appetite. However this patient was never in satisfactory condition due to being on prolonged box-rest for four weeks, and consequently this symptom and pruritus alone were not in themselves an indicator of a fatal disease.

After there was no response to various therapies, paraneoplastic pruritus was considered, but the examinations undertaken did not confirm the occurrence of a tumour until three weeks after admission to the hospital where there was a rapid deterioration of the the horse's condition.

The pattern of moth-eaten alopecia is important to notice and differentiate from other skin conditions that cause pruritus and alopecia. When comparing the photos of our patient with the patients in the article by Combarros et al. (2020) and Curnow (2020), the pattern of alopecia looks identical. It's unique presentation is not particularly simmlar to any other skin disease, so it could potentially be**pathognomonic** and help assist with ascertaining future and early correct diagnosis.

Serum was collected for examination of the proliferation marker, thymidine kinase, which can be significantly increased in the presence of lymphomas (Larsdotter et al. 2015), leukemia or multiple myeloma. This result was however negative, although this does not reliably exclude malignant tumour (Moore et al. 2021).

As it is unusual for small lymphocytes to be predominate in pleural effusion, small round cell lymphoma was suspected, but the tumour cells were large round cells, and so they were not perfectly compatible with the cells seen on the pleural effusion. Thus the cells in the effusion might have represented T-lymphocytes reacting to the neoplasia.

Histology confirmed a malignant round cell neoplasm which had infiltrated all of the submitted lymph nodes samples (mediastinum, spleen, stomach, kidney) as well as being present in the thymus and in the adipose tissue around the nodes. This location of the tumour and the changes in lymph nodes in the area of the digestive tract and kidneys would indicate mediastinal, thymic or thoracic lymphosarcoma, which most often affects geldings and stallions younger than 10 years of age (Fukunaga et al. 1993). Lymphosarcoma is the most common thoracic neoplasia in horses, occurring in 54-74% of cases (Sweeney and Gillette 1989, Mair and Brown 1993). Thoracic lymphosarcomas usually have a rapid course and the average time from the appearance of the first clinical signs to euthanasia is approximately four weeks (Mair and Hillyer 1991). These masses can be as large as 15-36 cm in diameter, and are often associated with large volumes of pleural effusion, which can dislocate the lungs dorsally, leading to compressive atelectasis of the lungs (Mair et al.1985, Sweeney and Gillette 1989, Meyer et al. 2006). Pleural effusion is caused by neoplastic infiltration of the mediastinal lymph nodes with reduced pleural lymphatic drainage (Sweeney 1992). Drainage of this fluid can sometimes be achieved with the placement of a permanently inserted catheter, however often without successas due to the rapid increase in the volume of fluid produced. The tumour in this patient was nodular, occured in the mediastinum, and had enlarged adjacent nodes which were adhered. Subsequently it was impossible to precisely circumscribe the tumour margins. Knottenbelt et at. (2015) describes this pattern as being typical of lymphosarcomas involving the mediastinum, but less so the case in thymic lymphosarcomas. However, the absence of immuno-positive staining for CD3 and CD79a did not support a diagnosis of T-or B-cell lymphoma. The second assumed possibility was histiocytic sarcoma, which albeit rare in horses, does metastasise quickly, affects the skin and subcutaneous tissue, parenchymatous organs, bone marrow and surrounding soft tissues. It can also metastasize to the lymph nodes of the mediastinum and also carries a poor prognosis (Beusker et al. 2010, Knottenbelt et al. 2015). However, this type of tumour did not completely match the description in this patient.

The exact identity of the tumour cells was ambiguous. They could be large lymphocytes (poorly differentiated, high-grade) or they could be histiocytes (disseminated histiocytic sarcoma). Other round cell tumour types and other cell lineages were possible but appeared less likely. The second examination confirmed the presence of lymphocytes.

But whatever the true identity of the cells, the lesion had disseminated widely and was also present in

the blood vessels, suggesting that further metastasis was likely and the prognosis would have been poor. Immunostaining was performed to try to distinguish B-cell lymphoma, T-cell lymphoma and histiocytic sarcoma. But poorly differentiated tumour cells do not react appropriately to immunostains, and additionally immunostains are not available for every possible tumour cell lineage. So while a definitive diagnosis remained unclear the second histological examination did indicate incidence of lymphoma. When we consider the case history, the comparison with previously peer-reviewed studies of similar cases, the clinical findings and also the confirmed presence of large amount of lymphocytes on histological and cytological samples, these findings confirm a diagnosis of an aggressive type of equine lymphoma.

The presentation of pruritus, self-mutilation and generalised moth-eaten alopecia could be the first clinical signs to suggest a diagnosis of neoplasia of the lymphatic system in the horse which is commonly only confirmed after a deterioration in the horse's general status with follow up cytology of the pleural or peritoneal fluid or upon taking a biopsy of a mass for histology and / or immunohistopathology or euthanasia.

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Table 1. Properties of increased pleural fluid

Parameter	Value
TP g/l	35.3
ALB (PHO) g/l	15.4

Parameter	Value
GLO g/l	19.9
ALB/GLO-Q	0.77
Cell Count / μ l	7789
Cholesterin (PHO) mmol/l	0.95
Triglycerides (PHO) mmol/l	0.23
Chol/Tri-Q	4.1
GLU mmol/l	3.75
LDH (PHO) U/l	294.2

Total protein < 25 g/l means transudate; > 30 g/l is considered exudate

Cell count < 1000/ μ l considered transudate; > 5000/ μ l considered exudate





















