Paraneoplastic bullous stomatitis in a horse

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- Bullous stomatitis is uncommon in horses. Differential diagnoses include autoimmune disorders, erythema multiforme, drug reactions, and various viral diseases.
- Systemic signs directly caused by the remote effects of malignancy are referred to as paraneoplastic syndromes.

A 6-year-old Tennessee Walking Horse gelding was admitted to the veterinary hospital for evaluation of weight loss, lethargy, anorexia, ataxia, and oral blisters. These problems had been noticed over a 2-month period and had started with decreased appetite and weight loss. Three weeks prior to admission, a round, firm mass on the right side of the midcervical region, under the horse's mane, was noticed by the owner. There was no history of injection or trauma in this area. Episodes of stretching, suggestive of mild abdominal pain, had been observed intermittently. Ten days prior to admission, weakness, ataxia, and anorexia had been noticed, as well as ulcers and blisters in the horse's mouth.

On admission, physical examination of the horse revealed moderate weight loss. The horse was afebrile, and heart and respiratory rates were normal. Tense blisters and ruptured bullae of various sizes were on the peripheral margin of the tongue, sublingual region, and mucous membranes lining the buccal cavity and lip. Palpation of the lesions elicited signs of pain. Lesions were not detected on the nasal mucosa, conjunctiva, coronary band, or the mucocutaneous junction of the penis or anus. The horse had bilaterally symmetrical tetraparesis and ataxia, characterized as grade 1/5 in the forelimbs and grade 3/5 in the hind limbs. A firm, intramuscular mass (9 × 5 cm) was located on the right side of the neck, lateral to the dorsal spinous process of the third cervical vertebra. Signs of pain were not evident when the mass was palpated. On rectal palpation, the rectal surface was rough and friable, and the rectum was immobile. After palpation, tiny flecks of blood were observed on the examination sleeve. The horse would assume a stretched body position several times a day, while looking at its flank. This behavior was most noticeable after the horse had been walked.

Initial differential diagnoses included autoimmune disease, chronic inflammatory disease, exposure to chemical vesicants, hepatic or renal disease, vesicular stomatitis virus infection, equine herpesvirus infection, or neoplasia. Initial laboratory abnormalities included leukocytosis (WBC, 16,500 cells/μl; reference range, 6,000 to 12,000 cells/μl) with mature neutrophilia (11,715 cells/μl; reference range, 3,000 to 6,000 cells/μl), anemia (PCV, 24%; reference range, 32 to 48%), hyperfibrinogenemia (1,000 mg/dl; reference range, 100 to 400 mg/dl), hypoalbuminemia (1.9 g/dl; reference range, 2.7 to 4.1 g/dl), and hyperglobulinemia (5.9 g/dl; reference range, 2.8 to 4.4 g/dl). Serum electrophoresis indicated a polyclonal gammopathy. Urinalysis revealed 4+/4+ protein and a few granular casts. Results of an agar gel immunodiffusion test for equine infectious anemia were negative.

Mature neutrophilia, anemia, hyperfibrinogenemia, and hyperglobulinemia were consistent with a chronic inflammatory process. Proteinuria and urinary granular casts were suggestive of glomerular or tubular disease. Additional diagnostic tests were conducted to further characterize the cause of the inflammation. Abdominocentesis yielded pale yellow fluid consistent with a transudate. Results of biopsy indicated that the rectal mucosal was histologically normal. Cerebrospinal fluid obtained from the lumbosacral junction was clear with normal amounts of protein and no WBC. Antithrombin III activity was 88% and results of a coagulation profile were within reference ranges, except for elevated fibrin degradation products (> 120 μg/ml). Results of Coombs' and antinuclear antigen testing were negative. Serum tested for *Borrelia burgdorferi* antibody titer had negative results at a 1:10 dilution by use of the immunofluorescent antibody test. Results of serum neutralization test for vesicular stomatitis virus were negative, and the titer for equine herpesvirus was less than 1:16. Fluid aspirated from an oral blister had negative results for viral cytopathic effects after 3 blind passages.

Microscopically, specimens of oral lesions had subepithelial clefts and vesicles (Fig 1). These clefts were located at the junction of the epithelium and the lamina propria (Fig 2). The basement membrane could not be identified with periodic acid-Schiff stain, indicating that this structure was destroyed or disrupted. Vesicles varied in size and contained RBC, neutrophils, fibrin, and serum. Eosinophils were not identified. The submucosa underlying these vesicles had scattered foci of collagen degeneration, hemorrhage, edema, moderate perivascular lymphoplasmacytic infiltrate, and a mild infiltrate of neutrophils. The overlying, elevated epithelium was normal. The

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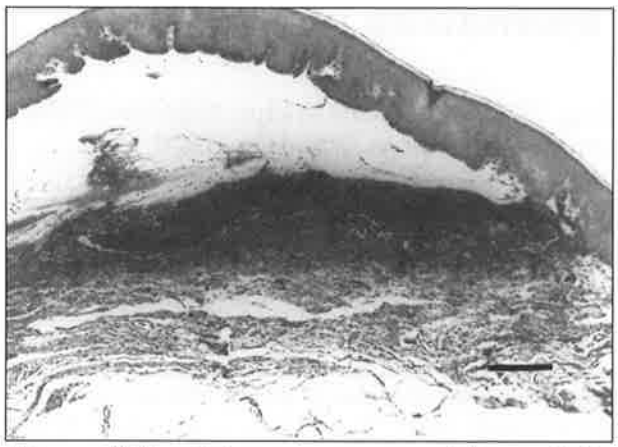

Figure 1—Photomicrograph of a section of a vesicle in the gingiva of a horse with bullous stomatitis. The large subepithelial vesicle contained a coagulum of RBC, fibrin, and serum that is evident here separating the gingival epithelium (top) from the propria/submucosa. H&E stain; bar = 200 μm.

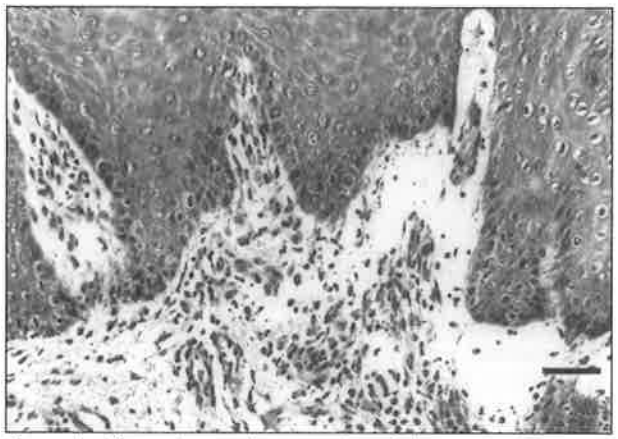

Figure 2—Photomicrograph of a section of the margin of the bulla depicted in Figure 1, illustrating the separation of the epithelium from the propria/submucosa at the basement membrane. H&E stain; bar = 30 μm.

histologic lesion was consistent with bullous pemphigoid.

Ruptured bullae were ulcers microscopically. The epithelium was lost and the defect was partially filled with immature inflammatory granulation tissue covered by a mixture of fibrin, RBC, neutrophils, and cell debris. The propria submucosa was infiltrated by neutrophils, lymphocytes, and a few histiocytes. The epithelium at the periphery of these ulcers was focally acanthotic.

Biopsy samples of oral lesions also were evaluated for autoantibodies in the epithelium or epithelial basement membrane. Results were equivocal. Results of direct immunofluorescence for equine IgG were suggestive of antibodies between epithelial cells. A distinct staining pattern was not observed for equine complement. Immunofluorescence was not observed along the basement membrane.

Ultrasonography of the cervical mass revealed a soft-tissue mass with a moderately dense fibrous capsule and fluid-filled center containing multiple hyperechogenic areas. Aspiration of the mass yielded dark, serosanguineous fluid containing RBC. Aerobic and anaerobic culturing of the fluid revealed no microbial growth.

The tentative diagnosis of the bullous stomatitis was bullous pemphigoid. Initial treatment consisted of prednisone (2.2 mg/kg of body weight, PO, q 12 h) with a gradual decrease in dose over 2 weeks to a maintenance dose (1.1 mg/kg, PO, q 48 h). The maintenance dose was administered for an additional 2 weeks. Trimethoprim/sulfadiazine (25 mg/kg, PO, q 12 hr) was administered for the first 14 days. The horse was stall rested, fed a moistened gruel, and the mouth was flushed twice daily with a 7% solution of NaCl. Attitude and appetite improved slightly; however, new oral blisters continued to form. The horse was discharged after 7 days.

Eight weeks later, the horse's condition had failed to improve and it was readmitted to the hospital. Oral blisters, ataxia, weight loss, and lethargy were still evident. The neck mass had enlarged and hardened to a bony consistency. Ultrasonography revealed a hyperechoic layer consistent with bone; this region previously had been occupied by a fibrous capsular layer. Aspiration of the mass was unsuccessful because of the dense osseous-like layer that prevented penetration of the needle. Radiographically, a large (11 × 6 cm) calcified mass was observed dorsolateral to the spinous processes of the second and third cervical vertebrae. Radiographic abnormalities in the dorsal spinous processes of the cervical vertebrae were not observed. Differential diagnoses included neoplasia, chronic inflammation, and calcified hematoma.

Laboratory analyses again revealed leukocytosis (WBC, 15,600 cells/μl), mature neutrophilia (11,076 cells/μl), anemia (PCV, 26%), hyperfibrinogenemia (1,100 mg/dl), and hyperglobulinemia (5.6 g/dl). Results of urinalysis were negative for protein, but a few granular casts were observed. Serum biochemical analyses revealed an elevation in γ-glutamyltransferase activity (102.5 IU/L; reference range, 2 to 29 IU/L), most likely attributable to the previously administered steroids.[1] Ultrasonography of the kidneys and liver revealed no abnormalities.

In an attempt to evaluate the integrity of the oral mucosa, manual pressure was applied, with the eraser end of a pencil, to a healthy area of tissue to elicit intraepidermal or dermoepidermal separation. Although no lesion developed at the eraser site, a large blister developed adjacent to the area where digital pressure had been applied to stabilize the horse's lip.

At the owners' request, surgery was performed to remove the mass. With the horse under general anesthesia, the mass was bluntly dissected from the surrounding structures. The horse was released from the hospital to the owners the next day. Procaine penicillin G (22,000 U/kg, IM, q 12 h) and phenylbutazone (2.2 mg/kg, PO, q 24 h) were administered for 5 days after surgery.

The excised cervical mass had been firmly attached to surrounding musculature and adjacent vertebral processes by broad bands of dense, fibrous connective tissue. The mass was ovoid and approximately 15 × 15 × 12 cm. On section, the periphery was composed of a 0.5- to 1.5-cm thick zone of osseous tissue. Centrally, the mass consisted of pale tan to red, homogeneous firm tissue.

Microscopic examination of a section of the mass revealed a densely cellular, poorly differentiated neo-

plasm composed of haphazardly organized, interlacing bundles and fascicles of closely packed, pleomorphic, moderately anaplastic spindle cells. Multiple blood-filled clefts and vascular spaces were lined by large, pleomorphic neoplastic cells. Individual neoplastic cells had a moderate amount of eosinophilic cytoplasm, surrounding a single, hyperchromatic, ovoid nucleus containing 1 to 3, variably sized, round, basophilic nucleoli and coarsely-clumped chromatin. Anisocytosis and anisokaryosis were moderate to marked. Mitotic activity was 1 to 3 per 400× field. The observation of vascular channels lined by anaplastic spindle cells in an otherwise poorly differentiated spindle cell neoplasm was consistent with hemangiosarcoma. The peripheral zone of osseous tissue was composed of multiple, regularly interconnected trabeculae of lamellar bone that separated spaces containing normal bone marrow.

The horse's attitude and appetite improved within 1 week after surgical removal of the mass. Oral lesions regressed. The ataxia, muscular weakness, and behavioral characteristics that had appeared to indicate abdominal pain disappeared. The horse gradually gained weight. The horse was reevaluated 14 months later. It appeared clinically normal except for having a small muscle defect at the surgical site.

Hemangiosarcoma, a malignancy of endothelial cells, is unusual in horses.[2-9] As in the horse of this report, hemangiosarcomas often appear as unexplained swellings, presumed to be hematomas.[2,3,8,9] The signs displayed by the horse of this report developed at the same time as the malignancy, and followed a parallel course; therefore, a causal relationship was considered. Systemic signs directly caused by the remote effects of malignancy are referred to as paraneoplastic syndromes.[10] This terminology must be differentiated from neoplasia-induced signs, which are signs associated with malignancy, but without a direct causal relationship.[11,12]

In the horse of this report, the most striking lesion was bullous stomatitis. Bullous and ulcerative skin diseases of horses are uncommon. Differential diagnoses include autoimmune disorders (eg, pemphigus foliaceus, pemphigus vulgaris, bullous pemphigoid, systemic lupus erythematosus), erythema multiforme, drug reactions, and several viral diseases. Oral ulceration also is uncommon and includes the same differential diagnoses except for pemphigus foliaceus.

Oral lesions in this horse were characterized histologically as subepidermal clefting. Disorders with this histological characterization include bullous pemphigoid, junctional epidermolysis bullosa, drug eruptions, and erythema multiforme. Bullous pemphigoid is a rare autoimmune, vesiculobullous, ulcerative disorder of skin or oral mucosa of human beings, dogs, and horses, which is characterized immunologically by detection of autoantibody against antigens at the basement membrane zone.[13-15] Clinically, horses with bullous pemphigoid have large vesicles or ulcers, prominent Nikolsky's sign (the outer layer of the skin is easily rubbed off by slight injury), severe systemic illness, poor response to treatment, and rarely immune deposits in the kidneys.[15] Junctional epidermolysis bullosa, erythema multiforme, and drug eruption were considered unlikely in this case because of the age of the horse, the presence of bullous lesions solely in the oral mucosa, the lack of previous drug administration, and lack of association with an infectious agent.

Contrary to the histologic characterization that was consistent with bullous pemphigoid, direct immunofluorescence findings in this horse were consistent with pemphigus. Pemphigus foliaceus is the most common autoimmune disease observed in horses.[13] Lesions usually begin on the face (especially periocular region and ears) or limbs, and may eventually become generalized. Lesions range from intact vesicles/pustules to crusts, scales, erosions, and epidermal collarettes. Diagnosis is based on histologic findings of subcorneal or intragranular pustular dermatitis with acantholysis. On the basis of the clinical description and histologic characterization of lesions from the horse in this report, pemphigus foliaceus as well as pemphigus vulgaris were ruled out. Reports of false positive staining for pemphigus, using direct immunofluorescence, have been seen with cases of equine dermatophilosis. In addition, pemphigus-like antibodies have been detected by indirect immunofluorescence testing in clinically normal horses and in a horse with lymphosarcoma.[16] The clinical features of this case, however, were compatible with an immune-mediated mucosal disease, lending more credence to findings by use of direct immunofluorescence.

Because growth of the mass coincided with development of clinical signs and removal coincided with remission of signs, a paraneoplastic disorder was diagnosed. Frozen serum, obtained on the initial admission, was analyzed for indirect immunofluorescence and immunoprecipitation. Immunofluorescence was performed by using monkey esophagus as an indicator of antibodies against epithelia, and murine urinary bladder as a marker for autoantibodies against desmoplakins.[17,a] Immunoprecipitation was performed according to the technique of Stanley et al,[18] with some modifications, by use of metabolically labeled keratinocyte culture extracts to detect circulating autoantibodies.[a] Sera from the horse, a human patient with proven paraneoplastic pemphigus, and control sera were used. The specifically immunoprecipitated polypeptides were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and identified by autofluorography. Indirect immunofluorescence on sections of monkey esophagus and murine urinary bladder indicated positive staining of the cell surface of keratinocytes and transitional epithelium of the bladder. These findings had been considered characteristic for pemphigus-like autoantibodies found in paraneoplastic pemphigus. The horse's antibodies detected polypeptides at 250, 230, 210, and 190 kd (Fig 3). These correspond with desmoplakin I and II (250 and 210 kd), the bullous pemphigoid 230 antigen (at 230 kd), and a 190-kd antigen that is not yet characterized. To date, these immunoprecipitation findings have been reported only in cases of paraneoplastic pemphigus in human beings.

Many of the clinical and laboratory features of the case reported here fit the criteria for paraneoplastic pemphigus.[12,19-24] The strongest evidence supporting this diagnosis included a refractory ulcerative stomatitis associated clinically with a neoplasm; the detection of antibodies bound to the cell surface of affected epithelium in a pattern resembling that of pemphigus; the detection of

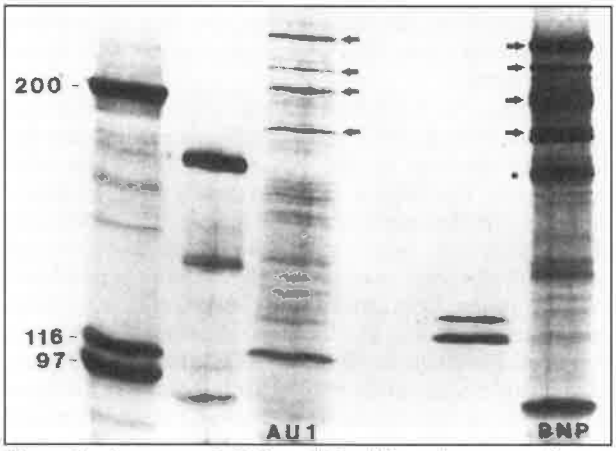

Figure 3—Immunoprecipitation obtained by using serum from a horse with paraneoplastic bullous stomatitis and metabolically labeled keratinocyte culture extracts. Serum obtained from the horse (lane AU1) was run in parallel with an index case (lane PNP) of known paraneoplastic pemphigus in a human being. The specifically immunoprecipitated polypeptides were separated on sodium dodecyl sulfate-polyacrylamide gel and identified by autofluorography. The left hand lane contains molecular weight standards at 200 kd, 116 kd, and 97 kd. Serum from the horse recognized polypeptides with a molecular weight of 250, 230, 210, and 190 kd, which migrated in an identical fashion to the polypeptides recognized by the index case. The only difference between the immunoprecipitation by the horse's antibodies and that by the human being's antibodies was that a strong diffuse band at approximately 170 kd was detected by the human patient's antibodies, but not by the horse's antibodies.

serum autoantibodies reactive with the cell surface of stratified squamous epithelia and other epithelia; and the detection of paraneoplastic pemphigus antigens by circulating autoantibodies, using immunoprecipitation with metabolically labeled keratinocytes. Therefore, 4 of the 5 criteria proposed by Anhalt et al[19] to define paraneoplastic pemphigus syndrome were fulfilled in this case. The only finding that was not consistent with the criteria of Anhalt et al[19] was the finding of subepithelial separation of the oral mucosa. The cases reported by Anhalt et al[19] involved suprabasilar acantholytic lesions, typical of that found in pemphigus, and not subepidermal separation, which is seen with bullous pemphigoid.

We believe that the bullous stomatitis in this horse was a mucocutaneous form of paraneoplastic syndrome. The stomatitis and other abnormalities (friable rectal mucosa, signs of mild abdominal pain, ataxia, and glomerular or tubular nephritis) were possibly associated with this horse's immune response to tumor antigens, because signs ceased shortly after tumor removal.[20,25] The ataxia and paresis also were believed to be attributable, in part, to local inflammation of the cervical spinal nerves and meninges. The classification of this case as paraneoplastic pemphigus or paraneoplastic pemphigoid requires a better understanding of antigen reactivity and specific polypeptide immunoprecipitation in clinically normal horses and horses with bullous autoimmune skin diseases. Similar confusion still exists in the classification of human cases of paraneoplastic disease. The remarkable similarity between immunoprecipitation findings in this horse and those in human patients with paraneoplastic pemphigus was particularly interesting; however, the histologic findings supported a more traditional diagnosis of paraneoplastic pemphigoid.

[a]Oursler JR, Ariss-Abdo L, Labib RS, et al. Autoantibodies against desmoplakins: a component of the immune response against epithelia and tumor in paraneoplastic pemphigus (abstr). *Clin Res* 1991;39:195.

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