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Foxtail grass (*Setaria viridis*)-induced ulcerative stomatitis-gingivitis resembling viral vesicular stomatitis in horses

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ABSTRACT

Physical trauma, dietary factors, toxins, immune-mediated disorders, and viral infections are known causes of equine stomatitis. There are also few reports of grass awn-associated stomatitis in horses. Movement of 323 horses was restricted at a Hungarian racetrack because of a suspected vesicular stomatitis (VS) outbreak. Many horses were affected at the same time, and an infectious disease or common offending source was suspected. To establish the nutritional origin of the feed, botanical examination and a food provocation test were carried out. Two healthy adult horses were fed exclusively with the hay of concern for a two-week period. All horses at the racetrack and the experimental horses were clinically examined daily and tested for VS, viral arteritis, glanders, and equine herpesviruses. Biopsy samples were taken from the oral lesions to characterize the histological alterations. Botanical analysis revealed that more than 15% of the forage was foxtail grass *(Setaria viridis)*. The nutrient content of the forage was adequate for horse maintenance, while its mold and yeast counts were below the reference limits. Competition horses showed signs of depression, decreased appetite, and drooling and ulceration of the lips, tongues, and gingiva after four hours of exposure to the tested forage or hay. The horses in the feeding trial showed the first clinical signs of oral papules on day four, and then showed rupturing pustules, ulcers, and extensive granulation tissues on day seven. Experimental horses did not show signs of depression or loss of appetite. Stomatitis healed spontaneously when the hay was changed. Results of the serological tests, hematological tests, biochemical analysis, and gastroscopy did not show significant alterations. Biopsy samples from both the competition and the experimental horses invariably showed grass awns surrounded by reactive mixed inflammatory cells and granulomatous inflammation. Since differential diagnosis of ulcerative stomatitis include highly contagious viral diseases like VS, quarantine measurements are necessary during stomatitis outbreaks. Differentiation based exclusively on clinical signs is not recommended, although wedged plant particles along the periodontal gingival sulcus might be indicative of inappropriate forage source. Evaluation of the feed in case of a stomatitis outbreak should be immediately performed. In case of grass awn-associated stomatitis, differences are noticed in the severity of lesions possibly based on plant types and plant percentage in the hay. As such, the extent to which foxtail grass-contaminated hay causes ulcerative gingivitis may be increasing because of recent changes in climate.

1. Introduction

Physical trauma, dietary factors, toxins, immune mediated disorders, and viral infections are known causes of stomatitis in horses (McCluskey and Mumford, 2000). In herbivores, coarse pasture and roughage can cause laceration or abrasions on the mucosa of the lips, oral cavity, gingiva, tongue, and oropharynx (Bankowski, 1956; McCluskey and Mumford, 2000; Mohammadi and Sardari, 2009). There are few reports of grass awn-associated stomatitis in horses, and some reports are anecdotal (Turnquist et al., 2001; Mohammadi and Sardari, 2009). Viral causes of ulceration in the oral cavity of horses include vesicular stomatitis virus (VS), equine viral arteritis, herpesvirus (Vengust et al., 2008), and horsepox virus (contagious pustular stomatitis) (Monreal et al., 1995; Letchworth, 1996; McCluskey and Mumford, 2000). Because of the open borders within the European Union and the speed with which the agent of an infectious disease can be disseminated through movement of goods and people, the importance of recognizing the cause of stomatitis in horses cannot be under-

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estimated (Bankowski et al., 1956). Since VS is a notifiable disease and Europe is considered free from outbreaks, immediate quarantine measures are implemented if occurrence is suspected.

In September 2012, the National Food Chain Safety Office in Hungary introduced quarantine measurements for horses staying at a Hungarian racetrack during an international equestrian event (horse-racing and eventing) to prevent the spread of stomatitis. Participants arrived from many countries outside of Europe, including the United States where VS is endemic (OIE, 2012). Movement of 323 horses was restricted because of suspected VS outbreak. Since many horses were affected at the same time, an infectious disease or a common offending source was suspected. Horses were provided with different sources of water and concentrates, but they were fed with the same hay. The objective of this study was to elucidate whether hay was an offending source of the ulcerative stomatitis outbreak.

2. Material and methods

The study was permitted by the Animal Health and Welfare Directorate of the National Food Chain Safety Office (NFCSO). All 323 horses at the racetrack were examined daily and horses with clinical signs were isolated. Blood samples were taken from the jugular vein and tested for VS with virus neutralization for Indiana and New Jersey strains, and for viral arteritis by virus neutralization, real time polymerase chain reaction (PCR) for equine herpes 1, 4, (IDEXX RealPCR™ Tests, IDEXX Laboratories, Germany), and 2, 5 (Nordengrahn et al., 2002) and for glanders with complement fixation test (Bioveta, Czech Republic) in the accredited central laboratory of the NFCSO. Biopsy samples were taken under sedation (xylazine 0.3 mg/kg and butorphanol 0.02 mg/kg) and local anesthesia with lidocaine (10%) from the gingival alterations of the maxilla of two competition horses to characterize the histological alterations. Samples were collected in 10% formalin and evaluated with haematoxylin-eosin stain.

To establish nutritional origin of the feed, botanical examination, hay analysis, and a food provocation test were carried out. Sampling of the large round bales for nutrient analysis and botanical examination was carried out by selecting 10 representative bales and collecting two cores from the circumference of each. To sample the center parts of the bales, we reached inside each bale and removed two or three handfuls from different locations. All samples were put in a bucket and homogenized. To identify the plant species of the samples, macroscopic and microscopic examinations were performed. Hay analysis included the determination of dry matter (DM), ash, ether extract, crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). These measurements were carried out according to relevant ISO standards (ISO 5983 – 16472)[1,2,3,4,5,6]. Digestible energy (DE) was estimated from the chemical composition. Mold and yeast counts were performed according to ISO 21527[7] standard.

The provocation test was performed during April and May 2013 at the Large Animal Clinic of Szent Istvan University, Faculty of Veterinary Science. Between September 2012 and April 2013, the forage samples were stored in a dry, covered location nearby to avoid being subjected to weathering. Two healthy Hungarian warmblood horses, one gelding and one mare, aged 10 and 15 years, respectively, were fed exclusively with the hay of concern for a two-week period. Horses were kept in a 20×30 m sandy paddocks during the day and they were moved to individual boxes with wood shavings as bedding material overnight. Water was offered as needed. The two horses were weighed before and after the feeding period. The animals were subjected to daily clinical examination from the first day until the end of the second week after the cessation of the feeding trial. Blood samples were taken from the jugular vein on the first and 14th day for hematological (Abacus Junior Vet, Diatron, Hungary) and biochemical analyses (Olympus AU640, Germany), and to perform serological tests for VS, viral arteritis, glanders, and PCR for equine herpesviruses (EHV 1, 2, 4, 5). On the 10th day, representative samples of ulcerative lesions were collected from the gingiva for histopathology. Biopsy samples were taken from the oral cavity as described above. Esophagoscopy and gastroscopy (CV100, Olympus, Germany) was performed immediately after the biopsy sampling under the same sedation. Following the trial, horses were fed with good quality grass hay. The healing process was followed in the next two weeks by daily clinical examinations.

3. Results

3.1. Evaluation of the hay

Results of botanical and nutrient content analyses are listed in Table 1. The foxtail grass *(Setaria viridis)* identified in the hay had a dense hairy seed head with long spikelets (Fig. 1.). The mold count of the hay sample was 8.2×10^4 cfu/g, yeast count was 1×10^5 cfu/g, and aerobic bacterial count was $< 1 \times 10^5$ cfu/g.

3.2. Clinical findings

Twenty nine (9%) adult horses of different breeds (9.9% of thoroughbreds and 6% of warmbloods) and gender (11% of males and 6.5% of females) showed signs of decreased appetite (86.2%), depression (75.8%), drooling (65.5%), and ulceration of the gingiva (86.2%), lips (79.3%), and tongues (20.6%) at the racetrack. Racehorses and competition horses in contact with the hay contaminated with foxtail grass showed clinical signs after four hours of exposure. The horses in the feeding trial weighed 530 kg and 590 kg at the beginning and 554 kg and 612 kg at the end of the trial, respectively. These horses showed the first clinical signs of oral papules, mucus membrane erythema, and plant particles wedged along the periodontal gingival sulcus on the 4th day (Fig. 2). Clinical signs quickly worsened: bleedings and erosions occurred on the 5th day, then rupturing pustules appeared, ulcers were formed, and extensive granulation tissues were observed by the 7th day (Fig. 3). Similar alterations were visible on the lateral aspect of the tongues and lips (Fig. 4). Experimental horses were in good general condition, i.e., they did not show any signs of depression or loss of appetite. Salivation was observed only at the end of the second week. Comparisons of clinical findings are listed in Table 2. Both racetrack and experimental horses spontaneously recovered within two weeks after changing the hay. Ten days after finishing provocative feeding, there were no obvious abnormalities in the oral cavity other than some scarring and loss of pigmentation at the site of previous ulcerations.

3.3. Results of serological and PCR examinations

All serological results of blood samples for VS, viral arteritis, and glanders, including the quarantined horses at the racetrack and the

[1] ISO 5983:2005. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content. ISO, International Organisation for Standardization. http://www.iso.org

[2] ISO 5984:2002. Animal feeding stuffs – Determination of crude ash. ISO, International Organisation for Standardization. http://www.iso.org

[3] ISO 6492:1999 Animal feeding stuffs – Determination of fat content. ISO 6492:1999 Animal feeding stuffs – Determination of fat content

[4] ISO 6496:1999 Animal feeding stuffs – Determination of moisture and other volatile matter content. ISO, International Organisation for Standardization. http://www.iso.org

[5] ISO 13906:2008. Animal feeding stuffs – Determination of acid detergent fiber (ADF) and acid detergent lignin (ADL) contents. ISO, International Organisation for Standardization. http://www.iso.org

[6] ISO 16472. 2006. Animal feeding stuffs. Determination of amylase-treated neutral detergent fiber content (aNDF). ISO, International Organisation for Standardization. http://www.iso.org

[7] ISO 21527–2:2008. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 2: Colony count technique in products with water activity less than or equal to 0,95. ISO, International Organisation for Standardization. http://www.iso.org