Documentation of Proliferative Enteropathy in Foals

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Abstract:

Proliferative enteropathy (PE) caused by *Lawsonia intracellularis*, which is an obligate intracellular bacterium, has been reported in individual horses as well as outbreaks in groups of horses. Nevertheless, the prevalence of the disease in the equine population remains unknown. Our goal was to determine the occurrence of *L. intracellularis* in foals in the State of Minnesota, USA. To achieve this goal, a retrospective study was performed. The hypothesis was that *Lawsonia* infection existed as an undiagnosed entity among foals submitted to the Veterinary Diagnostic Laboratory in Minnesota. Case records of 78 foals submitted between 1990 and 1998 were reviewed. Two groups of foals were investigated. Group 1 included 44 foals representing the total number of foals ranging in age from 3-12 months which were submitted for enteric-related disorders. Group 2 included 34 foals that have the same age range but were submitted for problems unrelated to enteric disorders. Paraffin-embedded intestinal sections were examined using immunohistochemistry with a *L. intracellularis*-specific antibody. Two foals out of a total of 44 (5%) examined were identified as *L. intracellularis* positive in group 1. No *L. intracellularis*-positive cases were identified among foals in group 2. *L. intracellularis* was only detected in foals with intestinal disease. The disease may be misdiagnosed if *L. intracellularis*-specific testing such as immunohistochemistry is not included.

Introduction

Proliferative Enteropathy (PE) is an enteric disease that mostly affects weanling animals; however, on occasion adults may be affected. The disease has been described in multiple animal species including horse, pig, hamster, deer, dog, blue fox, guinea pig, rat, ferret, monkey, rabbit and birds (emu and ostrich)(6).

In horses, proliferative enteropathy was described first time in 1982 in a six-month-old Arabian foal(2). The report briefly described a history of anorexia, depression, and diarrhea. On presentation, the foal had symptoms of diarrhea and weakness. In addition, pathologic examination revealed increased

thickness of the small intestine and hyperplasia of the crypt epithelium. Probably the most significant finding was the description of curved, rod-shaped organisms in the apical cytoplasm of the crypt epithelial cells. Later, Williams and his group(13) used classical and molecular approaches to identify *Lawsonia intracellularis* as the causative agent of proliferative enteropathy in the horse. Following that report, several individual cases of proliferative enteropathy have been reported as well as an outbreak in a group of horses (1,3,5,11).

Clinical signs of proliferative enteropathy are not unique and may include depression, diarrhea, weight loss or poor weight gain and death in untreated cases. The causative agent, *L. intracellularis*, is an intracellular bacterium that is non-culturable in conventional media (7, 8). Therefore, the disease is difficult to confirm in live animals and can be easily misdiagnosed with another enteric diseases such as *Salmonellosis* or Potomac horse fever. The main goal of this study was to document the occurrence of proliferative enteropathy due to *L. intracellularis* in foals in the State of Minnesota, USA. To achieve this goal, a retrospective study was initiated. Immunohistochemistry (IHC) was used to diagnose *L. intracellularis*-infected foals among cases submitted to the Minnesota Veterinary Diagnostic Laboratory.

Materials and Methods:

Case records of 78 foals submitted to the Veterinary Diagnostic Laboratory between 1990 and 1998 were reviewed and grouped into two groups. Group 1 included 44 foals representing the total number of foals ranging in age from 3-12 months which were submitted for enteric-related disorders. Group 2 included 34 foals representing foals ranging in age from 3-12 months submitted for problems unrelated to enteric disorders.

Paraffin-embedded tissues of small intestine were examined using immunohistochemistry with a *L. intracellularis*-specific antibody. A positive control obtained from a previously diagnosed case of equine proliferative enteropathy as well as a negative control were included in the evaluation. Immunohistochemistry was performed on intestinal sections from selected cases using the streptavidin/biotin method with *L. intracellularis*-specific monoclonal and/or polyclonal antibody that were developed at University of Minnesota, Minnesota, USA(4, 9).

Results:

Immunohistochemistry analysis revealed two *L. intracellularis*-positive cases in group 1. On the other hand, immunohistochemistry testing detected no *L. intracellularis* positive cases within group 2 (foals less than one year of age without enteric disorders). In group 1, one case of proliferative enteropathy was an eight-month-old American Saddlebred colt submitted to the Veterinary Diagnostic Laboratory for intestinal disease. Unfortunately, no other information was available for the case. Immunohistochemistry analysis of small intestinal tissues showed extensive staining of *L. intracellularis* mostly in the apical portion of the cytoplasm of absorptive enterocytes on the villi (Fig 1a, b). In addition, a small number of organisms were scattered throughout the cytoplasm of glandular epithelial cells and in the lamina propria.

The other case of proliferative enteropathy in group 1 was a six-month-old filly of unknown breed that was submitted to the Veterinary Diagnostic Laboratory with a history of diarrhea and fever. *Salmonella spp* was isolated from the intestine and hemolytic *E. coli* was grown from the lung, kidney and liver. The filly was previously treated with trimethoprim sulfonamide drugs and IV fluids. At necropsy a mild increase in the thickness of the small colon and a significantly thickened and edematous large colon were described. Additionally histopathologic examination revealed mild diffuse non-suppurative inflammation of the mucosal surface of the small colon while the submucosa of the large colon was edematous. Immunohistochemistry indicated the presence of a heavy infection with *L. intracellularis* in random sections of the small intestine (Fig 1c). Organisms were mostly located in the apical part of the cytoplasm of enterocytes in the crypts with a few organisms migrating through/between enterocytes into the lamina propria. A few organisms were detected in inflammatory cells in the submucosa.

Discussion :

The use of *L. intracellularis*-specific antibody to detect antigen in the intestinal tissues is a definitive diagnostic test for proliferative enteropathy(13). In this study, immunohistochemistry was used to test the available intestinal tissues obtained from cases within the population sample. A major obstacle faced in this retrospective study was the tissue sampling from the foals examined since intestinal sections were taken at random during a routine post mortem. Since proliferative enteropathy is characterized by the presence of multiple layers of enterocytes in infected crypts. Such lesion was not detected after re-evaluation of the sections using H&E staining due to the poor condition

of the tissue. Despite the fact that increased thickness of the small and large colon was described in the original pathology report of case 2, this lesion was attributed to *Salmonella spp*. However, *L. intracellularis* was present in large numbers in the crypts of the intestinal tissue which could have contributed significantly to the outcome. It is possible that the intestinal lesions described in case 2 resulted from a mixed infection of *Lawsonia* and *Salmonella*. Such mixed infections have been reported in swine(12). In case 1, intestine lesions may have been entirely due to *Lawsonia*. Therefore, based on these findings, proliferative enteropathy may be underdiagnosed or misdiagnosed if the appropriate testing is not performed.

The *Lawsonia* positive horses were 6 and 8 months of age and died in October and December, respectively. This is the age at which most cases of proliferative enteropathy in horses have been reported in the literature. We have detected a higher prevalence of proliferative enteropathy seropositivity in horses during the months of October, November and December (unpublished data). It is unclear whether this reflects age predisposition or change in weather or management practices.

Despite the fact that our study detected only two *Lawsonia* positive horses, this number represented approximately 5% of the diseased population. Examination of the records of the diseased population in this study showed that the most commonly isolated bacterial pathogen was *Salmonella spp*. Following *Salmonella*, *L. intracellularis* represented the second most commonly diagnosed bacterial pathogen in the examined diseased group. This study represents a first attempt to describe the occurrence of proliferative enteropathy in horses retrospectively. Prior to this study, proliferative enteropathy has been reported in individual cases or in outbreaks of horse farms. In swine, abattoir surveys have shown that 5-20% of animals may have proliferative enteropathy(10). The percentage of proliferative enteropathy in affected horses in this study is within the range reported for swine and was found with a frequency high enough to consider proliferative enteropathy as a differential diagnosis of enteric diseases in horses under one year of age.

Immunohistochemistry analysis of intestinal tissues from horses with undiagnosed enteric disease that were older than 12 months of age failed to detect positive cases (unpublished data). This limited number of animals studied was similar to those in the group 1 except for their age and the failure to assign a specific diagnosis to the enteric disease. The foals in group 1 represented all foals submitted with enteric disease including those with a

diagnosis assigned and those without a diagnosis assigned. Failure to detect *L. intracellularis* infection in a limited number of older horses supports the observation that this disease mostly affects foals under 12 months of age. In addition, the intestinal sections of foals that had no enteric disorders in group 2 failed to show any positive staining using immunohistochemistry with *L. intracellularis* antibody. These findings suggest that *L. intracellularis* is a pathogen of young horses and may be detected in the intestines of foals with enteric disorders but this organism is not commonly found in foals asymptomatic for enteric disease.

In conclusion, utilizing immunohistochemistry, it was possible to document *L. intracellularis* infection among the horse population retrospectively. Documentation of this disease among the equine population in Minnesota indicates the emergence of a new enteric disease in horses. Proliferative enteropathy should be considered in the differential diagnosis in foals with nonresponsive enteric disease. Specific testing using immunohistochemistry may be required to appropriately diagnose the disease after postmortem examination.

Acknowledgments

Support was provided by the University of Minnesota Equine Center with funds from the Minnesota Agricultural Experimental Station, the Minnesota Racing Commission and contributions from private donors. Special thanks to Jan Shivers for assistance with the IHC testing.

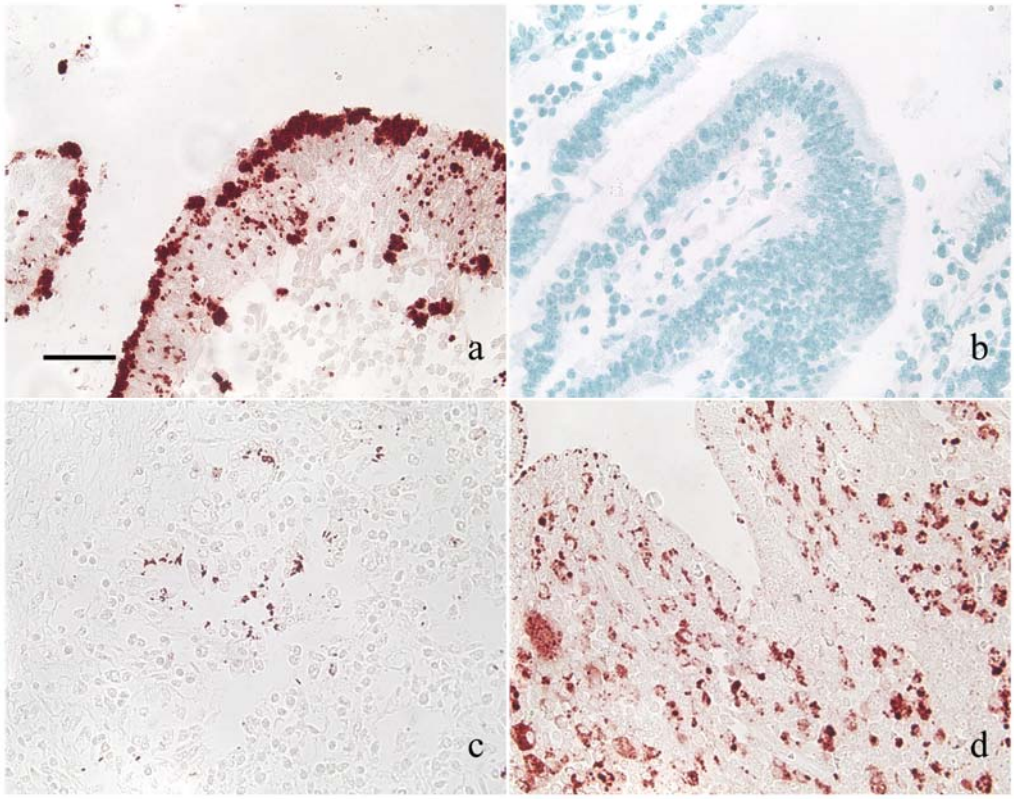

Fig. 1. Immunohistochemistry detection of *L. intracellularis* infection in the intestine utilizing monoclonal antibody a) case 1 showing specific staining in the apical cytoplasm of enterocytes of villi. b) case 1 negative control (treated with the same staining protocol except the primary antibody is substituted by mouse ascites fluid), c) case 2 showing specific staining in the apical part of cytoplasm of enterocytes of crypts and d) positive control (obtained from a known *Lawsonia*-positive case). Scale bar = 50 μm.

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دراسة توثيقية للاعتلال المعوي التكاثري في الأمهار

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المخلص :

تم وصف مرض الاعتلال المعوي الذي تسببه بكتيريا *Lawsonia ntracellularis* في حالات فردية وكذلك في مجموعة من الأمهار ولكن لا يزال مدى انتشار هذا المرض في الخيل غير معلوم. كان الهدف من هذه الدراسة معرفة ماذا كان هذا المرض موجود في ولاية منيسوتا. افترض أن هذا المرض موجود في الخيل في هذه الولاية لكن لم يتم تشخيصه من قبل في الحالات التي تمت إحالتها إلى المعمل التشخيصي البيطري. تمت معاينة ٧٨ حالة للخيول التي تمت إحالتها إلى المعمل في الفترة من ١٩٩٠ إلى ١٩٩٨ م. تم التحري عن مجموعتين من الخيل. اشتملت المجموعة الأولى على ٤٤ مهرا هي عبارة عن مجموع الأمهار التي تتراوح أعمارها بين ٣ إلى ١٢ شهرا وتمت إحالتها لأسباب مرضية تتعلق بالجهاز الهضمي. المجموعة الثانية عبارة عن ٣٤ مهرا تتراوح أعمارها أيضا بين ٣ إلى ١٢ شهرا وتمت إحالتها لأسباب لا تتعلق بالجهاز الهضمي. تم اختبار الأنسجة المعوية من عينات كانت محفوظة في الشمع وذلك باستخدام الكيمياء النسيجية المناعية باستخدام أجسام مناعية مضادة للبكتيريا. تم تشخيص حالتين من ضمن المجموعة الأولى أي ما نسبته ٥٪ حيث تبين إصابتها بـ *L. intracellularis*. لم نتمكن من تشخيص أي حالات موجبة ضمن المجموعة الثانية. يمكن القول ان هذه البكتيريا تصيب الأمهار وتتسبب في أعراض معوية. كما يمكن القول أن هذا المرض يمكن أن لا يشخص بشكل دقيق إذا لم يتم استخدام الاختبارات المحددة له.