Fecal egg counts after anthelmintic administration to aged horses and horses with pituitary pars intermedia dysfunction

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Objective—To determine effects of pituitary pars intermedia dysfunction (Cushing’s disease) and age on fecal egg count and time to egg reappearance after anthelmintic treatment in horses residing in similar environments.

Design—Cross-sectional study.

Animals—29 healthy horses (4 to 35 years old) and 13 horses with PPID (13 to 33 years old).

Procedures—Fecal egg counts were performed by use of a modified Wisconsin flotation method at 2-week intervals before and after ivermectin treatment.

Results—Horses with PPID had higher fecal egg counts before and 8, 10, and 12 weeks after ivermectin treatment, compared with counts for site-matched healthy horses. There was no difference in the period for < 90% reduction in fecal egg counts between the 2 groups. Age did not affect fecal egg counts at any time point.

Conclusions and Clinical Relevance—For similar environmental conditions, horses with PPID were more likely to have higher fecal egg counts than were healthy horses. Therefore, horses with PPID may need to have a more aggressive parasite prevention program than do healthy horses. Age did not affect fecal egg counts or time to egg reappearance after anthelmintic treatment, which suggested age alone does not likely require special consideration when designing a parasite control program for adult horses. (J Am Vet Med Assoc 2010;236:330–334)

Pituitary pars intermedia dysfunction (ie, Cushing’s disease) is a chronic progressive disease typically observed in aged horses. Classic clinical signs include hirsutism, polydipsia, polyuria, muscle atrophy, weight loss, and laminitis. Chronic infections, including sinusitis, foot abscesses, and dermatophils, are also common and have been attributed to compromised immune function resulting from increased plasma concentrations of anti-inflammatory hormones, including α-MSH, ACTH, and cortisol. An increased susceptibility of horses with PPID to endoparasitism has been suggested by several investigators, and frequent anthelmintic administration has been recommended as long-term supportive treatment in the management of horses with PPID. Despite this recommendation, the authors are not aware of any data to support a greater susceptibility to endoparasites and a shorter egg reappearance period in horses with PPID.

The effect of advanced age on immune resistance to parasites has not been adequately studied. In humans and mice, resistance to intestinal parasitism is predicted by the balance between Th1 and Th2 cytokines. Resistance is associated with predominantly a Th2 response, whereas a Th1 response imparts susceptibility. Aged mice have a greater susceptibility to infection by *Trichuris muris*, compared with the susceptibility of young control mice, and susceptibility is associated with predominately a Th1 response. Comparisons of parasite load or egg reappearance period between young and aged horses are lacking.

The objective of the study reported here was to determine whether the strongyle egg reappearance period is shorter and egg counts are increased in horses with PPID, compared with results for healthy horses residing in similar environments. In addition, this study was conducted to determine whether healthy aged horses have increased strongyle egg counts and a shorter interval to egg reappearance after anthelmintic treatment than do healthy adult horses.

Methods and Materials

Animals—Thirteen horses with PPID (13 to 33 years old) were identified from the Stillwater, Okla, area on the basis of clinical signs. Twelve of the PPID horses had evidence of or a history of incomplete or delayed shedding of the coat. Other indicators of PPID included weight

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<th>Abbreviations</th>
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<td>EPG</td>
<td>Eggs per gram</td>
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<td>MSH</td>
<td>Melanocyte-stimulating hormone</td>
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<td>PPID</td>
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loss, laminitis, excessive sweating, supraorbital fat deposits, or behavioral changes. Control horses (n = 29; 4 to 33 years old) were selected on the basis of a lack of clinical signs of PPID. Whenever possible, control horses were selected from the same farms as the PPID horses to minimize differences attributable to management. A medical history was obtained from each owner; information collected included date of the last anthelmintic treatment and the product used. All horses had access to pasture throughout the study period. All samples were collected in accordance with institutional animal care and use committee guidelines following approval of the Oklahoma State University Animal Care Committee and informed written consent from owners.

**Endocrine testing**—Disease status was confirmed in all horses via evaluation of plasma α-MSH concentrations measured by use of a radioimmunoassay in a sample obtained between April and June. A reference range was determined on the basis of results for 60 clinically normal horses from which samples were also obtained between April and June. The reference range (+3 SD, mean ± SD, 12.1 ± 5.7 pmol/L) was calculated as the mean ± 3 SD. All horses with PPID had a plasma α-MSH concentration ≥ 45 pmol/L.

**Fecal egg counts**—An initial fecal egg count was performed for each horse by use of a modified Wisconsin flotation method that involved sugar centrifugation of 5 g of feces/sample. Minimum detection limit was 1 EPG. After the initial fecal egg count, the body weight of each horse was estimated by use of a weight tape. Ivermectin (0.2 mg/kg [0.091 mg/lb], PO) was administered to each horse. Fecal egg counts were performed at 2-week intervals from 2 until 12 weeks after anthelmintic treatment. Four horses in the control group were removed from the study after 8 weeks (2 because of relocation and 2 because of a lack of owner compliance), and 2 additional horses in the control group were removed after 10 weeks because of a lack of owner compliance.

**Statistical analysis**—The relationship between age and fecal egg count before and after anthelmintic treatment was assessed by calculating the Spearman coefficient of correlation for all 29 control horses and for the largest population of control horses (n = 8 horses) on a single farm. The relationship between fecal egg counts before and after anthelmintic treatment in all horses was calculated by use of the Spearman coefficient of correlation. Reduction in fecal egg count was calculated at 6, 8, 10, and 12 weeks after anthelmintic treatment for all horses with an initial fecal egg count > 0 EPG by use of the following equation:

\[ \text{Reduction} = \frac{\text{fecal egg count before anthelmintic treatment} - \text{fecal egg count after anthelmintic treatment}}{\text{fecal egg count before anthelmintic treatment}} \]

The proportion of PPID horses with a reduction in fecal egg counts of < 90% or those reaching a threshold of ≥ 200 EPG was compared with the proportion of control horses with those same results by use of a Fischer exact test. Age of horses with and without PPID was compared by use of a t test.

Forward stepwise multiple linear regression was performed on transformed (log$_{10}$) fecal egg count data to assess the impact of age, disease status, and interval since anthelmintic administration on log$_{10}$ EPG data collected for all horses between 4 and 12 weeks after anthelmintic administration. To account for differences between strongly populations and management practices among farms, the effects of disease status and interval since anthelmintic administration on log$_{10}$ EPG data were assessed in 13 matched pairs of horses by use of a complete block design with repeated measures; there were 4 values for time interval (6, 8, 10, and 12 weeks after treatment) and 2 values for disease (PPID or control horses). Each PPID horse was paired with the healthy horse closest in age from the same farm for statistical analysis. The effect of disease at each value for time interval since anthelmintic treatment was evaluated further by use of simple main effects. For all statistical analyses, significance was designated at values of P < 0.05.

**Results**

The PPID horses included 6 geldings and 7 mares, with 8 Quarter Horse–type horses (Quarter Horse, Appaloosa, Paint, or Quarter Horse–crossbred horses), 3 Arabians, 1 Tennessee Walking Horse, and 1 Pony of America. Control horses comprised 21 geldings and 8 mares, with 23 Quarter Horse–type horses, 2 Arabians, 2 Thoroughbreds, 1 Saddlebred, and 1 Icelandic Pony. Horses with PPID were significantly (P = 0.01) older than the control horses (mean ± SD, 23.3 ± 5.9 years vs 16.7 ± 8.6 years, respectively). Mean plasma α-MSH concentration of horses with PPID was 158.8 ± 111 pmol/L, whereas the mean plasma α-MSH concentration of the control horses was 11.9 ± 5.2 pmol/L.

All horses in the study resided on 12 farms located within 48 km (30 miles) of Stillwater, Okla. There were 10 farms with 2 or 3 study horses enrolled, 1 farm with 6 horses enrolled, and 1 farm with 14 horses enrolled. All samples were collected within a 13-week period between May 15 and August 25.

Medical history, including last date of anthelmintic administration, anthelmintic product used, or both, was available for 27 of 42 horses. Eight horses had been dewormed within 60 days, 11 between 60 and 90 days, and 4 between 90 and 180 days before onset of the study. Last date of deworming was unknown for 19 horses. Ivermectin was the last product used in all horses in which product information was reported (n = 26 horses).

Resistance to ivermectin was not detected. All horses in the study had a reduction of 99% to 100% for fecal egg counts and a negative result for a fecal egg test (< 1 EPG) at 2 and 4 weeks after treatment.

We did not detect a correlation between age and fecal egg counts in control horses before (r = –0.06; P = 0.75 [n = 29 horses]) or 6 (r = –0.24; P = 0.21 [29]), 8 (r = –0.13; P = 0.51 [29]), or 12 (r = –0.10; P = 0.64 [23]) weeks after treatment. Among control horses from a single farm, there was no correlation between age and fecal egg count before (r = 0.20; P = 0.62 [n = 8]) or 12 weeks after (r = 0.05; P = 0.93 [8]) anthelmintic treatment. There was a significant (P = 0.01 at 6 weeks after treatment and P < 0.001 at 8, 10, and 12 weeks after treatment) correlation between initial fecal egg count and fecal egg count after ivermectin treatment.

Multiple linear regression was used to assess the impact of age, disease, and interval since anthelmintic administr-
tion on fecal egg count (Figure 1). Logarithmically transformed data for fecal egg count and the residuals associated with the regression model were approximately normally distributed, as determined by inspection of frequency histograms and normal probability plots, respectively. Results of forward stepwise regression indicated that interval since anthelmintic administration ($P < 0.001$) and PPID ($P = 0.002$) predicted an increase in fecal egg count but that the interaction term between PPID and age ($P = 0.001$) predicted a decrease in fecal egg count. Age of horse did not have a significant ($P = 0.15$) effect on fecal egg count.

The proportion of PPID horses with a reduction in fecal egg count of < 90% was higher than the proportion of control horses with a reduction in fecal egg count of < 90% for all time points (Figure 2). However, the proportions did not differ significantly.

Fecal egg count was < 200 EPG for all horses at 6 weeks after treatment, and at 8 weeks after treatment, only 3 horses (2 PPID horses and 1 control horse) had a fecal egg count ≥ 200 EPG. At 10 weeks, significantly ($P = 0.01$) more horses with PPID (6/13 horses) reached a threshold of ≥ 200 EPG, compared with the number of control horses (2/25 horses) that reached that threshold. By 12 weeks after anthelmintic treatment, there was no significant ($P = 0.50$) difference in the number of horses with ≥ 200 EPG.
between the PPID horses (6/13 horses) and control horses (7/23 horses; Figure 3).

Fecal egg counts for horses with PPID were compared with counts for site-matched control horses that were of similar age (range, 13 to 33 years and 6 to 35 years for PPID and control horses, respectively \( P = 0.06 \)). Analysis indicated that disease status \( (P = 0.02) \) and interval since ivermectin administration \( (P < 0.001) \) both significantly affected the number of EPG. Fecal egg counts were significantly higher in PPID horses than in matched control horses at 8, 10, and 12 weeks after anthelmintic administration but not at 6 weeks after anthelmintic treatment \( (P = 0.39; \text{Figure 4}) \).

**Discussion**

The study reported here was conducted to evaluate the effect of PPID and age on endoparasitism in horses. Endoparasitism was assessed by counting strongyle eggs in fecal samples by use of the modified Wisconsin flotation method. This method reportedly has greater sensitivity and efficiency of egg recovery at wide ranges of egg counts with a lower coefficient of variation, compared with results for dilution (modified McMaster) or gravitational (standard vial) methods.9,11 It is generally accepted that most strongyle eggs seen in horse feces are from small strongyles, which have a complex life cycle.12 Small strongyles have a prepatent period of between 6 and 12 weeks; however, they may persist for several years in an encysted, hypobiotic state in the wall of the cecum and large colon. As a result, fecal egg counts are not considered an accurate reflection of total worm burden in an individual horse. Despite these limitations, fecal egg counts are still considered the best antemortem test to evaluate worm burden and to monitor response to anthelmintic treatment.12

In the study reported here, horses resided on several farms and date of the last anthelmintic treatment varied among the farms. Therefore, pretreatment egg counts alone were not considered to be a suitable measurement to assess the interaction between PPID and fecal egg counts. To synchronize the interval from anthelmintic treatment until evaluation via fecal egg counts, all horses received ivermectin at the onset of the study. Other investigators13–15 have suggested that a failure to reduce egg counts by > 80% is consistent with anthelmintic resistance. No resistance was detected in the present study because all horses had reductions of fecal egg counts of 99% to 100% and a negative result for a fecal egg count (< 1 EPG) at 2 and 4 weeks after anthelmintic administration.

Median fecal egg counts were greater for horses with PPID, compared with results for control horses, before and 6, 8, 10, and 12 weeks after treatment with ivermectin (Figure 1). To assess the influence of disease, age, and interval since anthelmintic treatment on fecal egg counts, a linear regression model was developed. Although PPID was correlated with horse age, age alone did not affect fecal egg counts independent of PPID and was therefore excluded from the final model. Horses with PPID had higher egg counts, compared with results for control horses, when interval since ivermectin treatment was taken into account. Interest-ingly, although age did not independently affect fecal egg counts, the interaction of age and PPID was predictive of lower fecal egg counts. The opposite directions of the effects of PPID and age indicated that despite the fact that horses with PPID were significantly older than were the control horses, multicollinearity between age and PPID cannot explain the increase in fecal egg counts for horses with PPID (ie, horses with PPID had higher egg counts than did control horses, but this increase in fecal egg count was not attributable to the fact that horses with PPID were older). Differences in environment or management also cannot explain the higher egg counts detected for the horses with PPID because site-matched PPID horses had higher egg counts than did control horses when location was accounted for by use of the block design (Figure 4).

It has been suggested9 that determining the interval until egg reappearance by use of a percentage of pretreatment fecal egg count (fecal egg count reduction) is more statistically robust and conservative than is use of the interval to reach an absolute threshold. Although the fecal egg reduction method has been most commonly used to assess alterations in the response of parasite populations to anthelmintic treatment,9,10,11 it has also been used to assess the influence of host factors on interval until egg reappearance.9 In the present study, the proportion of horses with a reduction in fecal egg counts of < 90% was consistently higher for the PPID horses than for the control horses, although the proportions did not differ significantly. These results are difficult to interpret because of the small number of horses in the study and the fact that the control horses typically had low fecal egg counts throughout the study.

Thresholds for fecal egg counts are widely used by equine practitioners for diagnosis and surveillance of strongyles.16 Although there is not a consensus as to a biologically relevant cutoff threshold for health of a specific horse or pasture hygiene, most equine practitioners make treatment decisions on the basis of a cutoff value of 200 EPG.12,16 In the study reported here, 6 of 13 horses with PPID reached a threshold of ≥ 200 EPG by 10 weeks after anthelmintic treatment, which was significantly \( (P = 0.01) \) different from the proportion of control horses (2/23) that reached the threshold. At 12 weeks after treatment, there was no change in the number of PPID horses that had reached the threshold but an additional 5 control horses had ≥ 200 EPG.

Similar to all infections, parasitism in horses is the result of a balance between an animal’s exposure and its immunity. Differences in pasture management and hygiene, animal stocking density, and weather may contribute to pasture parasite load and therefore parasite exposure for a horse. In this study, horses resided on several farms within a 48-km radius. All horses had access to pasture, and samples were obtained during the same 15-week period. For each horse with PPID, an environmental control horse was selected (ie, the healthy horse closest in age to the horse with PPID from the same farm). Given similar environmental conditions, horses with PPID were more likely to have higher fecal egg counts than were healthy horses. These results, along with the strong correlation between fecal egg counts before and after anthelmintic treatment, sug-
suggested that horses with PPID have a greater susceptibility to parasitism than do healthy horses. Because a nonterminal design was used for the study, it was not possible to determine whether differences in fecal egg counts were a result of a difference in the absolute parasite load or a difference in the percentage of parasites actively shedding eggs versus those entering an arrested state. Additional studies are needed to elucidate the mechanism by which PPID results in an increase in fecal egg counts. Regardless of the cause of the increase in fecal egg counts, horses with PPID shed more eggs throughout the study than did control horses, which suggested that, at a minimum, they may pose a risk for pasture hygiene when not managed properly.

Although secondary infections are a common sequela of PPID, the exact mechanism of the immunosuppression in horses with PPID is unknown. It is likely that the high circulating concentrations of anti-inflammatory hormones (ACTH, α-MSH, and cortisol) suppress immune responsiveness. However, these hormones typically shift immunity toward a Th2 bias, which is not consistent with an increase in susceptibility to parasites. Additional studies are necessary to elucidate the mechanism responsible for increased susceptibility to parasites in horses with PPID.

To our knowledge, the study reported here is the first in which investigators evaluated whether aged horses are more susceptible to intestinal parasites than are younger adult horses. In this study, control horses ranged from 4 to 35 years of age, with 11 of 29 horses ≥20 years. No relationship was detected between advanced age and fecal egg counts before or after an anthelminthic treatment, and when control horses from a single farm were assessed, there was no correlation between age and fecal egg counts; however, the number of horses in that analysis (n = 8 horses) was quite small. Ideally, a study with a larger number of horses of a broad age range maintained on the same pasture should be performed to confirm the finding that older horses do not require special consideration when designing a parasite control program.

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