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Original Research

Comparison of the Immunologic Response to Anthelmintic Treatment in Old Versus Middle-Aged Horses

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ABSTRACT

The purpose of this study was to evaluate whether aged horses demonstrate statistically higher fecal egg counts (FECs) compared with middle-aged horses and to investigate systemic expression of proinflammatory cytokines in old and middle-aged horses treated with moxidectin compared with horses treated with pyrantel pamoate. Old horses (n = 21) and middle-aged horses (n = 19) were blocked by levels of inflammation and randomly allocated to one of the following treatment groups: group 1 (n = 8) old treated with moxidectin gel; group 2 (n = 9) old treated with pyrantel pamoate paste; group 3 (n = 4) old receiving no treatment; group 4 (n = 8) middle-aged treated with moxidectin gel; group 5 (n = 7) middle-aged treated with pyrantel pamoate paste, and group 6 (n = 4) middle-aged receiving no treatment. Fecal samples were collected on day 0 and again 14 days after treatment to determine FECs and presence of *Strongylus vulgaris* by polymerase chain reaction. Peripheral blood was also collected on day 0 and again at days 3, 5, and 14 after deworming for inflammatory cytokine analysis, along with routine hematological analyses. Results indicated that old horses have significantly higher FEC than middle-aged adults. Fecal egg counts declined significantly after anthelmintic treatment in both age groups of horses. Inflammatory markers exhibited differences between age groups and by anthelmintic treatment. In summary, this study provided evidence of different inflammatory and immunologic reactions to anthelmintic treatment in old horses. Moreover, the higher FECs found in the old horse group may have practical implications for parasite management routines on farms with representation of this age group.

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1. Introduction

Horses older than 20 years of age constitute about 15% of the equine population, and this number is ever increasing [1]. Many of these aged horses remain actively involved in equestrian sports and reproductive capacities as stallions and brood mares. As a result of the growing geriatric horse population, an increased demand for veterinary care and management is needed for these geriatric horses [2–4].

Advancing age in horses, as with other species, is eventually associated with a decline in body condition, muscle tone, and immune function [5–7]. Immunosenescence in the aged individual is characterized by changes in various aspects of cellular and humoral immunity, in particular, a decline in lymphoid cell numbers and function [8–11]. Somewhat paradoxically, advanced age is also associated with increased production of proinflammatory cytokines and other inflammatory mediators [12–14]. In

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fact, elderly people have twofold to fourfold increases in plasma or serum levels of inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α), and other inflammatory mediators, such as C-reactive protein [13]. This phenomenon of increased production of inflammatory cytokines in the elderly has been termed "inflamm-aging," and we have recently shown this phenomenon in the aged horse [15–18].

As part of the care and management of horses, parasite control is of importance. The tradition has been to apply frequent anthelmintic treatments to all horses year-round [19–22], but ever-increasing levels of anthelmintic resistance in equine parasites have forced the equine industry to take a more surveillance-based approach for parasite control [23–25]. Anthelmintic treatment has been documented to evoke an inflammatory response in horses, and the extent of this response has been associated with the anthelmintic drug class used [26–28]. Furthermore, one study with murine disease models suggests anti-inflammatory properties of macrocyclic lactones [29]. This is consistent with one equine study that demonstrated no detectable local or systemic inflammatory response to moxidectin treatment [26]. However, it remains unknown whether old horses elicit different immunologic responses to deworming. It is often claimed that old horses might be more susceptible to parasite infection and thus could exhibit higher fecal egg counts (FECs). Although some studies do suggest this [30], most field surveys have not found higher egg counts in older horses [31–33]. Given the changes in the immune system with age in horses and the fact that the immune system is important in regulating tolerance to parasites, it still remains to be determined the effect of age on levels of strongyle FECs and the possible inflammatory responses to anthelmintic treatment in geriatric horses.

Thus, the aims of this study were to (1) determine whether FECs differ significantly between geriatric and middle-aged horses, (2) compare the inflammatory cytokine production after deworming in old and middle-aged horses, and (3) evaluate whether systemic expression of proinflammatory cytokines differs between horses treated with moxidectin or pyrantel pamoate.

2. Materials & Methods

2.1. Study Design

A total of 21 old horses (20–33 years) and 19 middle-aged adult horses (5–15 years) of mixed sex and mixed breeds were used in this study. All horses were situated on the University of Kentucky North Research Farm and subject to the same management and feeding schedules. Furthermore, horses were kept on the same paddocks and pastures for 6 months before the study. Within each age group, horses were ranked according to the magnitude of their % interferon gamma (IFN-γ) levels being produced by peripheral blood mononuclear cells (PBMCs) and then randomly allocated to one of the treatment groups. Old horses were allocated to one of the three treatment groups: group 1 (n = 8) were treated with moxidectin gel (400 μg/kg, Quest gel, Zoetis LLC, Kalamazoo, MI); group 2 (n = 9) received pyrantel pamoate paste (6.6 mg base/kg, Strongid P, Zoetis LLC, Kalamazoo, MI), and group 3 (n = 4) served as controls receiving no treatment. Middle-aged horses were allocated to one of the three treatment groups: group 4 (n = 8) were treated with moxidectin gel (400 μg/kg, Quest gel); group 5 (n = 7) received pyrantel pamoate paste (6.6 mg base/kg, Strongid P), and group 6 (n = 4) served as controls receiving no treatment. All anthelmintic treatments were carried out on the same day across all groups.

2.2. Sample Collections

Fecal samples were collected rectally before anthelmintic treatment on day 0 and again 14 days after treatment to determine FECs and presence of *Strongylus vulgaris* by polymerase chain reaction (PCR). All samples were immediately kept at temperatures below 5°C and analyzed within 5 days after sampling. Peripheral blood was also collected before deworming on day 0 and again at days 3, 5, and 14 after deworming for inflammatory cytokine analysis.

2.3. Inflammatory Cytokine Production by PBMCs

Heparinized blood samples were collected aseptically from the jugular vein. peripheral blood mononuclear cells were purified by Ficoll–Paque Plus (Amersham Biosciences, Piscataway, NJ) gradient centrifugation, washed in sterile phosphate-buffered saline (PBS), pH 7.2 (Gibco, Grand Island, NY), resuspended in c-RPMI 1640 (Gibco) media supplemented with 2.5% fetal equine serum (FES, Sigma Aldrich, St. Louis, MO), 2 mM L-glutamine (Sigma) and 100U/mL penicillin, streptomycin (Sigma), and 55 μM 2-mercaptoethanol (Gibco). Isolated PBMC (3×10^6/mL) from each horse was placed in 24-well plates and incubated with c-RPMI-2.5% FES media alone or c-RPMI-2.5% FES stimulated with phorbol myristate acetate (PMA) and ionomycin (PMA; 25 ng/mL; Sigma) and (ionomycin; 1 μM; Sigma). All wells received brefeldin A (10 μg/mL; sigma) and were incubated at 37°C 5% CO_2 for 4 hours. After the incubation, cells were placed into duplicate wells of a 96-well V-bottom plate, fixed in 2% paraformaldehyde (Sigma), and stored overnight at 4°C. The fixed cells were then washed once in PBS saponin buffer. Intracellular staining for IFN-γ was performed. Briefly, fixed cells were washed in PBS saponin buffer (PBS supplemented with 1% fetal bovine serum; Sigma and 0.1% saponin; Sigma and 0.1% sodium azide; Sigma) and then stained with CC302 mouse antibovine IFN-γ fluorescein isothiocyanate (FITC) (Serotec, Raleigh, NC) at a concentration of 1 μg/mL in PBS saponin buffer and incubated on ice for 30 minutes. CC302 antibody has been previously shown to cross-react with equine IFN-γ [34]. After the incubation, cells were washed twice in PBS saponin buffer and resuspended in FACS flow (Becton Dickinson). Intracellular staining for TNF-α was performed using a mouse antiequine TNF-α monoclonal antibody (HL801, kindly provided by Dr. Rob MacKay, University of Florida). The TNF-α antibody-labeled cells were incubated for an additional 30 minutes on ice with FITC-conjugated goat $F(ab')_2$ antimouse IgG (H + L) (Caltag Laboratories, Burlingame, CA). All IFN-γ and TNF-α stained samples were resuspended in FACS flow for flow cytometric

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