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Research paper

Molecular survey of trichostrongyle nematodes in a *Bison bison* herd experiencing clinical parasitism, and effects of avermectin treatment

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ABSTRACT

North American bison (*Bison bison*) producers face many challenges, including the potential clinical and economics problems caused by trichostrongyle nematodes within their herds. Little is known about the prevalence, intensity, geographical distribution and clinical significance of these parasites in commercial bison herds, even from regions where bison production has become popular. This study involved a large herd of bison from eastern South Dakota that was experiencing clinical parasitism due to a temporary over-stocking problem. After documenting fecal egg counts (FECs) and trichostrongyle genera present among the 3 main age-categories (i.e. adults, yearlings, calves) of bison during this heavily infected grazing season, the effects of doramectin treatment on the different age groups was also evaluated. This is the first bison study using PCR to identify genera of trichostrongyles in fecal samples. Virtually all 103 bison fecal samples from all 3 age classes were shedding trichostrongyle eggs by the end of the season, and the mean FECs were 34 eggs/g (EPG) among the cows, 125 EPG in the yearlings, and 186 EGP among calves. Based upon this heavily-infected herd, there is evidence that the susceptibility of bison to trichostrongyles is more similar to beef cattle than to sheep. Other parasites such as *Moniezia, Nematodirus, Trichuris,* and coccidians were also identified in these samples. All but 3 of the 51 samples analyzed with PCR shown at least 1 trichostrongyle genera. *Ostertagia* was detected in 68.6% of the samples, *Cooperia* in 80.39%, *Haemonchus* in at least 73% and *Trichostrongylus* in 16% of the herd. Most commonly, bison were infected with combinations of *Haemonchus/Ostertagia/Cooperia*. After treatment with doramectin, the mean FECs dropped by 99.9% for all of the bison age classes.

1. Introduction

Published information on North American bison (*Bison bison*) infectious diseases, especially parasites, is extremely inadequate, and primarily limited to qualitative observations on small numbers of bison from single populations (Tessaro, 1989). For that reason, the prevalence, geographical distribution and clinical significance of most bison parasites cannot be widely interpreted (Tessaro, 1989), even though the commercial production of bison has become a viable industry in the United States and Canada (Woodbury et al., 2014). Approximately half of the 400,000 bison located in North American are found in the United States. (Gates and Ellison, 2010). South Dakota, the leading producer of bison, and its neighboring states produce more than half of all bison in the U.S.A. (Gates and Ellison, 2010; USDA, 2014). Therefore, there is a particular need for a greater understanding of the parasites and their effects in bison from this region.

Studies have shown that bison are parasitized by the various protozoan, trematode, cestode, nematode, and arthropod parasites which also occur in other ruminants, especially domestic cattle, goats and sheep (Dies and Coupland, 2001; Reynolds et al., 2003; Tessaro, 1989; Woodbury et al., 2014). With their direct life-cycles, coccidian protozoans and trichostrongyle nematodes are ubiquitously distributed within virtually all herds of ruminant livestock from the Northern Great Plain (Dies and Coupland, 2001; Hildreth et al., 2007; Sutherland and Scott, 2010; Van Vureni and Scott, 1995; Zaugg et al., 1993). Little is known about the clinical significance of coccidians in bison, but clinical problems (including fatalities) have clearly been noted from trichostrongyles (Reynolds et al., 2003; Wade et al., 1979). Trichostrongyle nematodes are generally the

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most significant parasite problem in grazing cattle, sheep and goat herds, even in temperate climates where subclinical parasitism is the rule. For example, recent studies involving South Dakota cattle have shown that these nematodes decrease weight gains in stocker cattle by 6.6 kg during the grazing season (Epperson et al., 2001; Mertz et al., 2005). For this reason, the focus of this project was on trichostrongyle nematodes.

Species from 6 trichostrongyle genera are most commonly present within North American livestock herds, and most trichostrongyle populations consist of combinations of 2 or more genera. *Ostertagia* and *Cooperia* seem to be the 2 most common genera in U.S.A. cattle, particularly within the more northern states (Harmon et al., 2009; Malczewski et al., 1996; Stromberg et al., 1991). *Trichostrongylus* and *Oesophagostomum* can occasionally be found at times in certain herds, but their more restricted prevalence and distribution limits their importance to cattle (Gibbs and Herd, 1986). The relative importance of *Haemonchus* in cattle from temperate regions is less clear, and appears to be changing (Waller and Chandrawathani, 2005). *Haemonchus* has been recently found in 69% of 13 cattle herds from South Dakota (Harmon et al., 2009), and is known to cause significant clinical problems in sheep from this region (Grosz et al., 2013). *Haemonchus*, *Teledorsagia* and *Trichostrongylus* are the genera most commonly found in sheep and goats from the U.S.A. Northern Great Plains (Herd, 1986). Host specificity varies among the various trichostrongyle species: some are strongly adapted to bovine (e.g. *Ostertagia ostertagi*); others more adapted to bovine than to ovine (e.g. *Cooperia oncophora*, *C. surnabada*, *C. punctata*, *Trichostrongylus longispicularis*, *Haemonchus placei*); still others with no special host preference among ruminants (e.g. *Trichostrongylus axei*, *Ostertagia leptospicularis*); others more adapted to ovine than to bovine (e.g. *Cooperia curticei*, *Trichostrongylus vitrinus*, *Trichostrongylus colubriformis*, *Haemonchus contortus*); and finally those that are strongly adapted to ovine (e.g. *Teledorsagia circumcincta*, *Oesophagostomum venulosum*, *Chabertia ovina*) (Borgsteede, 1981; Hoberg et al., 2004).

In cattle, *O. ostertagi* is generally considered to be the most pathogenic species of trichostrongyle. In 3 captive bison herds from New York state, fatalities were attributed to ostertagiosis in 9 animals, and clinical signs (including severe diarrhea, emaciation, unthrifty coats, anemia, and weakness were noted in some of the other animals (Wade et al., 1979). In addition to *O. ostertagi*, other trichostrongyles recovered from these herds included: *T. axei*, *Trichostrongylus lerouxi*, *C. oncophora*, *H. contortus* (or possibly *H. placei*), *Oesophagostomum* sp. and *Nematodirus helvetianus*. In 2 reviews of bison parasites recovered in necropsy studies (Hoberg et al., 2001; Knapp et al., 1993), 10 species of trichostrongyles are listed, including: *Ostertagia ostertagi*/*O.lyrata*, *Ostertagia* (*Orloffia*) *bisonis*/O.*cf kazakhstanicab*, *O. leptospiralis*, *T. circumcincta/trifurcata*, *C. oncophora*, *C. surnabada*, *T. axei*, *C. ovina* and *Oesophagostomum radiatum*. Collectively, these results indicate that bison are susceptible to both trichostrongyle species that are strongly adapted for bovine and also those that are strongly adapted for ovine. This raises the possibility that bison may be parasitized by most ruminant livestock trichostrongyle species.

Antemortem diagnosis of gastrointestinal helminths in livestock has traditionally been based upon the microscopic enumeration of eggs excreted in the host feces. Because the various genera of trichostrongyles have eggs that are morphologically indistinguishable, further differentiation of these eggs has historically required eggs to be cultured to the third larval stage, which are then morphologically distinguishable at the genus level (Van Wyk and Mayhew, 2013). Thus far, there have been no copra-cultured surveys to identify trichostrongyles in bison. Advances in molecular biology have more recently provided a variety of diagnostic tools useful in differentiating trichostrongyle infections in cattle, sheep and goats even beyond the genus level based upon DNA isolated from excreted eggs or cultured larvae (Gasser et al., 2008). PCR analysis has been used to determine the trichostrongyle genera present in eggs isolated from cattle and sheep fecal samples (Bott et al., 2009; Grosz et al., 2013; Harmon et al., 2009; Learmount et al., 2009; Roeber et al., 2012; Sweeny et al., 2012). Yet, there have been no attempts to use these PCR technologies to identify trichostrongyle genera from naturally infected bison. One objective for this study was to evaluate the effectiveness of a standard PCR method used to identify the 4 most common trichostrongyle genera (i.e. *Haemonchus*, *Cooperia*, *Ostertagia*, and *Trichostrongylus*) in cattle (Zarlenga et al., 2001) for diagnosing these trichostrongyle genera in bison herds. This same standard PCR assay was used in 2005 to determine the genera of trichostrongyles present in 239 calves from 13 cattle herds in eastern South Dakota (Harmon et al., 2009), and is based upon genus-specific sequences within the interspatial regions of ribosomal DNA. In the present bison study, a second set of *Haemonchus* PCR primers (von Samson-Himmelstjerna et al., 2002) was also used to verify the presence of this genus.

During the grazing season of this study, a significant portion of the pasture used by the herd became unavailable due to a road construction project. This created a temporary overcrowding problem within the herd which then resulted in clinical parasitism problems later in the season. Thus, it was also possible to observe the fecal egg counts and genera of trichostrongyles among the 3 main age-categories (i.e. adults, yearlings, calves) within a heavily infected bison herd. As with virtually all macroparasites, trichostrongyles have been shown to be aggregated within sheep and cattle herds (Polley and Bickis, 1987; Stear et al., 2007). The level aggregation depends on numerous parasite and host factors, including the age and susceptibility of the host (Stear et al., 2007). The heavy level of parasitism observed in the Flandreau bison herd also provided an opportunity to evaluation the degree to which aggregation exists within the 3 age-categories of bison.

The injectable and pour-on formulations of ivermectin have already been evaluated for the treatment of trichostrongyles in bison (Marley et al., 1995; Woodbury and Lewis, 2011), but there have been no published studies evaluating doramectin. The third objective this study was to use a standard fecal egg count reduction test (FECRT) to assess the efficacy of an injectable formulation of doramectin (Dectomax®) against trichostrongyles in bison (Wood et al., 1995).

2. Materials and methods

2.1. Study area, herd characteristics and initial sample collections

The commercial bison herd observed in this study is located near the city of Flandreau (approximately 44° 2′ 52″ N, 96° 35′ 47″ W), in Moody County, South Dakota. During the summer of 2010, the herd was composed of 91 cows, 5 bulls, and 84 subadults (calves, yearlings, and 2-year old), and their grazing was restricted to a 23.5 ha of rolling pasture that quickly became over-grazed. The primary plant species in both pastures was Kentucky bluegrass (*Poa pratensis* L.), but warm- and cool-season native grasses were also present in smaller amounts. Canada thistle (*Cirsium arvense* [L.] Scop.), plumeless thistle (*Carduus acanthoides* L.) and bull thistle (*Cirsium horridulum* Michx.) had become very prevalent. In an attempt to mitigate nutrition problems during that summer, hay (rolled out onto the ground) and grain (oats and corn distributed into feed bunks) were given daily throughout much of the grazing season. Even with this added nutrition, the animals were in poor nutritional health by August, with body condition scores of 3–4 on a 9 point scale, and many were showing mild signs of clinical parasitism (e.g. rough hair-coat, bottle-jaw). By mid-summer, the herd was also experiencing fly problems, and a few animals