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EFFICACY OF IVERMECTIN POUR-ON AGAINST *OSTERTAGIA OSTERTAGI* INFECTION AND RESIDUES IN THE AMERICAN BISON, *BISON BISON*

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ABSTRACT: Sixteen American bison, *Bison bison*, were artificially infected with 10^5 infective stage larvae of *Ostertagia ostertagi* on 21 April 1993. At 42 days post-infection eight bison were treated with 0.5% ivermectin pour-on (500 μg/kg bodyweight) and eight treated with the carrier only. Bison were necropsied 17 and 18 days post-treatment (21 and 22 June 1993, respectively). Mean (±SE) of 5,413 (±1,716) adults and 565 (±305) immature *O. ostertagi* were recovered at necropsy from bison treated with the carrier. No *O. ostertagi* were detected in bison treated with ivermectin pour-on. Based on the levels of the ivermectin marker metabolite in liver and adipose tissue 18 days post-treatment, the established bovine withdrawal time of 48 days appears adequate to insure that violative residues do not occur.

Key words: American bison, *Bison bison*, *Ostertagia ostertagi*, ivermectin, tissue residuum.

INTRODUCTION

The American Bison Association has estimated that over 130,000 American bison (*Bison bison*) are present in North America (B. Ward, pers. comm.). Most of these are in private herds. The remainder of the bison in the United States are in Yellowstone National Park, Wyoming, the National Bison Refuge at Moiese, Montana, the Wichita Mountain National Refuge in Oklahoma, and Custer State Park, South Dakota. In addition, there are bison present in National Parks in Canada.

Although classified in the family Bovidae, there is a lack of information to support direct similarities between cattle and bison. Moreover, in a recent literature review of parasites of the American bison, Knapp et al. (1993) found only a single publication on chemotherapeutic studies with bison; Worley and Hansen (1960) used Dow-ET-57 (0,0-dimethyl 0-2, 4,5-trichlorophenyl phosphorothioate) and Dowco 109 (0-4 (tert-butyl-2-chlorophenyl) 0-methyl methyl phosphoroamidothioate) administered as feed additives. Only Dow-ET 57 significantly reduced nematode egg per gram counts.

Bison producers believe they must treat their animals for parasites to avoid associated economic losses which are observed with cattle. In particular, treatment for *Ostertagia ostertagi* is desired because, in large part, of the economic impact that this parasite has on infected cattle. However, there are no approved anthelmintics available for use with bison. Consequently, producers either abstain from treating bison or use anthelmintics illegally through extra-label use.

Our objectives were to evaluate the efficacy of ivermectin pour-on (Ivomec® Pour-on, Merck & Company, Rahway, New Jersey USA) against the brown stomach worm, *O. ostertagi*, in the American bison, *B. bison*, and determine the level of ivermectin marker residue in liver and adipose tissue.

MATERIALS AND METHODS

Eighteen 8- to 12-mo-old bison heifers were obtained from Turner Ranches, Gallatin Gateway, Montana. The animals were maintained at the Flying-D ranch located in Gallatin and Madison Counties, Montana (45°27′ to 45°33′N, 111°16′ to 111°31′W). The original herd was maintained on 52,600 ha and roamed free throughout the year, but was handled once annually. Consequently, the study animals had been exposed to parasites. Bison were provided a maintenance of diet of dry grass hay, fresh water and trace mineral ad libitum. Good Lab-

oratory Practice Standards were conducted for this study as listed in the Federal Register (Part IV), defined by the Environmental Protection Agency (40 CFR Part 160).

In a pilot study (19 March to 20 April 1993), we tested the infectivity of the laboratory culture of infective third-stage larvae (L_3) of *O. ostertagi*; results were used to determine the infection dosage for the efficacy study. Additionally, the pilot study provided an indicator of natural parasite infection. The efficacy study (21 April to 22 June 1993) was designed to measure the effectiveness of ivermectin pour-on against *O. ostertagi*.

For the pilot study, two bison were weighted and uniquely identified with eartags. One animal was infected by gavage with approximately 7.5×10^4 *O. ostertagi* third stage larvae (L_3) obtained from the U.S. Department of Agriculture (USDA), Agricultural Research Service, Helminthic Diseases Laboratory, Beltsville, Maryland (USA), and the second animal was administered a water placebo. Fresh fecal samples were collected from the ground from each of the two bison and eggs per gram of feces (EPG) counts were determined using the modified Wisconsin double centrifugation technique (Marley, 1992) on study days 0, 5, 10, 15, 20, 25, 29, and 31, post-infection (PI) or post-placebo. Both animals were killed by captive bolt pistol followed by exsanguination and necropsied 31 days PI or post-placebo. The abomasal and intestinal nematodes were collected, identified, and enumerated using the dilution-autodigestion technique (Williams et al., 1977) and by procedures described by Brauer (1983), respectively.

For the efficacy study, 16 bison were weighed, randomly eartagged from numbers 1 to 16 and artificially infected by gavage with approximately 10^5 *O. ostertagi* L_3 (study day 0). The bison were ranked in ascending order of weights and assigned to two groups of eight bison each using the Gardiner and Wehr (1950) allocation method. Individual bison within each group were identified with a second colored and numbered eartag unique for each group. The ivermectin pour-on and carrier were supplied by Merck & Co. On day 42 PI, all bison were weighed. To determine more accurate patency data prior to treatment, an additional 14 days were added to the efficacy study. On Day 44 PI, one group of bison was treated with 0.5% ivermectin pour-on formulation applied topically using a 35 ml syringe. The ivermectin was applied 2 to 3 cm above the skin surface along the mid backline between the distal portion of the cape and the tailhead of each animal at the label dosage recommended for cattle (1 ml/10 kg bodyweight or 500 μg/kg). The control group was treated similarly with the carrier at 1 ml/10 kg bodyweight. All bison had dry hair at the time of treatment and there was no precipitation such as rainfall or snowfall, within 24 hr of treatment. Haircoat condition and percentage shed per bison were determined and recorded at treatment. Type and degree of haircoat shedding was divided into five categories ranging from complete to incomplete shedding of the haircoat on the dorsum of the animal from the shoulders (inclusive of cape) to the tailhead.

Fecal samples were individually collected from the ground from at least six of the eight bison per group on an individual basis on days 0, 10, 20, 25, 30, 33, 37, 41, and 44 PI, and days 47, 50, 54, 57, 61, and 62 PI (post-treatment days 3, 6, 10, 13, 17, and 18, respectively). All bison were individually fecal sampled (rectally) on infection day 0, treatment day 0, and at necropsy, and EPG recorded. All 16 bison were killed by standard humane practice: captive bolt pistol followed by exsanguination. Four bison from each group were necropsied on day 61 and the remaining four from each group were necropsied on day 62 of the study (post-treatment days 17 and 18, respectively), and examined for gastrointestinal parasites. Aliquots of diluted abomasal and intestinal contents were examined and total worm counts were determined by extrapolation. Samples of liver and adipose tissue were collected from each animal. The high performance liquid chromatography (HPLC) method of Markus and Sherma (1992) was used to determine the level of 22, 23 dihydroavermectin B_{1a} (ivermectin marker residue) in liver and adipose samples. The limit of detection of this method is 5 ppb in liver and 2.5 ppb in adipose tissue. Carcasses of the ivermectin-treated bison were incinerated.

Data were analyzed using Wilcoxon two-sample test (rank sums) (SAS Institute, 1985). Differences were considered significant at $P \leq 0.01$. Data are reported in mean ± SE of EPG and adult (and immature) numbers of *O. ostertagi* per bison group defined.

RESULTS

Based on the fecal examinations, non-infected bison in the pilot study had negligible egg counts throughout the 31-day-period. In contrast, the bison infected with 7.5×10^4 *O. ostertagi* L_3 had few eggs detected until 29 and 31 days PI when EPG's of 130 and 150, respectively, were detected; this was evidence for patency of the experimental infection. On post-mortem examination, the non-infected bison

TABLE 1. Mean (±SE) number of eggs per gram (EPG) recovered from American bison infected with 10^5 infective stage *Ostertagia ostertagi*.

	EPG at infection	EPG at treatment	EPG at necropsy[a]	Efficacy (%)
Ivermectin-treated group	5 ± 2.4	107 ± 64	0	100
Non-treated group	5 ± 1.8	75 ± 22	169 ± 95	NA[b]

[a] 17 and 18 days post-treatment.
[b] NA, not applicable.

had 20 adult *O. ostertagi* while the infected bison had 6,240 adults and 2,800 immature worms present. From these results, we estimated an infection establishment rate of 12% of the infective larvae developing to the adult and immature stages.

Data for the infected bison in the pilot study were included in the efficacy study data, in the non-treated group analysis. There were no significant differences on infection day 0 ($P = 0.8845$), or treatment day 0 ($P = 0.9616$), in EPG between the two groups of bison (Table 1). Conversely, at necropsy significant differences ($P = 0.0003$) were detectable between EPG for bison treated with ivermectin and bison treated with the carrier (Table 1).

Significant differences in adult ($P = 0.0003$) and immature ($P = 0.0010$) *O. ostertagi* mean numbers were detected at necropsy between the two groups of bison. Non-treated bison had 5,413 (±1,716) adults and 565 (±305) immatures recovered. No *O. ostertagi* adults or immatures were recovered from bison treated with ivermectin.

The average recovery of ivermectin marker residue from liver and adipose tissue administered with 100 ppb was 62% and 66%, respectively. Incurred ivermectin marker residue levels in the liver samples ranged from 6.5 to 54.8 ppb (mean ± SE = 32.1 ± 5.4 ppb), and in the adipose tissue from 1.5 to 16.7 ppb (mean ± SE = 6.8 ± 1.7 ppb). There was not a significant ($P > 0.05$) correlation between haircoat coverage on the animals and levels of ivermectin in adipose ($r = -0.20$) or liver ($r = 0.61$) tissue.

DISCUSSION

Our goal was to infect the bison with enough *O. ostertagi* larvae to reach EPG counts of approximately 200, and adult (and developing immature) counts of about 10^4. Because the dose used for the pilot study did not reach these counts, bison in the efficacy study were infected with 10^5 infective larvae in an attempt to increase the egg and worm counts. A single species artificial infection was used to avoid confounding results that might have occurred relative to species competition.

Based on the pilot study, we estimated that patency of *O. ostertagi* for the one artificially-infected bison occurred approximately 29 days PI. Patency in the 16 bison in the efficacy study was estimated to occur about 22 days PI. Ivermectin in the pour-on and injectable formulations for cattle reach maximum blood levels about 10 to 12 days post-treatment, and the ivermectin in the pour-on formulation may persist in the blood for about 21 days (J. E. Holste, pers. comm.). Necropsy of the bison was therefore scheduled at 17 and 18 days post-treatment to take maximal advantage of the anthelmintic activity period of the ivermectin treatment. The results of the efficacy study were indicative of a high level of efficacy (100%) of ivermectin in the removal of *O. ostertagi* infection in American bison.

The current U.S. Food and Drug Administration established safe levels of total ivermectin residues in cattle and reindeer liver and adipose are 50 and 100 ppb, respectively (CFR 21.556.344). In cattle, the marker tissue is liver in which the iver-

mectin marker residue concentration which corresponds to the safe level is 15 ppb. For cattle, the liver is the marker tissue because it has the slowest depletion rate (Chiu and Lu, 1989). Following subcutaneous administration of 0.3 mg/kg ivermectin to cattle, ivermectin marker residue concentrations were 454 ppb at 2 days, and 11 ppb at 28 days post-treatment (Tway et al., 1981). Liver samples taken from cattle 14 days post-treatment with 500 μg/kg ivermectin pour-on contained 27 ppb ivermectin marker residue (J. L. Cox, pers. comm.), which is similar to the 32 ppb mean for the eight bison in this study. Thus, the 48-day withdrawal time established for cattle after ivermectin pour-on treatment should be appropriate for bison.

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