PEER REVIEWED

A fecal egg count reduction test evaluating macrocyclic lactones using cattle treated 118 days earlier with saline, albendazole in combination with doramectin, or an extended-release formulation of eprinomectin

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Abstract

A fecal egg count reduction (FECR) test was conducted on stocker cattle treated 118 days earlier with saline injection (S); 0.09 mg/lb (200 mcg/kg) BW doramectin injection concomitantly with 4.54 mg/lb (10 mg/kg) BW albendazole oral suspension (DA); or 0.45 mg/lb (1 mg/kg) BW eprinomectin extended-release injection (ERE), and then continuously grazed by treatment group in groups of 4 until the start of the current study. In total, there were 8 S, 12 DA, and 12 ERE grazing groups (pastures of origin). Three animals from each pasture were randomly allocated for injection with ivermectin, doramectin or moxidectin, all at the rate of 0.09 mg/lb (200 mcg/kg) BW. Fecal samples were obtained at days -1 and 15, with treatments on day 0. Additionally, coprocultures were conducted on individual fecal samples collected on days -1 and 15.

On day -1 of the FECR test, the arithmetic mean strongyle eggs per gram of feces counts (EPG) across all pastures of origin were 412, 570, and 321 for the ivermectin, doramectin, and moxidectin-treated cattle, respectively. Day 15 egg counts in the same order were 177, 335, and 28. Using the above arithmetic means and by standard equation, the overall mean FECR percentages were 57.0, 41.2, and 91.2 for ivermectin, doramectin, and moxidectin, respectively. Lowest FECR test percentages were seen for animals from the ERE pastures. Coproculture larvae populations harvested on both days -1 and 15 were primarily *Haemonchus placei* and *Cooperia punctata*, regardless of animal's pasture of origin or FECR test treatment.

Key words: cattle, field trial, effectiveness, anthelmintics

Résumé

Un test de réduction du compte d'œufs dans les fèces a été mené chez des bovins en élevage qui ont été traités 118 jours auparavant avec soit une injection de saline (S), soit avec une injection de doramectine à la concentration de 0.09 mg/lb (200 mcg/kg) de poids corporel administrée en parallèle avec une suspension orale d'albendazole à la concentration de 4.54 mg/lb (10 mg/kg) de poids corporel (DA) ou soit avec une injection à libération continue d'éprinomectine à la concentration de 0.45 mg/lb (1 mg/kg) de poids corporel (ERE). Après ce traitement, les bovins ont été mis au pâturage sans interruption par groupe de traitement incluant quatre bovins jusqu'au début de l'étude. Au total, il y avait huit groupes e bovins pour le traitement S, 12 pour le traitement DA et 12 pour le traitement ERE au pâturage (pâturages d'origine). Trois bovins dans chaque pâturage ont reçu de façon aléatoire une injection d'ivermectin, de doramectine ou de moxidectine toutes à la concentration de 0.09 mg/lb (200 mcg/kg) par unité de poids. Des échantillons fécaux ont été recueillis aux jours -1 et 15 (jour 0 étant le jour du traitement). De plus, des coprocultures ont été faites sur des échantillons de fèces d'origine connue aux jours -1 et 15.

Au jour -1 du test de réduction du compte d'œufs fécaux, la moyenne arithmétique du compte d'œufs de strongyles par gramme (OPG) de fèces peu importe le pâturage d'origine était de 412 chez les bovins traités avec l'ivermectin, de 570 chez les bovins traités avec la doramectine et de 321 chez les bovins traités avec la moxidectine. Dans le même ordre, les comptes moyens au jour 15 étaient de 177, 335 et 28. En se basant sur ces moyennes arithmétiques et sur des équations standards, la moyenne globale de réduction du compte d'œufs fécaux était de 57.0% chez les bovins traités avec l'ivermectin, de 41.2% chez les bovins traités avec la doramectine et de 91.2% chez les bovins traités avec la moxidectine. Les plus petites valeurs du pourcentage de réduction ont été observées dans les pâturages du traitement ERE. La coproculture aux jours -1 et 15 a révélé principalement des populations larvaires de *Haemonchus placei* et de *Cooperia punctata* et ce peu importe le pâturage d'origine ou le traitement pour le test de réduction du compte d'œufs fécaux.

Introduction

At present, cattle in the replacement and stocker phases of production in the United States (US) receive no, repeated, or suppressive anthelmintic treatments. This gradation of intervention generally parallels producer appreciation for the detriment that is consistent with infection by parasitic nematodes. Unfortunately, the more chemical control placed on parasitic nematodes, the greater the selection pressure for anthelmintic resistance (AR). In fact, it appears that AR is the current status quo in parasitic nematode burdens found in all phases of cattle production, but most apparent in stocker/background cattle.[22] Resistance to the macrocyclic lactones (MLs), as opposed to anthelmintics of the other classes, has been documented most often,[11,17] and is considered resultant from decades of reliance on MLs to minimize worm burdens. Selection for AR is most demonstrated for *Cooperia punctata* and *C oncophora*, nematodes that have a high incidence in younger animals and shown to be dose limiting at the time of initial ivermectin availability, thereby requiring the least amount of selection in "jumping" from susceptible to resistant (e.g. "window of escalation"[16]). *Haemonchus placei* also exhibits AR, a nematode of high incidence and populations in animals of all ages in the southern tier of the US.[22] Therefore, some degree of AR, especially relative to the MLs, is common in younger animals. A currently unanswered question is whether an increase in ML resistance occurs at a detectable rate over 1 grazing season wherein suppressive anthelmintic intervention is employed (e.g. eprinomectin extended-release injection). In an attempt to address this question, the current study was conducted in stocker cattle after they received varied degrees of anthelmintic intervention previously in the grazing season.

Materials and Methods

Cattle used in the current fecal egg count reduction (FECR) study were those available at the end of a season-long study conducted in Hope, Arkansas to determine the effectiveness of eprinomectin extended-release injection[a] (0.45 mg/lb; 1 mg/kg BW) vs a concomitant injection of doramectin[b] (0.09 mg/lb; 200 mcg/kg BW) with albendazole oral suspension[c] (4.54 mg/lb; 10 mg/kg BW), or a saline injection in the control of stocker calf nematodiasis.[24] In the preceding study, 4 animals were assigned to each of 32 treatment-specific, Bermuda grass pastures: 8 saline (S), 12 combination treatment (DA), and 12 eprinomectin extended-release (ERE) pastures, and then maintained on their respective "pastures of origin" for 118 days. At the termination of the grazing study, 1 animal per pasture of origin was removed for necropsy, thereby making available the remaining 3 head per pasture for this FECR study (32 pastures, 3 head per pasture). The 3 animals from each pasture were allocated on a random basis to receive ivermectin[d] (IVM), doramectin (DRM) or moxidectin[e] (MXD), all as commercially available injectables given at the rate of 0.09 mg/lb (200 mcg/kg) BW. At treatment, each animal was weighed, and the dose (rounded to the next higher 0.1 ml) was given subcutaneously behind the right shoulder.

Fecal samples were collected from each animal on day -1, treatments were given on day 0 (18 Sept 2013), and post-treatment fecals were obtained from each animal on day 15 (03 Oct 2013). Nematode egg counts were performed on each fecal sample using 1-gram subsamples homogenized in saturated magnesium sulfate, sieved, centrifuged to 1 coverslip, and inspected microscopically per standard laboratory procedure.[21] For fecal samples shown to have a nematode egg per gram of feces (EPG) count > 20, and for which sample at least 20 grams of feces remained, a coproculture was conducted for the propagation, harvest, and counting of infective 3rd stage larvae, all according to standard procedures.[4,12] A minimum of 20 and a maximum of 100 larvae were identified and counted per coproculture.

For the entire duration of the FECR study, all animals grazed together as a single group. All were healthy throughout, displayed no adverse effects of medication, and were maintained according to procedures approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol #13049). After the fecal samples were obtained on day 15, the animals were relinquished back to the owner for immediate transfer to feed yard or other backgrounding facilities.

Fecal egg counts are presented as arithmetic means, but analysis of variance was performed with Proc Mixed of SAS[f] on individual animal fecal egg count reduction percentages or log-transformed fecal egg count data with differences noted at $P < 0.05$ and $P < 0.10$ levels of significance. The analysis accounted for pasture of origin, study treatment, and the interaction between the 2 following a Tukey-Kramer adjustment. Fecal egg count reductions were calculated from group arithmetic means as opposed to back-transformed, geometric means as the latter are prone to overestimate anthelmintic effectiveness.[6,7,15] Fecal egg count reductions are also presented as a group mean of all individual animal egg count reduction percentages, a method that gives equal weight to each animal in an experimental group as opposed to basing effectiveness interpretation on the animals with the highest egg counts.[5,18]

Results

The EPG ranges and arithmetic means are in Table 1 for all pasture of origin and treatment group combinations. Egg count data for individual animals was removed due to a 0 egg count on day -1 (1 animal), a lack of fecal sample on either day -1 or 15 (2 animals), or an EPG count on day 15 that was inexplicably higher than the day -1 count (1 animal). Significant differences between mean egg counts on day -1 or between mean egg counts on day -1 and day 15 were not seen ($P < 0.05$ or 0.10) regardless of pasture of origin, treatment group or combination thereof. On post-treatment day 15, mean egg counts for moxidectin-treated calves were

lower than counts for the doramectin-treated calves when comparisons were made for calves originating from the ERE pastures or for calves from all pastures of origin combined ($P < 0.10$ and $P < 0.05$, respectively).

Treatment group, mean fecal egg-count reduction percentages based on either treatment group, arithmetic means on days -1 and 15, or the mean of individual animal fecal egg count reductions are in Table 2. Overall, there was good agreement in reduction percentages between the 2 methods of calculation. Significant differences in mean FECR percentages based on individual animal FECR percentages were seen between the 3 treatment groups (MXD > DRM = IVM) when all pastures of origin were combined and for animals originating from the ERE pastures (MXD = IVM ≥ DRM). Regardless

Table 1. Arithmetic means (AM) and ranges for EPG counts on days -1 and 15.

Pasture of origin†	FECRT treatment*	N	Day of study‡			
			-1		15	
			AM	range	AM	range
ALL	IVM	32	412	4 – 2733	177[a,b]	0 – 1545
ALL	DRM	31	570	5 – 2724	335[b]	0 – 2193
ALL	MXD	28	321	3 – 1509	28[a]	0 – 387
CONTROL	IVM	8	545	8 – 1968	170	0 – 528
	DRM	8	439	11 – 1413	203	0 – 574
	MXD	6	290	25 – 468	18	0 – 107
COMBINATION	IVM	12	152	8 – 528	93	0 – 244
	DRM	12	611	6 – 2724	286	0 – 1551
	MXD	10	350	15 – 1029	9	0 – 50
ERE	IVM	12	585	4 – 2733	266[x,y]	0 – 1545
	DRM	11	619	5 – 2295	483[x]	0 – 2193
	MXD	12	313	3 – 1509	49[y]	0 – 387
CONTROL	ALL	22	437	8 – 1968	---	---
COMBINATION	ALL	34	372	6 – 2724	---	---
ERE	ALL	35	502	3 – 2733	---	---

*All treatments as injectable, subcutaneous, and at the rate of 0.09 mg/lb (200 mcg/kg) BW; IVM = ivermectin (Ivomec®, Merial Limited); DRM = doramectin (Dectomax®, Zoetis); MXD = moxidectin (Cydectin®, Boehringer Ingelheim)

†Control = saline injected; Combination = oral albendazole (Valbazen®, Zoetis) + doramectin (Dectomax®, Zoetis) injectable; and ERE = injectable extended-release eprinomectin (LongRange™, Merial)

‡Means in the same column and of the same pasture of origin by treatment combination with unlike superscripts are different [^{x,y} ($P < 0.10$), ^{a,b}($P < 0.05$)]

Table 2. Measurements for fecal egg count reductions.

Pasture of origin‡	FECRT treatment*	N	Based on individual animal FECR %'s		Based on group arithmetic means
			x	range	
ALL	IVM	32	57[a]	-93 to 100	57
ALL	DRM	31	48[a]	-176 to 100	41
ALL	MXD	28	94[b]	55 to 100	91
CONTROL	IVM	8	67	11 to 100	69
	DRM	8	72	27 to 100	54
	MXD	6	95	69 to 100	94
COMBINATION	IVM	12	44	-93 to 100	39
	DRM	12	61	32 to 100	53
	MXD	10	94	76 to 100	97
ERE	IVM	12	64[a,b]	24 to 100	55
	DRM	11	17[b]	-176 to 100	22
	MXD	12	92[a]	55 to 100	84

*All treatments as injectable, subcutaneous, and at the rate of 0.09 mg/lb (200 mcg/kg) BW; IVM = ivermectin (Ivomec®, Merial Limited); DRM = doramectin (Dectomax®, Zoetis); MXD = moxidectin (Cydectin®, Boehringer Ingelheim)

‡Control = saline injected; Combination = oral albendazole (Valbazen®, Zoetis) + doramectin (Dectomax®, Zoetis) injectable; and ERE = injectable extended-release eprinomectin (LongRange™, Merial)

[a,b]FECR % group means based on individual animal FECR %, within the same pasture of origin category, with different superscripts, are different $P < 0.05$

of pasture of origin or method of effectiveness calculation, neither the IVM or DRM treatment group had mean FECR percentages above 80%, whereas the MXD group had values > 90% except for the FECR percentage based on group EPG means for the ERE pasture of origin.

The percentages of animals in each pasture of origin by treatment group combination which had ≥ 80% fecal egg-count reductions (the most lenient measure for effective nematocidal activity according to current literature[10]), is presented in Figure 1. Regardless of pasture of origin, > 83% of the animals treated with MXD had their egg counts decreased by ≥ 80%, whereas < 63% of the IVM or DRM-treated animals had egg counts reduced by ≥ 80%. Additionally, regardless of treatment, the lowest incidences of animals experiencing ≥ 80% egg-count reductions were seen for animals originating from the ERE pastures.

The average coproculture, larvae percentages for all fecal samples cultured for days -1 and 15 are shown in Figure 2. These data are heavily weighted to the avermectin-treated animals (included in these data: day -1; 17 IVM, 9 DRM, and 3 MXD-treated calves, and day 15; 20 IVM, 21 DRM, and 4 MXD-treated calves). *Cooperia punctata* and *Haemonchus placei* were the most prominent species relative to the abundance of coproculture larvae (and correspondingly, to fecal eggs), an observation that was consistent for all dates, pastures of origin and treatment groups. Larvae of the above 2 species accounted for 77% of the day -1 (pre-treatment) harvests, and 97% of the day 15 (post-treatment) harvests. Percentages of total coproculture harvests decreased due to treatment for all species except for *H. placei*, a species whose mean percentage of the total went from 44% on day -1 to 81% on day 15.

The mean EPG counts for all animals in the FECR test were 438 on day -1 and 185 on day 15. These counts, multiplied by each species' mean percentage of total coproculture larvae count (Figure 2) yields calculated, species-specific EPG counts for all animals on days -1 and 15 (Table 3), a set of data that provides more definition regarding which nematodes were the most refractory to ML activity (as measured by overall worm burden fecundity). These calculated EPG counts, however, are primarily reflective of those animals treated with either ivermectin or doramectin, as only those animals had EPG counts of sufficient magnitude to allow for successful coproculturing at post-treatment. As shown in Table 3, percent reductions of calculated, species-specific EPG counts ranged from 100% for *O. ostertagi* to 22.8% for *H. placei*. Percent reductions for *C. punctata*, *O. radiatum*, *T. axei*, and *C. oncophora* were all below 90%.

Discussion

In this study, ML-treated stocker calves with prior, same-season anthelmintic interventions of varied intensities were generally shown to yield FECR percentages of moxidectin ≥ ivermectin ≥ doramectin. Several molecular (mode of

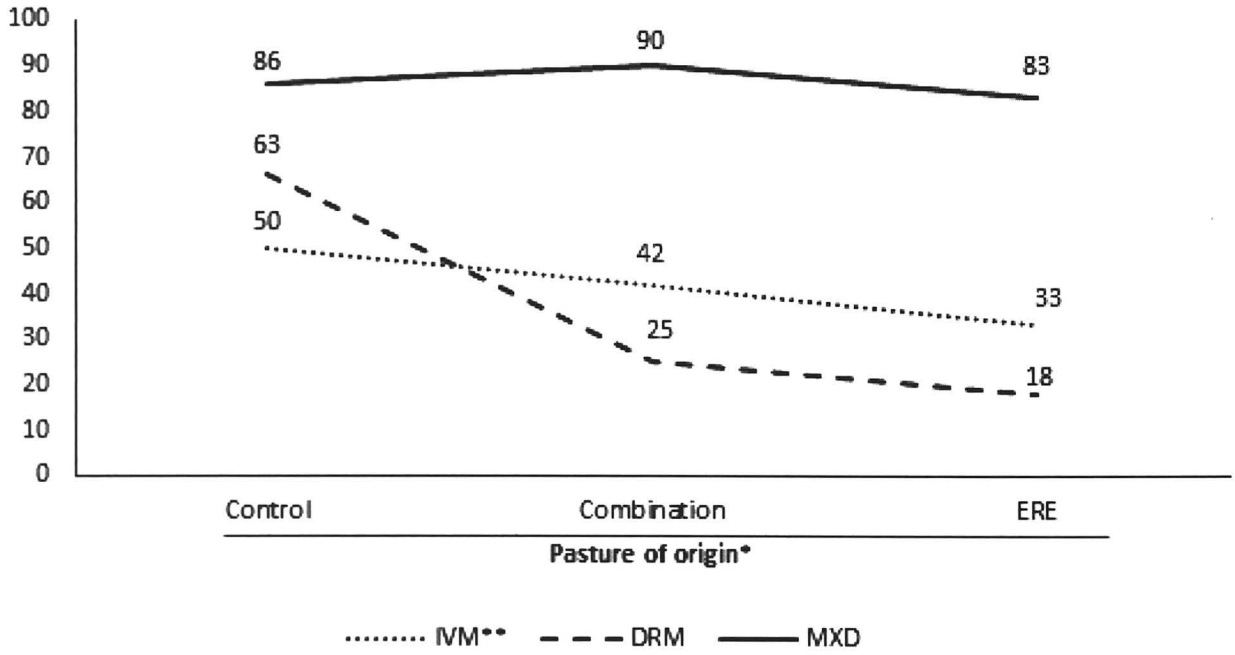

Figure 1. Percent of animals for each treatment group X pasture of origin combination with FECR % ≥ 80%.
*Control = saline injection; Combination = oral albendazole (Valbazen®, Zoetis) + injectable doramectin (Dectomax®, Zoetis); ERE = injectable extended-release eprinomectin (Long Range™, Merial Limited)
**IVM = ivermectin (Ivomec®, Merial Limited); DRM = doramectin (Dectomax®, Zoetis); MXD = moxidectin (Cydectin®, Boehringer Ingelheim)

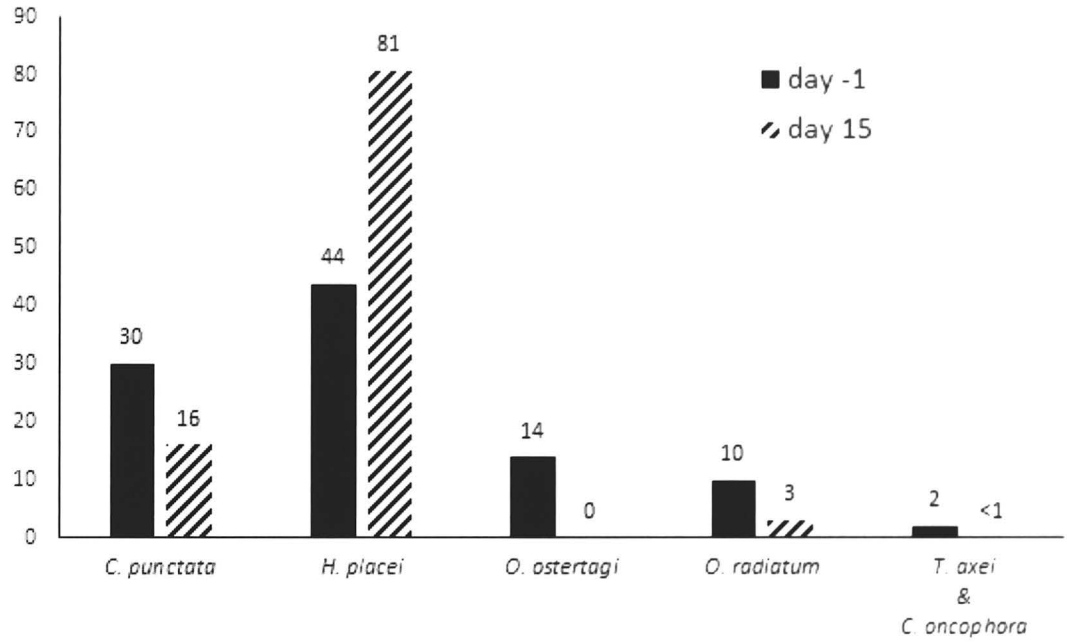

Figure 2. Mean coproculture L3 percentages across all animals for days -1 and 15 of the FECR test.

Table 3. Calculated species-specific mean EPG counts on days -1 and 15 as calculated from the mean EPG and coproculture larvae counts from all animals.

Species	x EPG on day		% reduction
	-1	15	
H. placei	193	149	22.8
C. punctata	131	30	77.1
O. radiatum	44	5	88.6
O. ostertagi	61	0	100.0
T. axei & *C. oncophora*	9	<1	>88.9
All combined	438	185	57.8

action) elements have been identified which confer greater nematocidal effectiveness to moxidectin (a milbemycin) than to ivermectin or doramectin (avermectins).[1,2,14] Additionally, others have demonstrated ivermectin to be more nematocidal than doramectin in tests conducted both *in vivo* and *in vitro*.[9,13]

Unique to the current study was the testing of anthelmintic efficacies at the end of the claimed period of effectiveness for the eprinomectin extended-release injection.[8] These results indicate that animal treatment with the eprinomectin extended-release formulation earlier in the grazing season does appear to decrease subsequent effectiveness of related, macrocyclic lactones, and that *H. placei* can be the nematode most refractory to ML treatment.

In discussing the results of this study, part of the title from a presentation given at the 2007 meeting of the American Association of Veterinary Parasitology should be cited; "Can sharp anthelmintic decisions be made using a blunt diagnostic tool?"[19] Coprology is an inherently flawed procedure for gaining perspective on the degree of anthelmintic resistance, activities, dynamics, and sizes of worm burdens. However, despite the flaws, it remains the only tool ("gold standard") for routine assessment of worm burden activity at the farm level in animals we cannot euthanize. Admittedly, additional procedures can be performed on fecal eggs and cultured larvae to gain more information, but ultimately, at best, only the eggs of actively fecund females are counted and utilized. All other gastrointestinal, non-egg laying parasitic nematodes (immature adults, temporarily non-fecund adult females, males, larvae, arrested stages, etc.) are not represented by the eggs. In addition to the fecal egg count being of dubious merit, the FECR test is further compromised by species-specific variations in nematode response to anthelmintics. For example; 1) anthelmintic activity against arrested nematode burdens cannot be assessed with a short-lived FECR test,[20] 2) adult female cooperiads that survive ML treatment temporarily become non-gravid,[3] and 3) adult *Haemonchus* spp females which survive ML treatment become more fecund than non-exposed females.[23] Considering the above, levels of anthelmintic effectiveness for all MLs used in this study may be overstated for the cooperiads, primarily *C. punctata*, and underestimated for *H. placei*.

Conclusions

Within the context of this study, heavily infected stocker cattle administered an ML at the end of a grazing season appeared to receive effective anthelmintic intervention in the order of moxidectin ≥ ivermectin ≥ doramectin. *Cooperia* and *Haemonchus* spp nematodes were the most abundant and the

most resistant to anthelmintic action. Use of eprinomectin extended-release treatment earlier in the season appeared to depress efficacies for all MLs tested. Given the results of this study, it is highly recommended that producers utilize the FECR test to insure that treatment results in effective anthelmintic intervention. In addition, methods should be used to address the degree of anthelmintic resistance that is common in stocker/backgrounder cattle operations, such as combinations, alternating dewormers, and non-chemical means of control.

Endnotes

[a]LongRange™, Merial, Duluth, GA
[b]Dectomax® Injectable Solution, Zoetis, Florham, NJ
[c]Valbazen® Suspension, Zoetis, Florham, NJ
[d]Ivomec® Injectable Solution, Merial Limited, Duluth, GA
[e]Cydectin® Injectable Solution, Boehringer Ingelheim Animal Health, St. Joseph, MO
[f]SAS Institute, Cary, NC

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