The impact of controlled release capsules of monensin on postcalving haptoglobin concentrations in dairy cattle

Rex G. Crawford, Ken E. Leslie, Randal Bagg, C. Paul Dick, Todd F. Duffield

Abstract

This study was designed to assess the impact of a controlled release capsule (CRC) of monensin, administered prior to calving, on postcalving haptoglobin levels. The role of disease on haptoglobin levels was also studied. The study population consisted of 1010 cows from 25 Holstein dairy herds near Guelph, Ontario. Monensin CRC or placebo capsules were randomly assigned within each herd 3 wk prior to the expected calving date. Serum from week 1 and week 6 postcalving was submitted for quantification of haptoglobin concentrations. Haptoglobin results were analyzed for associations with treatment, health data, and individual cow factors up to 95 d in milk. Haptoglobin concentrations were higher in week 1 than week 6 ($P < 0.05$). In univariate analysis, several diseases were significantly associated with haptoglobin concentrations. However, occurrence of disease appeared to be a confounding factor in the data interpretation. Thus, the analysis was stratified by the presence or absence of disease. There appeared to be associations between factors other than clinical disease contributing to increased haptoglobin levels in both clinically healthy and unhealthy cattle. Haptoglobin served as a good indicator of inflammatory disease. Monensin CRC treatment was associated with increased haptoglobin concentrations in clinically unhealthy cattle, perhaps reflecting a better ability to respond to disease challenge. The lower haptoglobin concentrations in monensin CRC treated cattle that were clinically normal may be a reflection of reduced subclinical disease.

Résumé

Cette étude avait pour objectif d'évaluer l'impact de l'administration d'une capsule de monensin à libération contrôlée (CRC) durant la période pré-vêlage sur les niveaux d'haptoglobine en période post-vêlage. Le rôle de maladie sur les niveaux d'haptoglobine a également été examiné. La population étudiée était formée de 1010 vaches de race Holstein provenant de 25 troupeaux laitiers situés près de Guelph en Ontario. À l'intérieur de chacun des troupeaux des capsules de monensin CRC ou des placebos ont été distribuées de façon aléatoire 3 semaines avant la date prévue de vêlage. Des échantillons de sérum prélevés 1 et 6 semaines post-vêlage ont été analysés pour mesurer les concentrations d'haptoglobine. Les résultats des concentrations d'haptoglobine ont été analysés pour évaluer une association possible avec le traitement, l'état de santé et des facteurs liés à chaque animal jusqu'à 95 jours en lait. Les concentrations en haptoglobine étaient plus élevées à la première semaine qu'à la sixième semaine ($P < 0,05$). Par analyse univariée, une association significative a été trouvée entre les concentrations d'haptoglobine et plusieurs maladies. Toutefois, la fréquence de maladie semblait être un facteur confondant dans l'interprétation des résultats. Donc, l'analyse a été stratifiée selon la présence ou non de maladie. Il semble y avoir une association entre des facteurs de risque autres que les maladies cliniques qui contribuent à une augmentation des niveaux d'haptoglobine autant chez les animaux en santé que les malades. L'haptoglobine s'est avérée un bon indicateur de maladie inflammatoire. Un traitement au monensin CRC était associé avec une augmentation des concentrations d'haptoglobine chez des animaux cliniquement malades, démontrant peut-être une meilleure capacité à répondre à un processus pathologique. Les concentrations d'haptoglobine plus faibles chez les animaux cliniquement normaux traités au monensin CRC sont peut-être le reflet d'une maladie sous-clinique.

(Traduit par Docteur Serge Messier)

Introduction

Studies have shown that the controlled release capsules (CRC) of monensin (Rumensin; Elanco Animal Health, Guelph, Ontario) resulted in improved transition cow health (1,2) with a decreased incidence of subclinical ketosis (3). Immune function is thought to be impaired by subclinical ketosis (4). Thus it might be expected that monensin would have a positive influence on immune function in transition dairy cows. Stephenson et al (5) demonstrated that cows treated with monensin CRC had improved neutrophil function. Large-scale studies that investigate neutrophil or lymphocyte function are difficult to conduct because of labour requirements and the need for fresh blood samples.

Recently, there has been renewed interest in the use of acute phase proteins for the measurement of inflammation. In the bovine, haptoglobin appears to be one of the best indicators of acute phase proteins (6). Haptoglobin is a stable protein that survives freezing

Department of Population Medicine, University of Guelph, Guelph, Ontario N1G 2W1 (Crawford, Leslie, Duffield); Elanco, a division of Eli Lilly Inc., Research Park Centre, Guelph, Ontario N1G 4T2 (Bagg, Dick).

Address all correspondence and reprint requests to Dr. Todd Duffield; telephone: (519) 824-4120 ext. 54057; fax: (519) 763-8621; e-mail: tduffiel@uoguelph.ca

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and the automated assay is relatively inexpensive at CDN $6.50 per test (7). Haptoglobin is an acute phase protein synthesized in the liver in response to inflammation. It is partially responsible for sequestering hemoglobin, which serves as a source of iron for bacteria. Haptoglobin is recognized as a good indicator of acute infections in cattle (8). Elevated serum haptoglobin levels were found in cows diagnosed with severe mastitis, acute severe mastitis, and retained placenta. In addition, cows diagnosed with ketosis, chronic endometritis, and milk fever did not have an elevated serum haptoglobin concentration. A recently published study in feedlot calves indicated that haptoglobin was useful in the assessment of cattle that subsequently became ill with respiratory disease (9).

The objectives of this study were to assess the impact of monensin CRC on postpartum haptoglobin concentrations and to further investigate the effects on cattle with postpartum disease. It was hypothesized that haptoglobin concentrations might be lower in cattle treated with monensin because there would be a lower incidence of disease in cows with a higher energy status, but confounding could occur by a better immune response if treated cattle became sick.

Materials and methods

The study population consisted of 1010 cows from 25 Holstein dairy herds near Guelph, Ontario, and was conducted between 1995 and 1996. Herds were selected based on the willingness of owners to participate, proximity to Guelph, and the availability of Dairy Herd Improvement (DHI) records. Twenty-three of the 25 herds were clients of the Ruminant Field Service division of the Ontario Veterinary College. Herd size ranged from 25 to 160 lactating cows with rolling herd average milk production between 7000 and 10 000 kg/y (10). In the original trial, monensin CRC or placebo capsules were randomly assigned within each herd 3 wk prior to the expected calving date. Serum samples were obtained at enrolment and at weeks 1, 2, 3, 6, and 9 postcalving and were stored at −20°C. Serum from weeks 1 and 6 was submitted to the Animal Health Laboratory (AHL) at the University of Guelph for quantification of haptoglobin concentrations. The haptoglobin was analyzed using an automated serum analyzer (Hitachi 911; Roche Diagnostics, Indianapolis, Indiana, USA). The analyzer measures the hemoglobin binding capacity in g/L, using methemoglobin reagent made by the University of Guelph AHL according to the method described by Skinner and Roberts (11).

Because of prolonged freezer storage, 2 samples from each herd and sample day, for a total of 100 sera, were submitted for total protein analysis. These values were compared to original values conducted on fresh sera in 1995 and 1996, as an indicator of current sample quality. The mean total protein was 74.65 g/L for the 2003 samples and 72.97 g/L in 1995 and 1996. Total protein was 1.7 g/L higher in 2003 or 102% of the 1995 and 1996 levels, and were not significantly different. As such, sample quality was deemed appropriate.

Haptoglobin results were entered into a database (Access; Microsoft Corporation, Redmond, Washington, USA) that included cow, health, and treatment data. Haptoglobin levels, treatment group, parity, season calved, calving information (dystocia and twins), initial body condition score, and clinical disease in the first 95 d of lactation were included in the statistical analysis. Initial body condition score, collected at bolus administration approximately 3 wk prior to the expected calving date, was transformed to a categorical variable such that < 3.25 was considered thin, 3.25 to 3.75 fair, and > 3.75 fat. Data was exported to a data file (Excel; Microsoft Corporation) and imported into 2 computer programs (Statistix; Analytical Software, Tallahassee, Florida, USA, and SAS, version 8.0; SAS, Cary, North Carolina, USA) for statistical analysis.

Disease and cow level events were collected using on farm data collection sheets, veterinary records, and farm level computer systems. Removal was defined as cows that were sold or died within the first 95 d of lactation. Twins was defined as delivering 2 calves at parturition. Dystocia was defined as a calving requiring veterinary assistance. Retained placenta was defined as failure to pass fetal membranes with 24 h of calving. Milk fever was based on veterinary diagnosis of parturient hypocalcemia, but did not include cows treated by farm staff with calcium. Metritis was based on veterinary diagnosis of uterine inflammation occurring prior to 15 d in milk. Mastitis was defined as abnormal milk detected by milking staff at any point in the first 95 d in milk. Ketosis was based on veterinary diagnosis based on a reduced feed intake, testing positive on a milk ketone test, and the absence of any other disease. Displaced abomasum was a veterinary diagnosis of either a left or right displaced abomasum. Digestive disease was a veterinary diagnosis of a digestive disorder other than displaced abomasum or diarrhea. Respiratory disease was a veterinary diagnosis. Lameness was only recorded if the cow was treated for lameness or if swelling of a limb or joint was noted. Other disease was used to collect infrequent disease events including: udder edema, diarrhea, endocarditis, and traumatic reticuloperitonitis (1). For the purpose of this study, cattle were categorized as "diseased" or "non-diseased." Diseased cows had 1 or more of retained placenta, milk fever, metritis, mastitis, ketosis, displaced abomasum, digestive disease, respiratory disease, lameness, or other disease in the first 95 d of lactation. Cows without any of the above disease events during the first 95 d of lactation were categorized as non-diseased. Parity was divided into heifers (parity 1) and cows (parity 2 and greater).

Haptoglobin levels were transformed to the natural log of haptoglobin to normalize the data because of a right skew of the raw data. Variables were screened for association with haptoglobin levels using a one way simple analysis of variance (ANOVA). Models for haptoglobin concentration were then created for both the week 1 and week 6 samples using proc mixed (SAS). The approach was backward elimination using variables from the initial screen ($P < 0.25$) and forcing monensin CRC treatment as a fixed effect. Herd was also forced as a random effect to control for the common correlation of cows within the same herd to help account for management, feed, and environment. Interaction between treatment and all significant variables in the final models were assessed and data analysis was further stratified if any interaction terms were significant ($P < 0.10$). Because disease appeared to be a confounder of the data, analysis was performed with stratification on the basis of diseased versus non-diseased status.

Table I. Descriptive data on haptoglobin concentrations at weeks 1 and 6 stratified by parity and disease status (g/L)

Week	Disease status	Parity	*n*	Mean	Standard deviation	Minimum	Maximum
1	Non-diseased	Heifers	178	0.95	1.33	0	10.2
	Non-diseased	Cows	394	0.37	1.06	0	11.6
	Diseased	Heifers	54	1.23	1.29	0.04	6.8
	Diseased	Cows	271	1.05	1.48	0	13.3
6	Non-diseased	Heifers	176	0.20	0.21	0.04	1.55
	Non-diseased	Cows	378	0.20	0.29	0.01	2.81
	Diseased	Heifers	52	0.24	0.22	0.06	1.25
	Diseased	Cows	253	0.32	0.54	0.02	3.97

Table II. Week 1 model of factors associated with Log (haptoglobin) concentration in all cattle

Variable	Beta estimate	Standard error	*P*-value
Intercept	−2.3395	0.4071	0.0001
Twins	0.5775	0.2106	0.0062
Heifer	0.4473	0.1020	0.0001
Initial BCS	0.3414	0.1158	0.0033
Treatment	−0.06664	0.0084	0.4296
Removed	0.4554	0.1609	0.0048
Disease	0.4374	0.0926	0.0001
Random effect of herd	0.1251	0.0533	0.0094

BCS — Body condition score

$n = 884$

Results

Sera from 897 cows were available for analysis for week 1 and from 859 cows for week 6 postpartum. Cows that did not have haptoglobin analysis completed due to lack of sample or began the trial but were removed before week 1 were removed from the analysis. Cows that did not have a week 6 sample were excluded from week 6 analysis, including cows that were removed from the herd between weeks 1 and 6. The lack of sample was primarily caused by insufficient serum collection from some cows in the original sampling and no sample being frozen and available for haptoglobin analysis in 2003. The animals consisted of 26%, 28%, and 46% in parities 1 (heifers), 2, and greater (cows), respectively. There was a significant difference ($P < 0.0001$) in mean haptoglobin concentrations at week 1 versus week 6 (0.88 g/L and 0.24 g/L, respectively). Initial univariate data screening revealed that in week 1 parity (heifers versus cows $P = 0.0004$), season ($P = 0.0377$), disease ($P < 0.0001$), retained placenta ($P < 0.0001$), metritis ($P = 0.0016$), milk fever ($P = 0.0360$), mastitis ($P = 0.0365$), twins ($P = 0.0028$), and removal from the herd ($P = 0.0004$) were found to be significantly related to haptoglobin concentrations ($n = 897$). Univariate screening in week 6 indicated disease ($P = 0.0002$), metritis ($P = 0.0251$), mastitis ($P < 0.0001$), displaced abomasum ($P = 0.0050$), other disease ($P = 0.0088$), and removal from the herd ($P = 0.0004$) were significantly related to haptoglobin concentrations.

Table I illustrates descriptive data on haptoglobin concentrations for week 1 and week 6, stratified by parity and disease status. The final mixed procedure model for week 1 included disease, parity (heifer versus cow), body condition score, removal from the herd, twins, and treatment (Table II). Treatment was forced into the models, but the main effect was not significant. Because of the strong association between haptoglobin and disease, data analysis for week 1 ($P < 0.0001$) and week 6 ($P = 0.0002$) was stratified by postcalving disease status (diseased and non-diseased). Table III illustrates the final proc mixed model for non-diseased and diseased cattle in week 1. Because of the interaction between treatment and parity in week 1, the data was further stratified by parity as illustrated in Tables IV and V. The final model for week 6 included disease and removal (Table VI). Table VII illustrates the final models for week 6 for non-diseased and diseased cattle.

Discussion

There was clearly a significant decrease in haptoglobin concentrations from week 1 to week 6 postpartum. Parturition is generally an inflammatory event, as is the involution of the reproductive tract in preparation for rebreeding. It is, therefore, logical that mean haptoglobin levels would be higher at this time, even accounting for disease-related increases in haptoglobin, although our data does not preclude other sources of inflammation being the primary factor associated with increased haptoglobin levels in the 1st wk postcalving. The data available on dystocia in this data set was restricted to veterinary assisted dystocia with an incidence of 2.7%. Thus, there was limited power to assess the association between a difficult

Table III. Week 1 model of factors associated Log (haptoglobin) concentration in non-diseased and diseased cattle

Variable	Estimate	Standard error	*P*-value
Non-diseased[a]			
Intercept	−2.5527	0.5324	0.0001
Twins	1.0310	0.3880	0.0081
Initial BCS	0.3779	0.1534	0.0141
Heifer	0.7078	0.1722	0.0001
Treatment	0.0593	0.1286	0.6449
Heifer*treatment	−0.4071	0.2318	0.0796
Random effect of herd	0.1664	0.0734	0.0117
Diseased[b]			
Intercept	-0.0769	0.1234	0.0001
Retained placenta	0.5615	0.1611	0.0006
Treatment	−0.1934	0.1471	0.1895
Heifer	−0.0125	0.2472	0.9597
Removed	0.7451	0.2035	0.0003
Heifer*treatment	0.8345	0.3623	0.0220
Random effect of herd	0.0304	0.0445	0.2470

BCS — body condition score

[a] $n = 560$

[b] $n = 324$

Table IV. Week 1 least squares (LS) means illustrating the variables affecting haptoglobin concentrations (g/L) in diseased heifers and cows

Variable	LS mean	Standard error	*P*-value
Diseased heifers[a]			
RP — Yes	1.56	0.4053	0.0625
— No	0.82	0.2663	
Treatment — CRC	1.53	0.3352	0.0224
— Placebo	0.83	0.3146	
Removed — Yes	1.71	0.4540	0.0556
— No	0.75	0.2430	
Diseased cows[b]			
RP — Yes	1.10	0.1785	0.0017
— No	0.62	0.1216	
Treatment — CRC	0.75	0.1444	0.1982
— Placebo	0.91	0.1462	
Removed — Yes	1.23	0.2174	0.0005
— No	0.56	0.9519	

RP — retained placenta; CRC — controlled release capsule; Removed — removed from herd

[a] Controlled for random effect of herd ($P = 0.08$), $n = 54$

[b] Controlled for random effect of herd ($P = 0.4636$), $n = 270$

calving and presumed increased inflammation indicated by increased haptoglobin. Further investigation should consider using a system of defining and possibly categorizing dystocia. Uchida et al (12) report that experimentally induced fatty liver was associated with increased haptoglobin levels, so part of this increase 1 wk postpartum could be caused by negative energy balance and subclinical fatty liver. As part of this study indicators of negative energy balance or fatty liver were not evaluated, so this is only speculation. No work has been done examining haptoglobin levels relationship with non-estrified fatty acids (NEFAs), betahydroxybutyrate, or varying degrees of triglyceride infiltration of liver in field conditions.

The results in week 1 agreed with results from Skinner et al (8), such that mastitis, metritis, and retained placenta were associated with increased haptoglobin levels. However, the current study differed in that we found milk fever associated with a significant increase in haptoglobin levels. This finding is likely due to the effect of hypocalcemia as a risk factor for other diseases, or the impact of parity on both haptoglobin levels and hypocalcemia. Milk fever was

Table V. Week 1 least squares (LS) means illustrating the variables affecting haptoglobin concentrations in non-diseased heifers and cows

Variable	LS mean	Standard error	*P*-value
Heifers[a]			
Treatment — CRC	0.75	0.6135	0.0697
— Placebo	1.05	0.6268	
Twins — Yes	1.60	1.2020	0.3281
— No	0.49	0.1478	
Cows[b]			
Treatment — CRC	0.50	0.2369	0.7001
— Placebo	0.47	0.2362	
Twins — Yes	0.81	0.4219	0.0149
— No	0.29	0.1138	

CRC — controlled release capsule

[a] Controlled for effect of BCS ($P = 0.1559$) and random effect of herd ($P = 0.1099$), $n = 173$

[b] Controlled for effect of BCS ($P = 0.0538$) and random effect of herd ($P = 0.0123$), $n = 387$

Table VI. Week 6 model of factors associated with Log (haptoglobin) concentration in all cattle

Variable	Beta estimate	Standard error	*P*-value
Intercept	−1.8956	0.0464	0.001
Disease	0.1784	0.0517	0.0006
Removed from herd	0.4651	0.1380	0.0008
Treatment	−0.0357	0.0484	0.4606
Random effect of herd	0.0122	0.0086	0.0774

$n = 859$

quickly eliminated as an independent factor when all significant diseases were modeled together.

There were temporal associations detected between disease and haptoglobin concentrations in the current data. Retained placenta had an effect on haptoglobin in week 1 but not week 6, and displaced abomasums had an effect in week 6 but not in week 1. Since median days for diagnosis of displaced abomasum was 11.5 (1), it is possible that the associations in week 6 were a result of inflammation resulting from surgical correction. Metritis had a direct effect in week 1, but the effect lingered to week 6, presumably in relation to continued uterine inflammation. The category of other disease was significant in week 6, presumably due to a higher incidence of other diseases further from calving. Mastitis at any point during the lactation caused significant increases in haptoglobin at both 1 and 6 wk postpartum. The duration of increased haptoglobin concentrations both before and after clinical mastitis has not been studied. Thus, it is difficult to speculate on temporal relations between haptoglobin and clinical mastitis.

Factors that increase inflammation around the time of calving also significantly increased haptoglobin concentrations. Parity was significantly associated with haptoglobin levels; heifers having higher mean haptoglobin than mature cows in week 1 (1.02 g/L versus 0.83 g/L). Twin birth was also associated with increased haptoglobin. Removal from the herd was significantly associated with higher haptoglobin, indicating that inflammatory disease was a risk factor for removal.

Haptoglobin appears to be involved in many physiologic mechanisms in cattle. It is known to increase in inflammation caused by irritants (6), as well as clinical disease known to cause inflammation (8). In feedlot calves, large numbers of calves have detectable or increased haptoglobin levels, although not all become clinically ill (13). The effect of subclinical disease on haptoglobin levels is very difficult to study, as is the duration of haptoglobin increase prior to clinical disease onset. Using haptoglobin levels as a prognostic indicator has been advocated by some authors (9), while others have difficulty showing a relationship between higher haptoglobin levels and poor prognosis (13). Data in the current study indicate that cows that were culled had significantly higher haptoglobin levels, but the study was not designed to interpret with certainty that the event causing increased haptoglobin levels was associated directly with being culled.

The relationship between glucocorticoids, stress, and fatty liver with haptoglobin is also not entirely understood. Dexamethasone administration plus fasting has been used to induce fatty liver in cattle, which subsequently had increased haptoglobin levels (14). The relationship of decreased feed intake, higher NEFAs, increased cortisol levels, uterine inflammation, and increased haptoglobin concentrations at parturition was identified by Uchida et al (15), but the interaction between different mechanisms was not determined. Uchida et al (12) determined that haptoglobin concentrations increase in fatty liver although the role of glucocorticoids is not certain. The role of fatty liver in haptoglobin production offers a possible

Table VII. Week 6 model of factors associated with Log (haptoglobin) concentration in non-diseased and diseased cattle

Variable	Beta estimate	Standard error	*P*-value
Non-diseased[a]			
Intercept	−1.8519	0.04605	0.0001
Treatment	−0.09987	0.05396	0.0647
Random effect of herd	0.01244	0.009223	0.0887
Diseased[b]			
Intercept	−2.8159	0.4008	0.0001
Treatment	0.1084	0.0928	0.2441
Mastitis	0.2797	0.0957	0.0038
Displaced abomasum	0.3255	0.1628	0.0465
Removed from herd	0.7537	0.2100	0.0004
BCS	0.2469	0.1142	0.0315
Random effect of herd	0.02323	0.0279	0.2022

BCS — body condition score

[a] $n = 554$

[b] $n = 305$

explanation for the non-diseased monensin treated heifers in week 1 having significantly lower haptoglobin levels than controls, as decreased negative energy balance, the primary trigger of fatty liver, is well established in monensin treated cattle. However, reduced occurrence of subclinical disease is an alternative reason for the trend to lower haptoglobin levels in treated non-diseased cattle found in week 6, as well as the results in week 1. The data becomes difficult to interpret with haptoglobin levels going in opposite directions depending on disease status among the treated heifers. The results are not unexpected, however, especially due to the lack of research into optimal haptoglobin responses and the association between immune suppression and haptoglobin response.

Murata and Miyamoto (16) showed impairment of lymphocyte blastogenesis in calves with increased haptoglobin levels following transportation. This work suggests that at some cutpoint, increased haptoglobin levels due to non-inflammatory triggers could cause immunosuppression. Immunosuppression caused by excessively high haptoglobin levels could explain the high haptoglobin levels associated with cows that were culled during the current trial.

No work has been done to establish what an ideal haptoglobin response to inflammation should be. We suggest that a smaller haptoglobin increase to a similar inflammatory event might indicate a suboptimal response and, therefore, reduced immune function. The literature does not offer evidence to either support or refute our suggestion that diseased heifers treated with monensin benefited from a higher haptoglobin response to disease in week 1, and could, therefore, be considered more immunocompetent than the control heifers. Haptoglobin levels were higher for heifers than cows in week 1 regardless of disease status and non-diseased heifers had similar levels to diseased cows. It is possible that treatment with monensin allowed diseased heifers to mount a haptoglobin response to disease over and above their baseline inflammation and that control heifers did not have the excess energy or immune function to respond in a similar manner. Alternatively, it is possible that the greater glucose supplied precalving by the monensin CRC treatment caused an elevated inflammatory response at calving because of slightly larger calves. However in our data, there was no difference in dystocias (1).

In conclusion, monensin CRC was associated with an increase in haptoglobin levels in diseased heifers at week 1, a decrease in haptoglobin levels in non-diseased heifers in week 1, and a trend toward decreased haptoglobin levels in non-diseased cattle in week 6. The dataset available does not allow for proof of our assertion that treatment allowed diseased heifers to respond more optimally to disease with higher haptoglobin increases. Subclinical disease is difficult if not impossible to measure, and is the suspected, although not proven, reason for decreased haptoglobin levels in non-diseased treated animals. The authors welcome further investigation to determine the optimal haptoglobin response and any association that energy balance may have on haptoglobin levels.

Further investigation is required to better understand the temporal changes in haptoglobin levels in different disease situations. Studying duration of haptoglobin increases and further examination of cows that have unexplained haptoglobin responses could lead to a better understanding of the potential relationships between immune function, haptoglobin concentrations, and disease.

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