

# Research Note: Evaluation of quinine as a chemoprophylactic candidate against histomoniasis in turkeys

L. C. Beer, B. M. Hargis, and C. N. Vuong<sup>1</sup>

Division of Agriculture, Poultry Science Department, University of Arkansas, Fayetteville, AR 72701, USA

**ABSTRACT** Histomoniasis, also commonly referred to as blackhead disease, is caused by the protozoan parasite *Histomonas meleagridis*. Since the removal of nitarsone in 2015, no approved prophylactics are available for mitigating histomoniasis. Disease incidence and high mortalities are frequently associated with turkey flocks, although infection of broiler breeders also occurs. Quinine is a naturally occurring alkaloid with antimalarial properties. *In vitro* assays have shown strong antihistomonal properties of quinine, leading to our hypothesis that quinine inclusion within the feed could prevent histomoniasis in turkeys. Selected concentrations of quinine were included within a turkey starter diet to evaluate effects on body weight gain (**BWG**), liver lesions, cecal lesions, and mortality of *H. meleagridis*-challenged turkeys. On day-of-hatch, poults were randomly assigned to either the basal diet or a quinine diet. Groups consisted of a non-challenged control (**NC**; basal diet), 0.022% quinine + challenge, 0.067% quinine + challenge, 0.2% quinine + challenge, or a positive-challenged control (**PC**; basal diet). On d 10,

challenged groups were intracloacally inoculated with  $10^5$  *H. meleagridis* cells/turkey, and lesions were evaluated on d 21 post-infection. Individual body weights were recorded on d 0, d 10, and d 31 to calculate the pre-challenge and post-challenge BWG. No significant differences ( $P > 0.05$ ) were observed between the d 0 to 10 pre-challenged BWG between quinine treatment diets and the basal diet. Similarly, no differences ( $P > 0.05$ ) were observed in post-challenge d10-31 BWG of the quinine dietary treatments as compared to the PC. Cumulative mortalities, liver lesions, and cecal lesions related to histomoniasis were not reduced ( $P > 0.05$ ) in any of the quinine treatment groups as compared to the PC. Although quinine successfully reduced *H. meleagridis* cells *in vitro*, results from the *in vivo* experiment indicated no reduction in histomoniasis severity as evidenced by similar lesions and mortality as the PC. Taken together, these data indicate that quinine inclusion within the feed at these concentrations and under these experimental conditions was not efficacious in the prevention or treatment of histomoniasis.

**Key words:** histomoniasis, *Histomonas meleagridis*, quinine, turkey, blackhead

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## INTRODUCTION

*Histomonas meleagridis*, the etiological agent of histomoniasis, is a protozoan disease primarily affecting turkeys and commonly resulting in high mortalities with no prophylactic drugs commercially available to mitigate outbreaks (Liebhart et al., 2017). Quinine is a naturally occurring cinchona alkaloid that has previously been shown to impair carbohydrate metabolism of the parasite *Plasmodium gallinaceum*, etiological agent of malaria in poultry, both *in vitro* and *in vivo* (Moulder, 1948). Following an intravenous injection, chickens exhibited quinine-free blood within 4 to 5 h;

however, the inhibitory effects of quinine incurred by *P. gallinaceum* lasted for 24 h postinjection (Moulder, 1948). This continued inhibition of the parasite's carbohydrate metabolism following blood clearance of quinine would further suggest that quinine acts directly upon *P. gallinaceum*, causing irreversible inhibition to the parasite rather than indirectly impacting phagocytic mechanisms (Moulder, 1948). Dietary concentrations of 0.5 or 1% quinine sulfate have resulted in decreased feed intake and diet rejection when chicks were allowed free access of 2 dietary choices of quinine-supplemented diet or basal diet (Ueda et al., 2002). Previous studies suggest that chicks are able to perceive the bitter taste of quinine similar to human perception, resulting in the decreased feed intake, especially at dietary concentrations higher than 0.2% quinine (Ueda and Kaidou, 2005). Feed intake of chicks was not significantly reduced when chicks were supplied with a 0.1% quinine diet (Ueda et al., 2002).

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<sup>1</sup>Corresponding author: [vuong@uark.edu](mailto:vuong@uark.edu)

Tyzzler (1923) evaluated large unspecified doses of quinine HCl injected intramuscularly or intravenously into turkeys but observed no mitigation of histomoniasis. No further evaluations of quinine against histomoniasis appear to have been conducted despite this alkaloid's marked antimalarial effect which we hypothesized could transfer antihistomonal properties to turkeys. Studies in chickens indicate taste aversion at high quinine concentrations, but the maximum tolerance in turkeys has not been established (Ueda et al., 2002; Ueda and Kaidou, 2005). *H. meleagridis* adapts to flagellated or amoeboid form depending on cecal lumen location or tissue invasion; therefore, quinine could potentially impair *Histomonas* metabolism before the parasite enters the liver via the hepatic portal vein following cecal degradation and translocation. As a chemoprophylactic compound, quinine is rapidly cleared in chickens with accompanying strong antimalarial properties; therefore, quinine should be further evaluated against other protozoal diseases, such as histomoniasis. The purpose of the present study was to evaluate quinine as a chemoprophylactic administered in feed against histomoniasis in turkeys at specified dietary concentrations.

## MATERIALS AND METHODS

### *Histomonas* Isolates and Culture

Isolates of *H. meleagridis* were obtained from field outbreaks in the southern United States (Buford, Georgia; **Bu** strain; isolated from infected chickens) and Northwest Arkansas (**PHL2017** strain; isolated from infected turkeys). Histomonads were grown according to previously described methods (van der Heijden and Landman, 2007; Beer et al., 2020). In brief, Modified Dwyer's Media (**MDM**) was comprised of Medium 199 (Product #12-118F, Lonza, Basel, Switzerland) supplemented with 10% heat-inactivated horse serum (Product #26050-088, Gibco, Life Technologies Corporation, Waltham, MA) and 1.6 mg/mL white rice flour (Arrowhead Mills, Boulder, CO) with an undefined bacterial population. Subculture occurred every 48 to 72 h into 25cm<sup>2</sup> tissue culture flasks (Product #10062-874, VWR International, Radnor, PA) containing fresh, supplemented MDM. Incubation occurred anaerobically at 40°C.

### *In Vitro* Assessment of Quinine

Three *in vitro* assays per *H. meleagridis* strain (Bu and PHL2017) were independently completed to evaluate selected concentrations of food-grade quinine HCl dihydrate (Product #W297607; Sigma-Aldrich, St. Louis, MO) on histomonad viability. Histomonads were revived from aliquots and propagated as described above with passages occurring fewer than ten times prior to each assay. Each tube contained a ratio of 250  $\mu$ L histomonads + 50  $\mu$ L of treatment + 700  $\mu$ L of MDM into sterile microcentrifuge snap-cap tubes (Product #20170-333; VWR). Initial histomonad seeding density

for each treatment was  $1.5 \times 10^5$  cells/tube. Treatments included the negative treatment control or final concentrations of either 0.022, 0.067, or 0.2% quinine reconstituted in sterile H<sub>2</sub>O. Each treatment was performed in triplicate. Incubation occurred at 40°C under anaerobic conditions for 20 h. Viable histomonads/mL were enumerated with a hemocytometer using Trypan blue dye exclusion (Product #15250-061, Gibco). A series of 10-fold dilutions of each treatment were subsequently plated on tryptic soy agar (**TSA**, Product #211822, Becton Dickinson, Sparks, MD) at 0 h and 20 h time-points to enumerate bacterial colony forming units (**CFU**). Plates were incubated anaerobically at 40°C. Additional assay treatment controls for bacterial enumeration included MDM control or final concentrations of either 0.022, 0.067, or 0.2% quinine in MDM to ensure no bacteria were introduced other than the deliberate seeding of bacteria from the *Histomonas* culture in treatment groups.

### Animal Trial and Diet

On day-of-hatch, a total of 200 female turkey poults were obtained from a local commercial hatchery, wing-tagged, and randomly allocated to battery cages at the University of Arkansas Poultry Health Laboratory. All animal handling procedures were in compliance with regulations of the University of Arkansas Institutional Animal Care and Use Committee (IACUC protocol #21094). A corn soy-based starter feed meeting NRC requirements (1994) and water were provided *ad libitum*. Quinine was incorporated into the basal diet at concentrations of 0.022, 0.067, or 0.2%. Groups consisted of a non-challenged control (**NC**; basal diet), 0.022% quinine + challenge, 0.067% quinine + challenge, 0.2% quinine + challenge, or a positive-challenged control (**PC**; basal diet). From d 0 to d 13, each group was allocated to 4 replicate cages of 10 turkeys. From d 14 to d 31, NC and PC groups each consisted of 8 replicate cages of 4 turkeys, while quinine feed treatment groups each consisted of 6 replicate cages of 4 turkeys.

### *H. Meleagridis* Challenge

The PHL2017 strain of *H. meleagridis* was selected to be utilized for experimental challenge. Viable histomonads/mL were enumerated with a hemocytometer as described above, and dilutions for the challenge inoculum were prepared with fresh MDM. On d 10, each poult in a challenged group received a total of  $10^5$  histomonads administered intracloacally with an animal gavage needle. The NC group received a sham-inoculation consisting of MDM.

### Lesion Scores and Body Weight Gain

All poults were individually weighed on d 0, 10, and 31 for calculation of pre-challenge and post-challenge body weight gain (**BWG**). Liver and cecal lesions were

recorded from all mortalities following challenge. On d 31, all remaining poultts were humanely euthanized and lesion-scored according to previously established methods (Beer et al., 2020). In brief, liver and cecal lesions were scored separately on a scale of “0” to “3” where a score of “0” indicates a healthy organ; “1” indicates the beginning of detectible lesions; “2” indicates intermediate histomoniasis lesions; and “3” indicates classical histomoniasis lesions. Individuals determining lesion scores were blinded to treatment groups.

### Statistical Analysis

Cell viability, bacterial growth, and BWG data were analyzed using JMP Pro 15 software (SAS Institute Inc., Cary, NC) with significant differences between treatment groups determined using ANOVA. Where applicable, means were further separated using Tukey’s multiple range test. Mortalities related to histomoniasis were analyzed using a chi-square test. Lesion score data were analyzed using the Proc Mixed Procedure in SAS 9.4 software with significance between mean lesion score values considered in comparison to the PC group. Using a chi-square test, each subgrouping of scores (“0” to “3”) were further compared between quinine feed treatment groups and the PC. Significance for all statistical analyses was set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### In Vitro Cell Viability Assessment

Addition of 0.2% quinine to *in vitro* cultures of Bu strain and PHL2017 strain significantly reduced ( $P < 0.05$ ) the growth of histomonads after 20 h of incubation as compared to the negative control (Table 1). The 0.067% quinine treatment significantly reduced ( $P < 0.05$ ) histomonads in all PHL2017 strain assays and in 2 of the Bu strain assays. The 0.022% quinine only reduced ( $P < 0.05$ ) histomonads in the third Bu strain assay, with no significant reduction of histomonads ( $P > 0.05$ ) in any other assays. Following 20 h of incubation, the 0.2% quinine significantly reduced ( $P < 0.05$ ) recoverable bacterial CFU/mL in all assays as compared to the negative control. Recoverable bacterial CFU/mL was significantly reduced ( $P < 0.05$ ) in the 0.067% quinine group for one Bu strain assay and two PHL2017 strain assays as compared to the negative control. There was no reduction ( $P > 0.05$ ) in recoverable bacterial CFU/mL in the 0.022% quinine group as compared to the negative control. These data suggest that the antihistomonal impact of quinine is not contributed solely to destruction of accompanying bacteria which is well known to impair *H. meleagridis* growth *in vitro* (Lesser, 1964).

### Performance, Mortalities, and Lesions

No significant differences ( $P > 0.05$ ) were observed in pre-challenge BWG between quinine treatments and the

**Table 1.** *In vitro* viability assays evaluating selected concentrations of food-grade quinine for antihistomonal properties against two different strains of *Histomonas meleagridis*.<sup>1,2</sup>

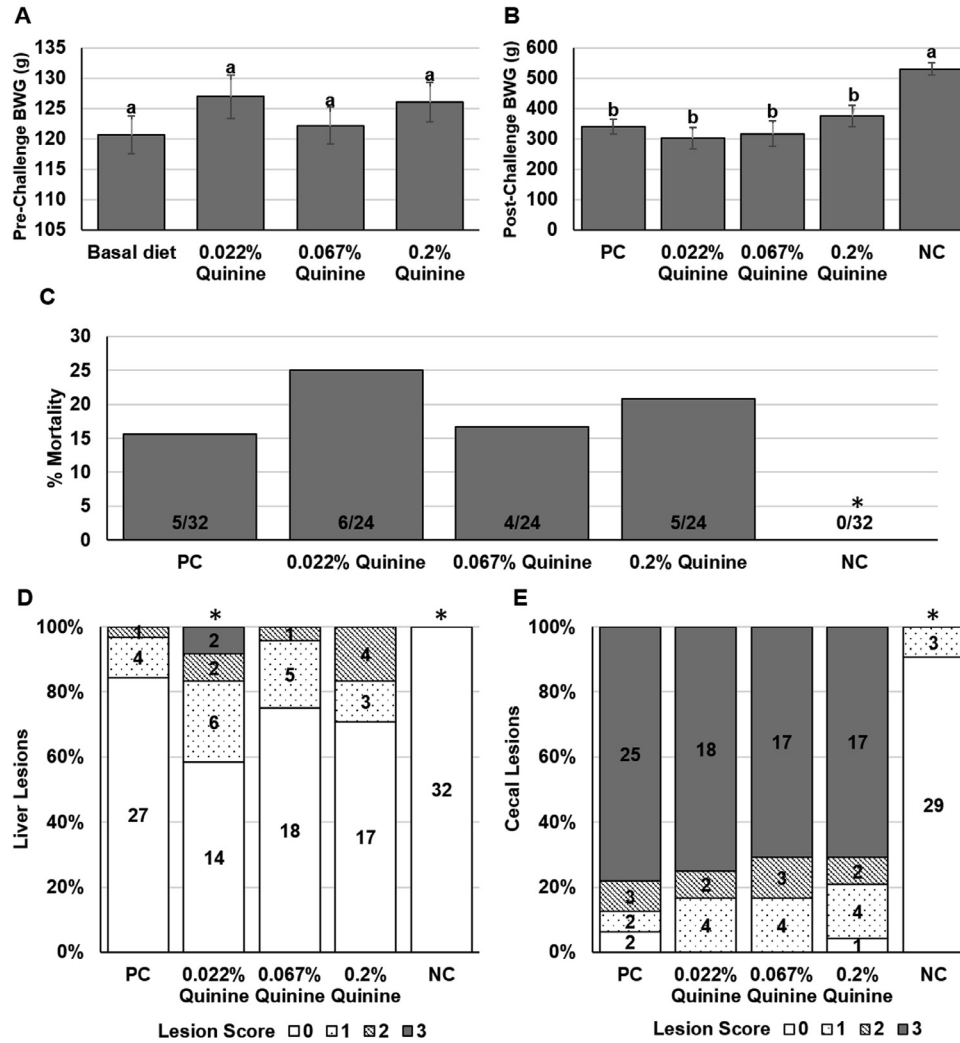
Treatment	Viability		
	<i>Histomonas</i> cells/mL (Log <sub>10</sub> )	Bacterial CFU/mL (Log <sub>10</sub> )	
Buford strain	20 h	0 h	20 h
Assay 1			
Negative control	5.44 ± 0.02 <sup>a</sup>	7.69 ± 0.12 <sup>a</sup>	8.72 ± 0.21 <sup>a</sup>
0.022% Quinine	5.24 ± 0.05 <sup>a</sup>	7.52 ± 0.14 <sup>a</sup>	8.65 ± 0.09 <sup>a</sup>
0.067% Quinine	4.13 ± 0.09 <sup>ab</sup>	7.46 ± 0.24 <sup>a</sup>	8.10 ± 0.10 <sup>b</sup>
0.2% Quinine	1.45 ± 1.45 <sup>b</sup>	7.46 ± 0.09 <sup>a</sup>	6.52 ± 0.04 <sup>c</sup>
Assay 2			
Negative control	5.30 ± 0.05 <sup>a</sup>	7.64 ± 0.11 <sup>b</sup>	8.37 ± 0.11 <sup>a</sup>
0.022% Quinine	5.19 ± 0.08 <sup>a</sup>	7.93 ± 0.02 <sup>a</sup>	8.20 ± 0.20 <sup>a</sup>
0.067% Quinine	4.57 ± 0.10 <sup>b</sup>	7.83 ± 0.12 <sup>ab</sup>	8.07 ± 0.07 <sup>a</sup>
0.2% Quinine	0.00 ± 0.00 <sup>c</sup>	7.95 ± 0.03 <sup>a</sup>	6.79 ± 0.26 <sup>b</sup>
Assay 3			
Negative control	5.29 ± 0.05 <sup>a</sup>	7.77 ± 0.12 <sup>a</sup>	8.43 ± 0.13 <sup>a</sup>
0.022% Quinine	4.91 ± 0.04 <sup>b</sup>	7.78 ± 0.04 <sup>a</sup>	8.16 ± 0.16 <sup>a</sup>
0.067% Quinine	3.45 ± 0.10 <sup>c</sup>	7.66 ± 0.06 <sup>a</sup>	8.40 ± 0.10 <sup>a</sup>
0.2% Quinine	0.00 ± 0.00 <sup>d</sup>	7.66 ± 0.12 <sup>a</sup>	6.30 ± 0.00 <sup>b</sup>
PHL2017 strain			
Assay 1			
Negative control	5.36 ± 0.05 <sup>a</sup>	8.27 ± 0.20 <sup>a</sup>	8.60 ± 0.00 <sup>a</sup>
0.022% Quinine	5.14 ± 0.06 <sup>a</sup>	7.91 ± 0.07 <sup>ab</sup>	8.83 ± 0.02 <sup>a</sup>
0.067% Quinine	4.08 ± 0.09 <sup>b</sup>	7.96 ± 0.06 <sup>ab</sup>	8.06 ± 0.06 <sup>b</sup>
0.2% Quinine	0.00 ± 0.00 <sup>c</sup>	7.87 ± 0.02 <sup>b</sup>	6.33 ± 0.20 <sup>c</sup>
Assay 2			
Negative control	5.38 ± 0.05 <sup>a</sup>	8.46 ± 0.09 <sup>a</sup>	8.77 ± 0.08 <sup>a</sup>
0.022% Quinine	5.39 ± 0.04 <sup>a</sup>	7.97 ± 0.03 <sup>b</sup>	8.94 ± 0.07 <sup>a</sup>
0.067% Quinine	4.79 ± 0.06 <sup>b</sup>	8.04 ± 0.15 <sup>ab</sup>	8.18 ± 0.09 <sup>b</sup>
0.2% Quinine	0.00 ± 0.00 <sup>c</sup>	8.05 ± 0.18 <sup>ab</sup>	6.20 ± 0.20 <sup>c</sup>
Assay 3			
Negative control	5.46 ± 0.01 <sup>a</sup>	7.40 ± 0.20 <sup>a</sup>	8.77 ± 0.07 <sup>a</sup>
0.022% Quinine	5.28 ± 0.03 <sup>a</sup>	7.73 ± 0.19 <sup>a</sup>	8.70 ± 0.10 <sup>a</sup>
0.067% Quinine	2.39 ± 1.20 <sup>b</sup>	7.46 ± 0.16 <sup>a</sup>	8.48 ± 0.18 <sup>a</sup>
0.2% Quinine	0.00 ± 0.00 <sup>b</sup>	7.58 ± 0.19 <sup>a</sup>	6.54 ± 0.16 <sup>b</sup>

<sup>a-d</sup>Data expressed as mean ± SEM. Statistical evaluation using ANOVA followed by post hoc Tukey’s range test. No common superscripts within a column indicate means differ significantly ( $P \leq 0.05$ ).

<sup>1</sup>Quinine HCl dihydrate (Product #W297607, Sigma-Aldrich, St. Louis, MO) utilized in *in vitro* assays; n = 3 replicates/treatment.

<sup>2</sup>Assay seeding density was 5.18 *H. meleagridis* cells/mL (Log<sub>10</sub>). Media controls were included to verify no initial bacterial contamination was introduced.

basal diet from d 0 to d 10 of age (Figure 1A). Previous research indicates that chicks can perceive the bitter taste of quinine, resulting in decreased feed intake when dietary concentrations are higher than 0.2% (Ueda and Kaidou, 2005). Similarly, in this current study, the comparable BWG between groups suggests that turkeys did not have an aversion to the highest feed inclusion rate of 0.2% quinine. The post-challenge BWG from d 10 to d 31 of age in quinine feed treatments was not significantly different ( $P > 0.05$ ) as compared to the PC group (Figure 1B). The PC group and all quinine feed treatments resulted in lower post-challenge BWG ( $P < 0.05$ ) than the NC group. Taken together, these data indicate that quinine inclusion in the feed at concentrations as high as 0.2% was not detrimental to pre-challenge BWG, suggesting that turkeys do not have taste aversion to this cinchona alkaloid at these tested levels. Levels higher than 0.2% quinine are known to reduce feed intake in chickens, but the specific threshold for dietary quinine inclusion has not been evaluated in turkeys (Ueda and Kaidou, 2005). Given this information, a



**Figure 1.** Effect of quinine on body weight gain (BWG) at (A) pre-challenge from d 0 to d 10 and (B) post-challenge from d 10 to d 31. BWG data expressed as mean  $\pm$  SEM and analyzed using ANOVA in JMP Pro 15. Different superscripts denote significance ( $P < 0.05$ ). (C) Percentage of mortalities associated with histomoniasis. No difference was detected between quinine treatments as compared to the positive-challenged control (PC) with a chi-square test. Cumulative lesion scores associated with histomoniasis from d 9 to d 21 post-challenge for (D) liver and (E) caecae. A lesion score of “0” indicates a healthy organ whereas a score of “3” indicates severe histomoniasis. Numbers within columns indicate the number of turkeys per evaluated lesion score. Statistical difference was detected by SAS Proc Mixed Procedure between mean lesion scores as compared to the PC ( $*P < 0.05$ ). PC and quinine groups were intracloacally challenged with  $10^5$  histomonads/turkey of *Histomonas meleagridis* (PHL2017 strain) on d 10. Negative control (NC) received sham challenge. Quinine obtained as quinine HCl dihydrate (Product #W297607; Sigma-Aldrich, St. Louis, MO).

higher inclusion rate of quinine could potentially be included within the feed but the impact on feed intake, growth, and histomoniasis severity in turkeys is unknown until further tests are conducted.

None of the quinine feed treatments effectively reduced ( $P > 0.05$ ) mortalities related to histomoniasis when compared to the PC group (Figure 1C). Moreover, there was no reduction ( $P > 0.05$ ) in liver or cecal lesions in the quinine feed treatments, regardless of inclusion, as compared to the PC group (Figure 1D,E). Interestingly, the 0.022% quinine feed treatment actually resulted in a higher average liver lesion as compared to the PC group ( $P < 0.05$ ). Although quinine exhibited antihistomonal activity *in vitro* against 2 different *Histomonas* strains, quinine was not effective for preventing histomoniasis when evaluated *in vivo* at the selected concentrations and experimental conditions, which is consistent with

previous research evaluating antihistomonal candidates (Thøfner et al., 2012).

Interestingly, *H. meleagridis* exhibits a flagellate nature rather than a solely amoebic form, which could be a possible reason antimalarial drugs, such as quinine, are ineffective at reducing severity of histomoniasis (Tyzzer and Fabyan, 1922; Tyzzer 1923). Moulder (1948) showed that intravenous injection of 20 mg/kg BW to *P. gallinaceum*-infected chickens resulted in parasite metabolism changes including: increased rate of glucose use, decreased rate of pyruvate use, lowered ratio of oxygen to glucose, and lowered oxygen uptake, presumably due to aerobic phase inhibition by quinine via the pyruvate oxidation step in the TCA cycle. Although *H. meleagridis* can adopt either a flagellated or amoeboid form, this parasite is cultured *in vitro* under anaerobic conditions. The



primary mode of action of quinine against *P. gallinaceum* appears to be via inhibition of the parasite's aerobic pathway, which could explain the lack of ameliorative effects when provided to *H. meleagridis*-infected turkeys. Further research with quinine as an antihistomonal would be discouraged unless delivery to the cecae could be ensured and mode of action further elucidated. This research note emphasizes the importance of *in vivo* evaluation for verifying *in vitro* results and emphasizes that dietary inclusion of quinine at the levels evaluated in this study were not efficacious for preventing histomoniasis in turkeys.

## DISCLOSURES

The authors have no conflicts of interest to report.

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