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Evaluation of the speed of kill, effects on reproduction, and effectiveness in a simulated infested-home environment of sarolaner (Simparica™) against fleas on dogs

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ABSTRACT

Four studies were conducted to evaluate the speed of kill, effect on egg production, and efficacy in a simulated infested-home environment of a novel isoxazoline, sarolaner (Simparica™, Zoetis), against fleas on dogs. Individually identified and housed, purpose-bred Beagles were used in each study and were allocated randomly to groups based on pretreatment parasite counts. In two speed of kill studies, groups of dogs infested with 100 fleas prior to treatment were treated orally with placebo or sarolaner tablets providing the minimum dose of 2 mg/kg and then re-infested with fleas weekly for five weeks post-treatment. Comb counts were conducted to determine the numbers of viable fleas at one to three, four, eight and 12 h after treatment and each subsequent infestation. In the egg production study, sarolaner- and placebo-treated dogs were similarly challenged with fleas and at 48 h after each infestation the dogs were housed for 20 h in cages allowing the collection and counting of all flea eggs produced during this period. Collected eggs were incubated to evaluate hatch and development to adults. The last study used dogs housed in a flea-infested simulated-home environment. Dogs were allocated to treatment with either placebo or sarolaner tablets providing a dose of 2 mg/kg once a month for three treatments. Flea infestations were assessed by comb counts (fleas were replaced on the dogs) on Days 14, 30, 44, 60, 74 and 90.

The speed of kill studies demonstrated that a single 2 mg/kg oral dose of sarolaner started killing fleas within three to four hours after treatment or subsequent re-infestations for up to a month, and achieved ≥98% control of fleas by eight hours after treatment or re-infestation for 28 days. In the study to assess effects on flea reproduction, a single oral treatment of sarolaner resulted in the complete cessation of egg-laying for 35 days. This rapid kill of fleas and inhibition of reproduction were confirmed in a simulated-home environment where the existing infestations were reduced by >95% within two weeks of the first treatment and eliminated from the dogs after two monthly doses.

1. Introduction

The cat flea, *Ctenocephalides felis felis,* is the major ectoparasite of companion animals and is found worldwide (Rust and Dryden, 1997). Adult fleas are the only life stage commonly found on the host; eggs fall from the pelage and the larvae develop in the environment, feeding mainly on the dried blood in adult flea feces (Dryden, 1989; Krämer and Menke, 2001). Adult fleas acquire a host, mate and about 24 h after the first blood meal, females start laying eggs with each female flea on the animal producing up to 40–50 eggs/day (Rust and Dryden, 1997). Effective flea control is dependent upon elimination of fleas from the animal and its environment. This can be achieved by combining an adulticide with compounds such as the insect growth regulators, lufenuron, pyriproxyfen and methoprene that disrupt the development of eggs and larvae (Dryden and Broce, 2002; Chin et al., 2005). In fact,

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effective control of infestations can be achieved with the use of a compound such as lufenuron alone, though the animal is still at risk of re-infestation from an external source. Alternatively, insecticides that have a rapid onset of activity can also disrupt the flea life cycle by killing adult fleas before they can lay eggs, thus reducing the environmental infestation level (Jacobs et al., 2001). For an ectoparasiticide to prevent egg production, residual activity must be sufficient to kill newly acquired fleas within 24 h, or produce sufficient toxicity to stop blood feeding and therefore egg production (Dryden et al., 2007).

Sarolaner is a new isoxazoline ectoparasiticide (McTier et al., 2016) with excellent efficacy (>99%) against fleas on dogs for 35 days following oral administration (Six et al., 2016). Here we report studies with an oral chewable formulation of sarolaner (Simparica™, Zoetis) evaluating speed of kill against existing flea infestations and post-treatment challenges, effects on flea reproduction and efficacy in a flea-infested, simulated home environment.

2. Materials and methods

The studies were conducted in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats (Marchiondo et al., 2013) and complied with Good Clinical Practices, (VICH guideline GL9) (EMEA, 2000). Study protocols were reviewed and approved by the local and/or Zoetis Institutional Animal Care and Use Committee. All dogs used in these studies had not been treated with an ectoparasiticide for at least 60 days, demonstrated good flea retention prior to treatment and were in good health at enrollment. Dogs were housed individually in enclosures that conformed to accepted animal welfare guidelines and ensured no direct contact between dogs. Masking of all studies was assured through the separation of functions. All personnel conducting observations or animal care, or performing flea infestations and counts were masked to treatment allocation.

Two studies were conducted to evaluate the speed of kill of sarolaner against existing infestations and re-infestations of cat fleas on dogs. The efficacy of the minimum dose of 2 mg sarolaner/kg bodyweight against fleas at 1, 2, 3, 4, 8, and 12 h after a single oral dose and weekly flea challenges up to 35 days was determined. Study 1 was conducted in Ireland and Study 2 in California, USA. Study 3 was an evaluation of the effects of treatment on flea reproduction conducted in California, USA, and Study 4 was a simulated flea-infested environment study performed in Texas, USA.

2.1. Animals

Individually identified, purpose bred Beagles of both sexes were used in these studies. All dogs had not been treated with an ectoparasiticide for at least 60 days, demonstrated good flea retention prior to treatment, and were in good health at enrolment. Dogs were individually housed in enclosures that conformed to accepted animal welfare guidelines and ensured no direct contact between dogs. Dogs were fed an appropriate maintenance ration of a commercial dry canine feed for the duration of the study. Water was available *ad libitum*. Forty eight dogs from 10 to 64 months of age and weighing from 9.1 to 17.2 kg were used in the Study 1. Sixty four dogs from 10 to 142 months of age and weighing from 8.0 to 18.6 kg were used in Study 2. Twenty dogs from 12 to 36 months of age and weighing from 8.4 to 12.4 kg were used in Study 3. Twenty four dogs from 6 to 8 months of age and weighing from 6.1 to 9.2 kg were used in Study 4.

2.2. Experimental design and methods

2.2.1. General methods

Dogs were acclimated to the study conditions for at least 7 days prior to treatment. The dogs were observed for general health at least once daily throughout the studies. A physical exam was performed on each dog by a veterinarian to determine health and suitability prior to inclusion in the trial. For infestations, approximately 100 cat fleas (*C. felis*, ~1:1 sex ratio) were applied directly to the dogs and allowed to disperse into the hair coat. Flea counts were performed by personnel trained in the standard procedures in use at the test facility. Protective gloves and clothing were changed between dogs and personnel conducting parasite or other observations were unaware of treatment assignments. The dogs were thoroughly combed to count and remove fleas. Fleas able to stand upright and/or move in a coordinated manner were considered live. Commercial fine-toothed flea combs were used. Dogs were systematically combed while standing using repeated strokes starting from the head and proceeding caudally along the dorsum. The dog was then placed in lateral recumbency and then on its back for combing of the sides and ventral surfaces. Dogs were combed until no fleas were recovered during a 5 min period. Each animal was examined for a minimum of 10 min.

On each treatment day (Day 0 for all studies and also Days 30 and 60 for Study 4), the dogs were fasted overnight and then offered their normal food ration ~20 min prior to dosing. In Study 1, dogs were administered a sarolaner tablet that was shaved and/or sanded to the appropriate size to deliver the minimum dose of 2.0 mg sarolaner/kg. Control dogs were dosed with a single placebo tablet. In Studies 2, 3 and 4, dogs treated with sarolaner were administered a single or combination of tablets from strengths of 5, 10, 20 or 40 mg to achieve a dose as near as possible to 2.0 mg sarolaner/kg without under dosing. Control dogs were dosed with similarly-sized placebo tablets. All treatments were administered by pilling to ensure complete dosing. Each dog was observed for several minutes to ensure the dose was swallowed and for any adverse events associated with administration, and then periodically for up to 2 h for any signs of emesis. Dogs were observed for general health and any reaction to treatment approximately 1, 3 and 6 h after each treatment, then at least once daily for the remainder of the study.

Study 1: Five days prior to treatment, a pool of suitable animals was infested with fleas. These dogs were then combed to count and remove fleas 24 ± 3 h later and the 48 dogs with the highest live flea counts were selected for inclusion in the study. The dogs were ranked by flea count into six blocks of eight and then the animals of each block were randomly assigned to one of four placebo-treated groups or four sarolaner-treated groups; resulting eight groups of six dogs each. All dogs were infested with fleas on Days −2, 7, 14, 21, 28 and 35. One placebo- and one sarolaner-treated group were assessed for infestations by combing to count and remove fleas at 1 h after treatment and after the Day 7 and 14 infestations and, then at 2 h after the subsequent flea infestations. Dogs in the remaining placebo- and sarolaner-treated groups were combed to count and remove fleas at 4, 8, or 12 h after treatment and subsequent infestations.

Study 2: Six or eight days prior to treatment, pools of suitable animals were infested with fleas. These dogs were then combed to count and remove fleas 24 ± 2 h later and the 64 dogs with the highest live flea counts were selected for inclusion in the study. The dogs were ranked by flea count into eight blocks and then randomly assigned to one of four placebo-treated groups or four sarolaner-treated groups to give eight groups of eight dogs. All dogs were infested with fleas on Days −1, 7, 14, 21, 28 and 35. Dogs from one of each of the placebo and sarolaner groups were combed to count

Table 1
Geometric mean flea counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo at various times after treatment and weekly infestations for Beagles treated orally with sarolaner tablets at 2 mg/kg (Study 1).

Count Day	Time of count							
	1 or 2 h[a]		4 h		8 h		12 h	
	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner
0	89.5	91.4 (0.0)	81.3	11.6* (85.7)	78.1	0.3* (99.6)	85.0	0.0* (100)
7	90.7	89.2 (1.6)	87.4	0.2* (99.8)	92.5	0.1* (99.9)	88.0	0.0* (100)
14	72.2	68.0 (5.8)	84.2	0.3* (99.6)	86.9	0.0* (100)	74.4	0.0* (100)
21	78.8	72.2 (8.4)	86.2	3.6* (95.8)	84.7	0.0* (100)	85.6	0.0* (100)
28	44.3	30.9 (30.1)	55.0	2.7* (95.1)	50.9	0.6* (98.9)	56.0	0.0* (100)
35	86.5	79.8 (7.7)	89.4	81.7 (8.3)	91.3	9.3* (89.8)	87.0	0.0* (100)

* Geometric mean counts are significantly lower than placebo; $P \leq 0.0001$. Percent efficacy is given in parentheses.
[a] 1 h on Days 0–14, 2 h on Day 21–35.

Table 2
Geometric mean flea counts for placebo and sarolaner-treated dogs and percent efficacy relative to placebo at various times after treatment and weekly infestations for Beagles treated orally with sarolaner tablets at 2 mg/kg (Study 2).

Count Day	Time of count							
	3 h		4 h		8 h		12 h	
	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner	Placebo	Sarolaner
0	63.7	27.8* (56.3)	67.1	6.4* (90.4)	69.9	0.0* (100)	60.7	0.0* (100)
7	76.4	6.9* (91.0)	81.0	12.6* (84.4)	90.9	0.0* (100)	87.9	0.0* (100)
14	66.8	52.6 (21.3)	72.2	14.1* (80.4)	73.5	0.2* (99.7)	81.9	0.1* (99.9)
21	77.6	25.5* (67.2)	82.1	36.6* (55.4)	83.3	0.3* (99.6)	83.3	0.0* (100)
28	69.4	61.1 (12.0)	74.5	44.3* (40.5)	84.3	1.7* (98.0)	86.2	0.8* (99.1)
35	76.7	74.5 (2.9)	79.4	58.3 (26.6)	81.9	3.1* (96.2)	80.7	3.5* (95.7)

* Geometric mean counts are significantly different to placebo; $P \leq 0.0217$. Percent efficacy is given in parentheses.

and remove fleas at 3, 4, 8 or 12 h after treatment and subsequent infestations.

Study 3: Seven days prior to treatment, a pool of suitable animals was infested with fleas. These dogs were then combed to count and remove fleas 24 ± 2 h later and the 20 dogs with the highest live flea counts were selected for inclusion in the study. The dogs were ranked by flea count and randomly assigned to either placebo control or treatment with sarolaner. There were 10 dogs in each treatment group. The dogs were again infested with fleas on Days −1, 5, 12, 19, 26 and 33. At 48 ± 2 h after each infestation, the dogs were placed in individual crates designed for the collection of flea eggs and held in these cages for approximately 20 h. Just prior to removal from the cages, the hair coat of each dog was vigorously rubbed to dislodge any retained flea eggs. The dog was then removed from the cage and combed to remove fleas. All flea eggs were carefully collected and counted. Two samples of up to 100 arbitrarily selected eggs from each dog were transferred to rearing containers with larval growth medium and placed in an incubator. At five days after collection, one container from each dog was inspected and viable larvae were counted. Thirty five days after egg collection the second rearing container was checked and adult fleas were counted.

Study 4: On Day −49, a pool of suitable animals was infested with fleas. These dogs were then combed to count and remove fleas 24 ± 2 h later and the 24 dogs with the highest live flea counts were selected for inclusion in the study. The dogs were ranked by flea count and randomly assigned to pens. The dogs were subsequently infested with fleas on Days −42, −35, 7, 37 and 67. On Days −20, −6, 0, 14, 30, 44, 60, 74 and 90 the dogs were combed to count and remove fleas. On all of these days except Days 0 and 90, all viable fleas (up to a maximum of 100) combed off each dog were replaced on that dog after combing. Fleas were not replaced on the animals on Day 0, so that the challenge evaluated was derived from the infested environment. Following the Day −6 flea counts, the dogs in each pen were ranked by flea count and randomly allocated to either placebo control or treatment with sarolaner oral tablet. There were 12 dogs in each treatment group.

2.2.2. Parasites

Study 1. The flea colony was initially established by the University of Cardiff, UK and has been maintained in Ireland since 1996. EU origin, field-collected fleas were last introduced to the colony about eight years prior to conduct of the study.

Studies 2 and 3. The flea colony was established in 1997 with wild fleas collected in Turlock, California, USA. Locally collected fleas from naturally infested animals have been periodically added to the colony, with the last introduction of wild fleas occurring two months prior to the start of study 2 and five months before Study 3 was initiated.

Study 4. The colony was initiated with fleas originally obtained from Kansas State University. Additional field collected fleas from California are introduced twice a year. The most recent introduction was approximately 5 months prior to the study.

2.3. Data analysis

Studies 1, 2 and 4. The individual dog was the experimental unit and the primary endpoint was live flea count. Flea and tick counts were transformed by the $\log_e(\text{count} + 1)$ transformation prior to analysis in order to stabilize the variance and normalize the data. Using the PROC MIXED procedure (SAS 9.2, Cary NC), transformed counts were analyzed a mixed linear model for repeated measures. The models included the fixed effects of treatment, day of study or time of count, and any interactions. Random effects included block, room, block within room, any interactions and error. Treatment means were compared with the relevant control at the two-sided significance level $\alpha = 0.05$. Percent efficacy, relative to the control group and based on geometric means, was calculated as follows:

$$\%\text{Efficacy} = \frac{(\text{Mean Control - Mean Treated})}{\text{Mean Control}} \times 100$$

In Study 3, the individual dog was the experimental unit and the primary endpoint was flea egg count. Secondary variables examined were egg hatch and adult flea emergence. Egg counts were transformed and analyzed as for flea counts (described above). The proportions of eggs hatching and adult fleas emerging were subjected to an arcsine square-root transformation and analyzed using a general mixed linear model at each time point. The model included the fixed effect of treatment and random effects of block and error. Testing was at the two-sided significance level $\alpha = 0.05$. Percent reductions were calculated from back-transformed means using the above efficacy formula.

3. Results

3.1. Efficacy

Study 1. Placebo-treated animals maintained flea infestations throughout the study (Table 1). Flea counts for sarolaner-treated dogs were not significantly different from placebo ($P > 0.05$) at one hour after treatment, one hour after infestations on Days 7 and 14, two hours after infestations on Days 21, 28 and 35, and at four hours after infestation on Day 35. Live flea counts at all other time points for sarolaner-treated dogs were significantly lower than the respective placebo group ($P \leq 0.0001$) on all post-treatment count days. Against the pre-existing flea infestation, efficacy was 85.7, 99.6 and 100% at four, eight and 12 h after treatment, respectively. For subsequent infestations, residual efficacy was >95% within four hours and >98% within eight hours after infestation for four weeks after treatment. At five weeks after treatment, 89.8% efficacy was achieved by eight hours after infestation. For all post-treatment challenges, 100% efficacy was attained by 12 h after infestation.

Study 2. Placebo-treated animals maintained good flea infestations throughout the study (Table 2). Flea counts for sarolaner-treated dogs were not significantly different from placebo ($P > 0.05$) at three hours after infestations on Days 14, 28 and 35, or at four hours after infestation on Day 35. Live flea counts at all other time points for sarolaner-treated dogs were significantly lower than the respective placebo group ($P \leq 0.0217$) on all post-treatment count days. Against the pre-existing flea infestation, efficacy was 56.3, 90.4, 100 and 100% at three, four, eight and 12 h after treatment, respectively. For subsequent infestations up to five weeks after treatment, residual efficacy ranged from 2.9 to 91.0% at three hours, 26.6–84.4% at four hours and was >95% at eight and twelve hours after infestation.

Study 3. Placebo-treated animals maintained flea infestations following each challenge with large numbers of flea eggs recovered from almost all dogs. The arithmetic mean egg counts ranged from 166 to 353 eggs/dog (individual dog range = 0–933) for the five weeks of the study. Flea egg counts for all sarolaner-treated dogs were significantly lower than the placebo group ($P \leq 0.0002$) on all post-treatment count days. There were no eggs collected from any sarolaner-treated animal throughout the study. The respective percentages of live larvae and adults that emerged from eggs collected from the placebo-treated dogs ranged from 30 to 60% and 40 to 60%. As there were no eggs to incubate, no live larvae or adults were obtained from any sarolaner-treated dog.

Study 4. Ongoing infestation of the dogs' environments was demonstrated by the Day 0 (pretreatment) counts and confirmed in subsequent counts for the placebo-treated animals (Table 3). On Day 0, dogs had a geometric mean pretreatment flea count of ~100 fleas/dog with individual counts ranging up to 402 fleas per dog, following infestation with a total of 200 fleas each from five to six weeks previously. As more fleas were recovered from the dogs than could have been expected to survive, this indicated that fleas were successfully breeding in the simulated environment.

Table 3
Geometric mean flea counts and ranges for placebo and sarolaner-treated dogs and percent efficacy relative to placebo for Beagles held in a flea-infested, simulated home environment and treated orally with sarolaner tablets at 2 mg/kg on Days 0, 30 and 60 (Study 4).

Count Day	Placebo		Sarolaner		
	Mean	Range	Mean	Range	% Efficacy
0	92.5	19–328	102.2	31–402	–
14	99.6	33–375	4.4*	0–35	95.6
30	54.6	7–470	0.8*	0–4	98.6
44	65.4	21–317	0.2*	0–2	99.6
60	40.9	5–354	0.0*	0–0	100
74	44.6	13–411	0.0*	0–0	100
90	15.5	0–358	0.0*	0–0	100

* Geometric mean counts are significantly different to placebo; $P < 0.0001$.

This was confirmed by counts of more than 300 fleas for individual placebo-treated animals on all counts after the initial treatment (Table 3) as all adult fleas were removed from the dogs on Day 0, and additional induced infestations of 100 fleas each were only conducted on Days 7, 37, and 67. Live flea counts for sarolaner-treated dogs were significantly lower than the placebo-treated dogs at all post-treatment counts ($P \leq 0.0001$). Reduction in flea infestations in sarolaner-treated dogs relative to placebo-treated dogs was 95.6, 98.6, and 99.6% on Days 14, 30 and 44, respectively, and 100% on all subsequent count days.

3.2. Dose acceptance

All animals were successfully dosed. In Study 4, a single dog regurgitated a complete sarolaner tablet directly after dosing on Day 0. The tablet was recovered immediately and successfully re-administered. A small number of dogs in both treatments were noted to either gag or cough following the administration of water after the tablet was dosed.

3.3. Health observations

There were no adverse events observed in any of the studies that were considered related to sarolaner treatment.

4. Discussion

Taken together, the results of these four studies demonstrate the rapid onset of efficacy of sarolaner against adult fleas starting within 3–4 h of treatment or re-infestation, and producing ≥98% reductions within 8 h through 28 days after treatment. This rapid and complete efficacy for a full month after a single dosing resulted in the complete cessation of flea reproduction on treated dogs for up to 35 days and was confirmed under simulated infested environment conditions.

In the first investigation of the speed of kill versus fleas (Study 1), by four hours after sarolaner had been administered at an oral dose of 2.0 mg/kg, flea counts were significantly lower in treated dogs ($P \leq 0.0001$) and efficacy was 85.7%. Similarly, flea counts were significantly lower in treated dogs than in placebo by four hours after infestation for post-treatment challenges up to four weeks after treatment ($P \leq 0.0001$) and efficacy was >95% through this period. At Day 35, a significant reduction in live fleas of 89.8% ($P \leq 0.0001$) was achieved by 8 h after infestation.

In Study 2, live flea counts were significantly lower in sarolaner-treated dogs than placebo within three hours of treatment and infestations on Days 7 and 21 ($P \leq 0.0001$) but not on Days 14, 28 and 35. Confirming the previous study, at four hours after treatment and post-treatment infestations for up to four weeks, sarolaner-treated dogs had significantly fewer live fleas than placebo dogs

($P \leq 0.0217$) with efficacies ranging from 40.5 to 90.4%. By eight hours after treatment and subsequent infestations efficacy was 96.2–100%.

These two studies confirmed that a 2 mg/kg oral dose of sarolaner started killing fleas within three to four hours after treatment or subsequent re-infestations for up to a month, and achieved ≥98% control of fleas by eight hours after treatment or re-infestation for 28 days following a single treatment. This rapid onset of effectiveness and consistent rapid kill of fleas through a month after a single treatment compares favorably with other topical and oral flea products (Everett et al., 2000; Schenker et al., 2003; Franc and Bouhsira, 2009). As complete kill was obtained within 12 h through 35 days, monthly treatment with sarolaner could be expected to have an impact on the ability of fleas to reproduce since female fleas require at least 24 h on the host prior to the initiation of egg laying (Dryden and Broce, 2002).

The impact of the rapid speed of kill of sarolaner on flea reproduction was confirmed in Study 3, where no flea eggs were recovered from one day after treatment or following weekly re-infestations through 35 days after treatment with a single oral dose of sarolaner.

For dogs held in a flea-infested simulated-home environment, monthly treatment with sarolaner resulted in a rapid reduction in flea burden on the dogs and complete eradication of the flea infestation within two months of treatment initiation. This efficacy resulted from the rapid speed of kill and cessation of egg-laying throughout the month long treatment period. Adult fleas newly emerging from the infested environment were killed before they could reproduce and contribute to re-infestation of the environment. The small number of fleas detected on sarolaner-treated dogs up to about six weeks after the initiation of treatment were likely newly emerged fleas derived from eggs laid prior to the first dosing; this is consistent with the flea lifecycle (egg to adult) which can take up to eight weeks to complete (Rust and Dryden, 1997). The complete lack of any live fleas on sarolaner-treated dogs for the last six weeks of the study was likely due to the rapid and consistent kill of adult fleas before they were able to lay eggs.

5. Conclusions

The studies reported here demonstrated that a single oral dose of sarolaner at 2 mg/kg provided rapid onset of activity and knockdown of existing infestations of fleas on dogs and quick and consistent kill of new infestations for at least one month. Fleas started dying within three to four hours after treatment or subsequent re-infestations for up to a month and ≥98% control of fleas was achieved by eight hours after treatment or re-infestation for 28 days. This rapid and consistent speed of kill was shown to completely suppress flea egg-laying for one month following a single treatment. The simulated infested home-environment study confirmed the ability of monthly treatment of the host with sarolaner to provide excellent control of existing environmental infestations of fleas.

Conflict of interest

The studies reported here were funded by Zoetis, Florham Park NJ., RHS, CB, SPM, SC, MRM and NS are current employees of Zoetis. LC, BG and DRY were independent investigators contracted for these studies. All authors assisted with the design and conduct of the studies, interpretation of the data and manuscript review. There were no conflicting interests that could have influenced the conduct and reporting of these studies.

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