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PAPER

Influence of dog-appeasing pheromone on canine maternal behaviour during the peripartum and neonatal periods

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Abstract

Background Parturition and the initial postpartum period are important moments in the reproductive cycle of dogs.

Methods A study assessed the effect of ADAPTIL, a dog-appeasing pheromone, on maternal behaviour during peripartum. Bitches were continuously exposed to ADAPTIL (n=20) or placebo (n=21) in double-blinded conditions from an average of about seven days before parturition up to 21 days postpartum. Differences in maternal behaviour in relation to the treatment were evaluated by the observation of specific activities through video recordings, such as the time spent by the bitch in close contact with the puppies, oronasal interaction and nursing duration and position. Videos were recorded at four time points (W0: within the first 48 hours of whelping; W1: one week after parturition; W2: two weeks after parturition; and W3: three weeks after parturition). In addition, the perception of breeders in relation to the quality of maternal care, puppies' wellbeing and overall relationship between the bitches and the puppies was evaluated using Visual Analogue Scale at the same time points. Moreover, the daily activity of the bitches was measured by using an electronic device (FitBark dog activity trackers, Kansas City, Missouri).

Results For all observed maternal behaviours, there was a steady decrease in levels as the puppies developed, independently of treatment. However, bitches exposed to ADAPTIL tended to nurse significantly more in lying position, while those exposed to the placebo nursed more in a seated position, especially at W1 (P=0.06) and W3 (P=0.005). According to the breeders, the attention scores of bitches towards puppies were significantly higher in ADAPTIL than in the placebo group at each time point (P=0.01). Moreover, a difference according to parity was observed (P=0.004), with greater attention score displayed by primiparous bitches exposed to ADAPTIL compared with placebo on W0 (P=0.02), W1 and W3 (P<0.001). The global mother–puppies relationship was also perceived as significantly better (P=0.0002) by breeders of bitches exposed to ADAPTIL, with significant differences at W2 (P=0.01) and W3 (P=0.001). The bitches' daily activity increased starting two days before the whelp, peaked during parturition and then gradually declined up until four days postpartum. There was a trend towards a difference in the activity level according to the treatment during the full study period (P=0.09) and at two days before parturition (P=0.07). Bitches exposed to ADAPTIL were more active compared with placebo in relation to the FitBark data.

Conclusion The use of ADAPTIL in maternity modulated maternal behaviours. Concerning the caregiver's view, bitches under the influence of ADAPTIL had greater and extended attention towards the puppies and they were eager to stay with the puppies for a longer time.

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Introduction

The maternal peripartum and the neonatal periods are transition stages that are essential for the survival of all newborn mammals. During these times, the female undergoes physiological and neuroendocrine changes that assure both the delivery of the offspring and the

expression of appropriate maternal behaviours that increase the likelihood of neonate survival.[1] Specifically, the female invests time and energy resources for in utero development, parturition and initial postnatal feeding through lactation.[2] For the progeny, the degree of developmental maturity at birth dictates the amount of maternal care necessary for the newborn to adapt to the environment. Domestic dogs, an altricial species, produce puppies that, at birth, are extremely immature. Newborn puppies are unable to regulate their body temperature, movements are limited to crawling, eyes and ears are closed, maternal stimulation is required for waste elimination, and feeding is requisite soon after birth. Therefore, adequate maternal care during the neonatal period is essential. Canine maternal behaviour has been recently reviewed,[3] but there are important factors that are still unclear. For instance, the impact of the average time to the first suckling on neonate survival has not been addressed.[4] Newborn piglets, like puppies, are also born with poor levels of energy. For pigs, research has shown that intake of colostrum[5] and milk is of utmost importance for proper development of the immune system and for obtaining adequate nutrition for continued growth and development.[6–8] Therefore, it is possible that poor maternal care soon after parturition will directly impact a puppy's immunological and nutritional needs, two key elements influencing survival rate.

Besides neonatal survival, the quality of maternal care might affect the temperament of puppies and later adult dogs, although further evaluations are still needed. The neonatal and transitional periods are followed by rapid development of the nervous system. This growth marks the beginning of the puppy's ability to engage in social interactions, both with the bitch and the caregiver. These interactions could have a profound and long-lasting effect on the puppy's disposition. However, knowledge of the effects of maternal parenting style on the cognitive and emotional development of domestic dogs is limited.[9 10] In addition, how the quality of maternal care during this specific time window influences the stress response and the potential for future behaviour problems in dogs is unknown.

Recent studies on maternal care and newborn development provide additional information for discussion. Foyer *et al*[11] studied the data of 503 German shepherd dogs from 105 litters bred at the Swedish Armed Forces and suggested that the degree of maternal investment modified the later behaviour of the offspring. For dogs with military aptitude, confidence and physical engagement of young adult dogs were influenced by parity of the bitch, as more experienced bitches most likely provided more mature maternal behaviour.[11] To better understand the impact of maternal behaviour in dogs, focus has been given to the observation and duration of mother–pup interactions such as physical contact, licking/poking and nursing (considering the duration and position of the dam). In these studies, all different interactions loaded into one principal component defined as maternal care or maternal behaviour. High levels of maternal care were characterised by bitches that passed more time in close contact and were more engaged in licking and grooming their puppies.[12–15] Higher maternal care was associated with better performance in temperament tests in young adult German shepherd dogs[12] and in beagle puppies of two months of age.[13] Controversially, higher scores for maternal care increased the manifestation of undesirable behaviours such as anxiety-related and separation behaviours.[14 16] Overmothered puppies were less likely to be selected as guide dogs[16] and showed low performance in behaviour tests at the age of two months when they were raised in household situations.[14] Indeed, maternal behaviour seems to play a key role in the offspring's ontogenesis,[12–14 16] which might be variable depending on the breed and the environment.

However, what are the essentials of maternal behaviour? Maternal bonding is a combination of external and internal factors associated with a pheromone-mediated experience initiated at parturition[17] as well as with hormonal changes, related to oxytocin, prolactin and progesterone levels.[18] Although stress is part of the whole preparation to birth, excessive stress could potentially affect maternal bonding. Overstressed females due to a threatening or disturbing situation might potentially stop the process of labour due to increased secretion of the stress hormone catecholamine, as described in human beings[19–21] and other mammals.[22] Therefore, stress can have a potential negative impact on the bonding process of the mother and the offspring.[2 23] Bitches generally search for a quiet, secluded place or the proximity of the owner to whelp, most likely to reduce stress and to ensure the protection of their puppies.[24–29] This natural behaviour of bitches might be indicative of the importance to diminish the overall stress around peripartum since the impact of high prepartum and postpartum stress levels has been suggested to be associated with abnormal maternal behaviours in other mammals.[30–32] In fact, during the peripartum period, the bitch may display behaviours due to overstress, which could profoundly affect the normal pathway of parturition and maternal responsiveness. Therefore, the use of a synthetic form of the natural dog-appeasing pheromone ADAPTIL (Ceva Santé Animale, Libourne, France), a well-recognised product to reduce stress in young and adult dogs,[33] may be interesting to consider in an attempt to minimise the consequences of overstress for some females. In adult dogs, ADAPTIL has shown a positive effect on improving conditions such as fear of loud noises,[34 35] and in reducing stress and anxiety due to separation,[36] during transportation[37] or when brought to the veterinary practice.[38] Since the use of ADAPTIL has been associated with relaxation in dogs in different contexts, its use during peripartum

could potentially modulate bitches' behaviour and help them to overcome any excessive stress situation.

To evaluate the benefits of a dog-appeasing pheromone plug-in diffuser (ADAPTIL® Calm) on the maternal behaviour of the bitch and the interactions with the puppies in peripartum, a randomised, double-blinded, placebo-controlled, multicentric field clinical study was conducted. The hypothesis was that bitches coping better with the stress in peripartum should be able to display higher quality of maternal care, through more interaction with their puppies.

Materials and methods

Subjects

Bitches and their respective puppies were recruited from professional breeders in France, contacted by phone through the client database of the Centre d'Etudes en Reproduction des Carnivores, Maisons-Alfort, France. The selection of animals was done from April to December 2018. The conditions of rearing were specific to each kennel, respecting the inclusion criteria: the presence of a well-defined maternity area (dogs should not live inside the house during the study period to reduce the influence of housing and proximity with owner) and a mortality rate lower than 15 per cent in the first 15 days postpartum based on the breeder's records (to exclude breeding kennels with sanitary problems). Pregnant bitches, both primiparous and multiparous, were recruited from breeds with no predisposition to dystocia (exclusion of brachycephalic breeds) and not previously exposed to a dog-appeasing pheromone product (ADAPTIL) during the current pregnancy. In general, bitches were transferred on average seven days before parturition and remained in the maternity area until at least 21 days postpartum. Enrolled bitches were not exposed to any behaviour modification product other than the one tested. Reproductive history and parturition data were collected using a questionnaire that was filled in by the breeders (online supplementary appendix 1). Depending on the breeding facility, bitches were housed either individually or separated by half walls. Neither the number of bitches in each maternity unit at any given time nor the potential positive or negative impact of the proximity of another bitch on the behaviour of a bitch and her litter was taken into account during the data analysis.

Treatment

Treatment was done using a plug-in device releasing a dog-appeasing pheromone product or a placebo. The diffusers were placed at least two days before parturition and continuously spread in the dogs' environment until the end of the third week postpartum. Allocation of treatment was randomised and double-blinded. All personnel involved in the study were blinded to treatment conditions (the study investigator, the breeders and their staff, and the statistician). The only exception was the sponsor representative, who allocated treatments according to a randomisation list while ensuring there was no concurrent utilisation of both treatments (ADAPTIL and placebo) in the same kennel at the same time, to prevent cross-contamination in the air. The change in treatment in two kennels was done respecting a washout period of at least three weeks to ensure sufficient cleaning of the surfaces and air renewal. One kennel received ADAPTIL and then placebo two months after the end of the first treatment, while the other received the placebo followed by ADAPTIL after three weeks. The plug-in diffusers and refill vials were identical and just differentiated by a unique numeric label. A single diffuser was used in individual maternities or in maternities divided by a half wall if the area was smaller than 70 m^2. The diffuser was plugged in at a height of around 1.5 m above the floor to optimise the diffusion. One plug-in diffuser and two refills labelled as 'A' and 'B' of the same identification number were provided to cover the full study period. The first refill was plugged at the first day of enrolment (at entrance in the maternity area) by the study investigator and then replaced by the second refill in the first 48 hours after parturition. All diffusers were removed at the end of the third week postpartum.

Analysis of maternal care

Maternal care was assessed through the observation of video recordings and Visual Analogue Scale (VAS) in four specific times. The VAS is a measurement instrument using a scale (0 to 10 cm) to quantify subjective characteristics or attitudes that cannot be directly measured that is anchored with two unipolar extremes, whose extremes represented points of a negative and positive outcome commonly used to describe pain levels in human medicine.[39] The first control (W0) was done within 48 hours after parturition and then at the first, second and third week postpartum (W1, W2 and W3, respectively) to compare different stages of the postpartum period.

The duration of the video and the time of day when the videos were recorded were variable in relation to the routine of the kennel and the recording material used. Nevertheless, all the recordings were done during daytime (between 09.00 and 17.00) and human presence was limited. The mean duration per recording was 75 minutes. Only two bitches had free access outside of the maternity area. In all other cases, the video was recorded when the bitch was present in the maternity area. The target duration per recording was set at one hour. The cameras were attached to the wall in close proximity to the whelping box to guarantee good visualisation of the bitches and puppies. The experimental unit was the bitch and her litter: puppies were not individually identified and maternal care was evaluated in relation to the whole litter. The variables

measured were based on the list of maternal behaviours previously described in the literature.[12 15 40 41]

- *Time inside the whelping box*: bitch with at least her two front legs inside the whelping box.
- *Time of contact with puppies*: bitch sitting or lying down in close proximity (body contact) to at least one puppy.
- *Nursing time and posture*: at least one puppy was attached to the nipple and the position of the bitch (sitting or lying down) was recorded. It was also observed if all puppies from the litter suckled during the video recording.
- *Oronasal interaction*: bitch interacting through licking, grooming and/or sniffing a puppy.

Continuous sampling was used to record each selected behaviour in each video and its duration (in minutes). Behaviours were not mutually exclusive. To take into account the different lengths of all individual recordings, all behaviours were expressed as a percentage of the recorded time per session.

A total of 187 hours, 57 minutes and 53 seconds of footage for the mother–puppy dyad were viewed multiple times and analysed by one single observer. No specific video coding software was used. The time of the beginning and end of all interactions between the bitch and the puppies were recorded and treated using an Excel table.

VAS scores were also used to assess the bitch's maternal behaviour towards their puppies as well as the puppies' overall behaviour, and the mother–litter relationship, from the breeders' perspective. All scales were completed by the same observer (the owner itself or one of their employees) in daily contact with the litter at all time points. All observers were experienced in the care of bitches and puppies. They assessed each VAS in blinded conditions, meaning they were unaware of the product their dogs had been exposed to. The VAS scores were assessed using a plain 100-mm line with two endpoints. Seven parameters were considered to qualify the behaviour of the bitch, the wellbeing of the puppies and the maternal–puppy relationship. At each assessment, corresponding to the four time points, the animal caregiver was asked to answer the VAS by placing a mark on each line corresponding to their perception of the behaviour or attitude based on their observation on that particular day (online supplementary appendix 2). No particular time duration was specified. Scores were calculated by measuring the length (in mm) of a 10-point maximum score, from the left end. For all VAS in this study, a score of 0 reflected an inappropriate behaviour while a score of 10 reflected a positive maternal attitude or highest wellbeing of the puppies.

Activity tracker data evaluation

To record the bitch's activity profile, an electronic activity tracker device (FitBark, Kansas City, Missouri) was attached to the collar at enrolment until the end of the trial. Individual data were uploaded and synchronised to a smartphone through the FitBark application. The daily information retrieved was divided into three main categories as 'play', 'active' and 'rest', corresponding to high activity, medium activity and no movement (standing still or sleep), respectively. 'Play', 'activity' and 'rest' were analysed for each individual animal. Then, 'active' and playing' times were combined into a single 'active-play' parameter, as opposed to 'rest' since it was more meaningful to group those parameters to better reflect the bitches' overall activity. In the Results section, 'activity' will refer to 'active+play' time.

Statistical analysis

Parameters were analysed using descriptive statistics by treatment groups. Quantitative variables were analysed using mean and sd, and categorical variables were analysed using frequency counts and percentages. A linear mixed model for repeated measures was used to analyse quantitative variables. The covariates were the time points, the treatment and their interaction as fixed effects. The dog number was used as a random effect. Covariates like the number of puppies per bitch or parity were tested in the statistical model. The second-order interaction was tested to detect a product effect according to time point and parity, or time point and number of puppies per bitch (comparing two litter sizes: (1; 4) vs (5; 10)). When there was an effect of parity, variables were then analysed according to parity (primiparous versus multiparous).

Contrasts were calculated to see significant differences between products. Since this was a pilot study, the significance threshold was set at 0.10, and all tests were two-tailed. All statistical tests were performed using the SAS software (V.9.3; SAS Institute).

Results

Forty-eight bitches and their respective puppies were recruited from 13 professional breeders. Seven bitches were excluded a posteriori due to a caesarean section (n=5), late abortion (n=1) and neonatal mortality of all puppies (n=1). Of the 41 bitches that completed the study (table 1), 20 were exposed to ADAPTIL (for a total of 113 puppies born and 105 puppies still alive at day 0 (D0) and 21 to the placebo (128 born puppies and 115 live pups at D0). Most of the facilities (n=11, corresponding to 24 bitches) were randomly allocated to one single treatment, with all their selected bitches exposed to either ADAPTIL or placebo. Two larger breeding facilities, raising more dogs, were ultimately exposed to both treatments at two different times, separated by a washout period of at least three weeks, to prevent environmental cross-contamination. The different breeds were as well randomly allocated to treatment. For the German shepherd dogs, a breed over-represented in the study (34.1 per cent), although an effort was done to balance the sample it was not possible. However, if breed effect had an impact, it should be towards the placebo group. For Bray *et al*,[15] the breed effect was observed as labrador retriever mothers spent

Table 1 Demographic information of the breeding facilities and selected bitches

Characteristics	Quantity
Number of different breeders selected for the study	13
Size of the breeding facilities	8–117 reproductive bitches
	▶ 10 breeders (77%) raising 8–43 reproductive bitches
Raised breeds	1–9 different breeds ▶ 10 breeders (77%) raising only one or two breeds
Breeds enrolled in the study	12
Females of each breed	
German shepherd dog	14 (34.1%)
Labrador	5 (12.2%)
Bichon	4 (9.8%)
Beagle	3 (7.3%)
Standard poodle	3 (7.3%)
Cocker spaniel	2 (4.9%)
Coton de Tulear	2 (4.9%)
Dachshund	2 (4.9%)
Golden retriever	2 (4.9%)
Shar-Pei	2 (4.9%)
Spitz	1 (2.4%)
Papillon	1 (2.4%)

more time engaging in maternal behaviour than German shepherd mothers. Therefore, the breed effect should be observed in the placebo group (five Labradors and five German shepherd dogs) against the ADAPTIL group (zero Labrador and nine German shepherd dogs).

Both groups were well balanced regarding the age of the selected bitches (mean age=3.1±1.3 in ADAPTIL and 3.8±1.5 in placebo) and parity (8 and 7 primiparous, 12 and 14 multiparous, respectively). Overall, more multiparous bitches (n=26, 63.4 per cent) than primiparous (n=15, 36.6 per cent) were enrolled in the study. Conversely, descriptive analysis a posteriori revealed that both groups were not equal regarding the general temperament of the bitches as qualified by their breeder. Twenty-four bitches (65.8 per cent) were considered as rather calm, versus 14 which were perceived as anxious/nervous (34.1 per cent), but with more anxious bitches in the treatment group than in the placebo group (n=9 and n=5, respectively). This difference was greater in the multiparous subpopulation, with 41.7 per cent of bitches exposed to ADAPTIL being generally qualified as nervous, versus 92.9 per cent of bitches in the placebo group being qualified as calm.

All maternities had a whelping box adapted to the size of each respective breed, with an exception of four small bitches (Coton de Tulear) which were housed in built-in boxes in two levels. Although not ideal, these four bitches were only lodged in this situation in peripartum up to the third week after whelping, and during this period they had access to an exercise area for at least three times per day.

On average, exposure to the diffuser in the maternity area before whelping was for 7.4 days, ranging from two to 20 days. There was no significant difference (P=0.26) between exposure time for the treatment groups (ADAPTIL, mean=8.3 days; placebo, mean=6.6 days). After whelping, bitches and their litters were continuously exposed to the diffuser until at least D21 postpartum.

Treatment groups were no different in terms of number of puppies born (ADAPTIL, mean=5.7 puppies (3–9); placebo, mean=6.1 (1–12)) or number of live puppies at the first day after excluding the stillborn (ADAPTIL, mean=5.4 puppies; placebo, mean=5.2). Due to the wide variability in litter sizes, the total number of puppies per bitch was considered when analysing the data by comparing small litter size (up to four puppies) versus larger litter (5–10 pups). However, this covariate had no effect on the different criteria assessed in this study.

Video observation

The amount of time spent on all activities significantly decreased in all bitches over the postpartum period (P<0.0001). This included the time spent in the whelping box, the time in contact, the oronasal interaction between bitches and their puppies, and the amount of time dedicated to nursing puppies (expressed as a percentage of total video-recorded time). No significant difference according to treatment was observed for any of these above criteria (see table 2). On average, bitches spent 68.1 per cent of their time nursing puppies just after birth, versus 40.4 per cent, 31.6 per cent and 19.7 per cent after one, two and three weeks, respectively.

However, in relation to the nursing position, a significant treatment effect was found over the whole study period: bitches exposed to ADAPTIL tended to nurse their puppies significantly more in a lying position, while placebo bitches tended to nurse significantly more in a seated position (seated: P=0.02; lying: P=0.009). The differences of least square means between both treatments were greatest at W1 (P=0.06) and W3 (P=0.005). In addition, although differences between groups were not significant, bitches exposed to ADAPTIL seem to have a more steady decrease in the proportional time of nursing at each time point (figure 1) and they were more frequently observed nursing all their puppies on average than the placebo group. Although video recording was intermittent and performed only once a week, this difference was noticed at each time point, despite the global decrease in feeding all puppies at the same time over weeks (table 2). No difference was observed based on the litter size covariate.

Owners' perception of maternal care

The VAS highlighted several significant differences in the maternal behaviour of bitches, as assessed by their breeders. The breeders' perception was unbiased, since they were blinded towards the treatment their dogs were exposed to during the maternity period. The attention displayed by mothers towards their puppies

Table 2 Evolution of maternal behaviours assessed through the video, according to treatment (n=41)

Behaviour	Treatment group	W0	W1	W2	W3
Mean time in the whelping box (sd)					
Time spent inside the whelping box (% of total video observation time)	Placebo	87.5 (19.6)	57.2 (36.5)	43.1 (31.0)	28.8 (36.0)
	ADAPTIL	90.8 (11.2)	49.1 (32.9)	45.3 (27.9)	34.5 (28.5)
Mean time in contact (sd)					
Time in contact with puppies (% of total video observation time)	Placebo	83.2 (22.3)	51.3 (35.9)	33.6 (27.4)	31.5 (34.5)
	ADAPTIL	84.7 (15.9)	45.6 (29.2)	48.0 (29.4)	33.0 (29.0)
Mean feeding time (sd)					
Total feeding time (% of total video observation time)	Placebo	67.0 (30.3)	39.9 (34.3)	24.9 (26.5)	15.9 (17.6)
	ADAPTIL	69.2 (23.2)	41.3 (27.7)	39.6 (23.0)	23.9 (20.9)
Total feeding time in seated position (% of total feeding time)	Placebo	16.0 (32.0)	40.9 (43.1)	34.9 (41.0)	31.7 (38.2)
	ADAPTIL	13.1 (25.8)	17.5 (30.3)	17.0 (32.3)	10.1 (25.9)
Total feeding time in lying position (% of total feeding time)	Placebo	84.1 (32.0)	58.7 (43.6)	62.0 (41.2)	51.9 (41.5)
	ADAPTIL	87.0 (26.0)	82.9 (30.5)	82.7 (32.2)	83.6 (26.1)
Feeding all puppies %					
Feeding all puppies (yes/no)	Placebo	81.0	81.0	70.0	57.9
	ADAPTIL	95.0	92.9	88.2	76.5
Mean time in oronasal interaction between mother and puppies (sd)					
Oronasal interaction (% of total video observation time)	Placebo	15.3 (17.3)	7.1 (5.4)	7.1 (5.6)	2.6 (2.6)
	ADAPTIL	14.7 (7.2)	6.6 (4.5)	7.0 (5.2)	5.2 (4.1)

W0, within the first 48 hours of whelping; W1, one week after parturition; W2, two weeks after parturition; W3, three weeks after parturition.

(through licking, nursing and so on) was significantly higher in the ADAPTIL group (W0=8.9±1.3, W1=9.2±0.8, W2=9.2±0.8 and W3=9.4±0.5) than the placebo (W0=8.3±1.9, W1=8.7±1.3, W2=7.7±2.1 and W3=8.1±1.8) over the study period (P=0.0004) and at each time point (P=0.01) (table 3A and figure 2). Moreover, a difference according to parity was observed (P=0.004), with greater attention scores displayed by primiparous bitches exposed to ADAPTIL compared with placebo at W0 (P=0.02), W2 and W3 (P<0.0001) (table 3B).

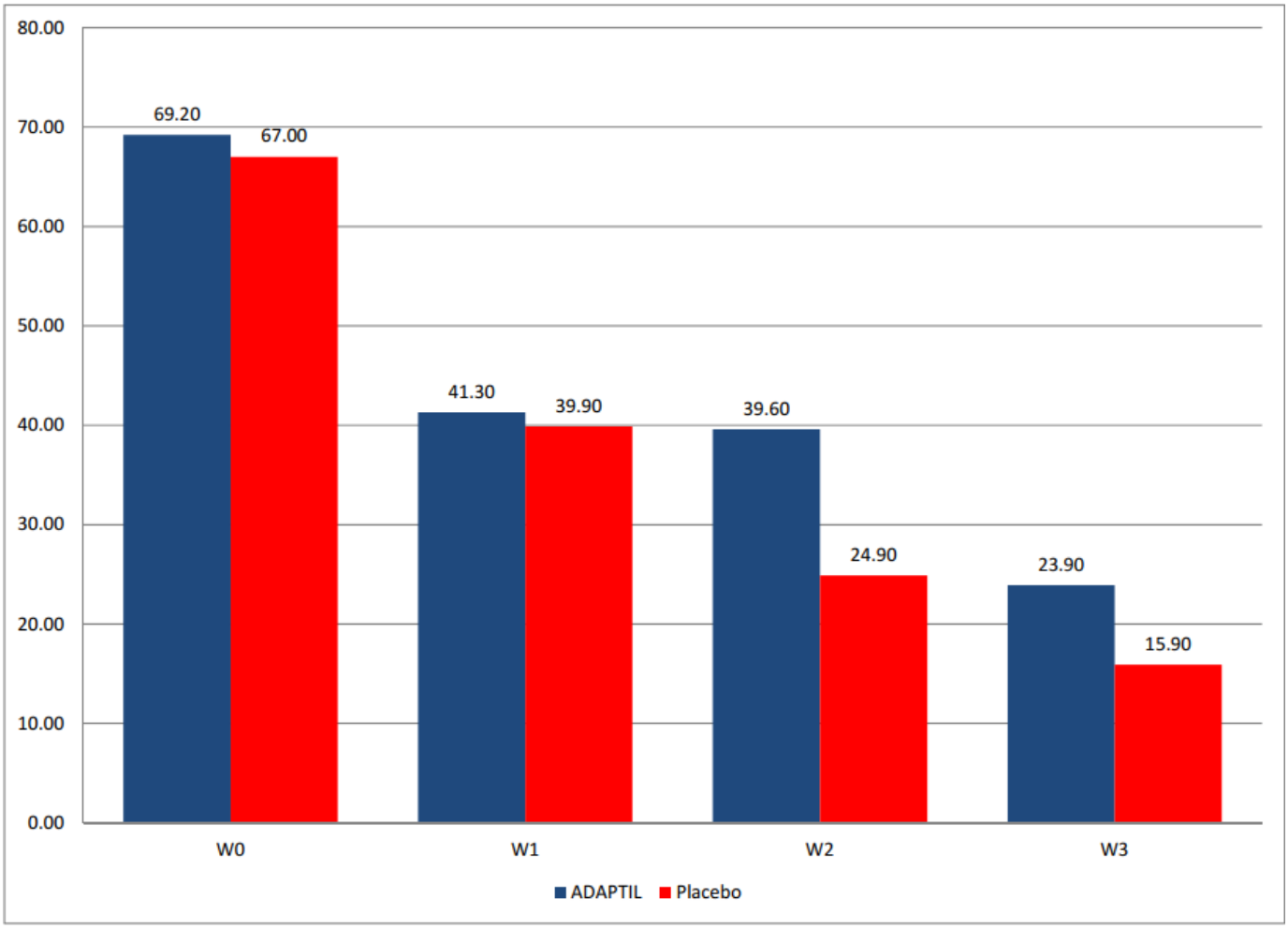

Figure 1 Proportional time dedicated to nurse (% of total video observation time) from the first 48 hours (W0) to weeks 1–3 (W1, W2 and W3) postpartum.

Table 3A Distribution of VAS scores according to treatment and time point (n=41) (mean values out of a total of 10 +sd)

VAS criteria	Treatment group	W0	W1	W2	W3
Mother parameters					
Mothers' attention	Placebo	8.3 (1.9)	8.7 (1.3)	7.7 (2.1)	8.1 (1.8)
	ADAPTIL	8.9 (1.3)	9.2 (0.8)	9.2 (0.8)	9.4 (0.5)
Contact to puppies	Placebo	8.7 (1.7)	7.9 (1.9)	7.3 (2.1)	7.1 (2.3)
	ADAPTIL	8.9 (1.5)	9.0 (0.8)	8.7 (1.0)	8.3 (1.8)
Global relationship	Placebo	8.6 (1.8)	8.6 (2.0)	8.2 (2.0)	7.7 (2.2)
	ADAPTIL	8.8 (1.3)	9.1 (1.1)	9.4 (0.6)	9.5 (0.5)
Puppies parameters					
Puppies' wellness	Placebo	9.2 (0.9)	9.3 (1.0)	9.4 (0.7)	9.4 (1.0)
	ADAPTIL	9.0 (1.0)	9.1 (1.1)	9.2 (0.9)	9.4 (0.5)
Puppies' whining*	Placebo	8.5 (1.7)	9.1 (0.8)	8.8 (0.9)	8.2 (1.5)
	ADAPTIL	8.6 (1.6)	8.7 (1.4)	9.0 (0.8)	8.8 (1.1)

*Higher score indicates the puppies did not whine frequently.
VAS, Visual Analogue Scale; W0, within the first 48 hours of whelping; W1, one week after parturition; W2, two weeks after parturition; W3, three weeks after parturition.

Table 3B Distribution of VAS scores according to treatment and time point, for primiparous bitches only (n=15) (mean values out of a total of 10 +sd)

VAS criteria	Treatment group	W0	W1	W2	W3
Mothers' attention	Placebo (n=7)	7.1 (2.7)	8.3 (1.0)	6.2 (2.4)	6.9 (2.1)
	ADAPTIL (n=8)	8.7 (0.9)	8.9 (0.8)	9.1 (0.6)	9.4 (0.6)
Global relationship	Placebo (n=7)	7.9 (2.5)	7.2 (2.5)	6.8 (2.5)	6.0 (2.2)
	ADAPTIL (n=8)	8.0 (1.4)	8.9 (1.0)	9.1 (0.5)	9.4 (0.6)

VAS, Visual Analogue Scale; W0, within the first 48 hours of whelping; W1, one week after parturition; W2, two weeks after parturition; W3, three weeks after parturition.

The time spent in close contact with the puppies was also significantly higher in the ADAPTIL group than in the placebo over the study period (P=0.02) and at each time point (P=0.08) (figure 3). Moreover, contact scores decreased over time in both groups, reflecting natural age progression where puppies have less need to be in close contact with their mother. However, despite this global decrease in contact, bitches exposed to ADAPTIL were observed by their breeders to spend more time in direct contact with their puppies than in the placebo group. Greater levels of interaction were observed at W1 (P=0.04), but even at two or three weeks of age (P=0.01 and P=0.02, respectively), when puppies are usually more vigorous and less reliant on their mother. The overall mother–puppies relationship was significantly higher in the ADAPTIL group over the study period (P=0.002) and at each time point (P=0.0002): breeders of bitches exposed to ADAPTIL rated them as behaving as 'better mothers', although a more relaxed and harmonious relationship than in the placebo group, at each time point (figure 4). Moreover, the global relationship scores slightly decreased over time in the placebo group, while they increased in the ADAPTIL group. The difference was significant at two weeks (P=0.01) and at three weeks (P=0.001) of age. A difference according to parity was also observed (P=0.001), with greater relationship scores displayed by primiparous bitches exposed to ADAPTIL compared

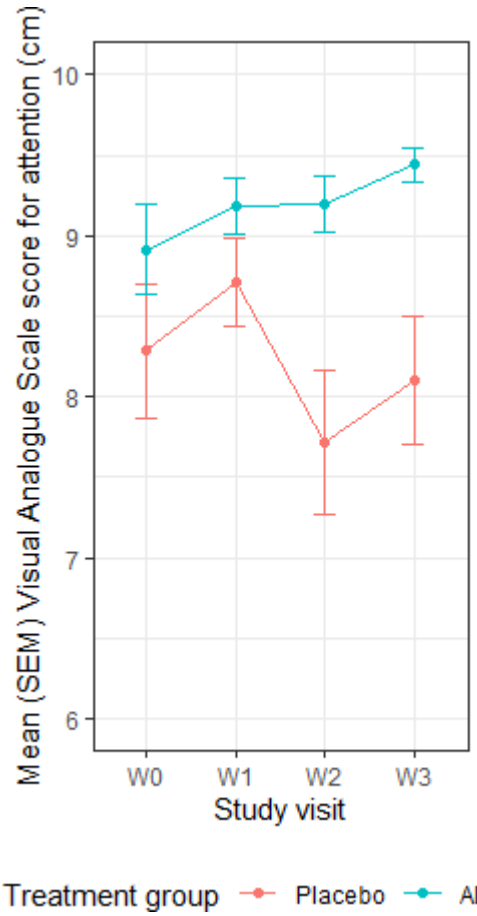

Figure 2 Mean Visual Analogue Scale score evolution for 'attention towards puppies' according to treatment and time. SEM, standard error of the mean; W0, within the first 48 hours of whelping; W1, one week after parturition; W2, two weeks after parturition; W3, three weeks after parturition.

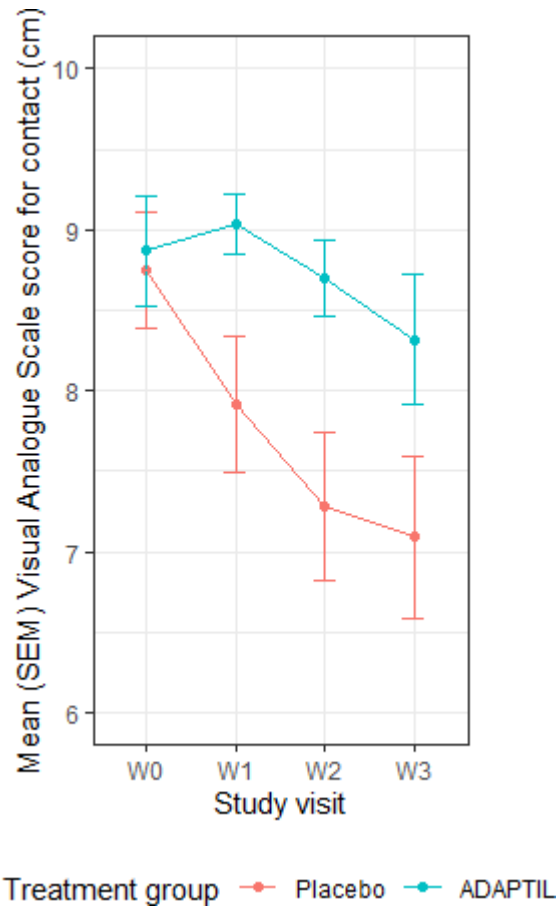

Figure 3 Mean Visual Analogue Scale score evolution for 'contact to puppies' according to treatment and time. SEM, standard error of the mean; W0, within the first 48 hours of whelping; W1, one week after parturition; W2, two weeks after parturition; W3, three weeks after parturition.

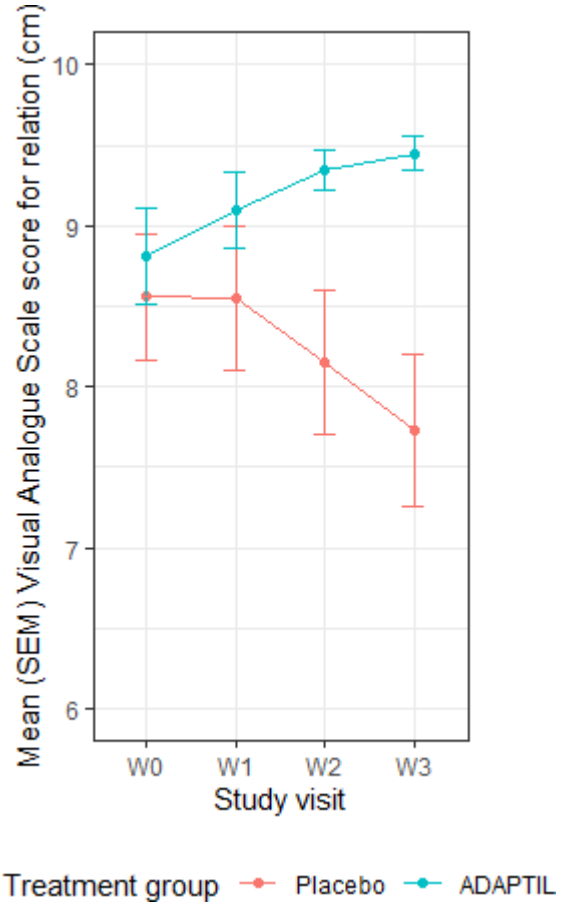

Figure 4 Mean Visual Analogue Scale score evolution for 'global bitch-puppies relationship' according to treatment and time. SEM, standard error of the mean; W0, within the first 48 hours of whelping; W1, one week after parturition; W2, two weeks after parturition; W3, three weeks after parturition.

with placebo at W1 (P=0.02), W2 (P=0.001) and W3 (P<0.0001) (table 3B). As opposed to the VAS scores focused on maternal behaviour, no significant differences according to the treatment were observed through the two scales assessing puppies. Altogether, scores in both groups were high (>9/10 for the VAS assessing global wellness) at each time point, meaning that puppy litters (as the experimental unit) seemed to feel well and comfortable.

Activity tracker data

The FitBark device was well tolerated by all animals with no complications or issues being reported. The collar bothered two bitches, based on the owner's description, during the first three days and then they adjusted to it. The system of recording was reliable; however, on three bitches the system failed to synchronise, with total data missing for two bitches and partial (10 days) in another one.

Overall, bitches from both groups increased their daily global activity on D-2, to reach a peak during parturition and then gradually decreased until four days postpartum. After that, the mother's activity level was quite stable until the end of the third week postpartum. Despite similar trends in both groups, during the full study period there was a significant difference in the global activity level of bitches according to the treatment, as expressed by the comparison of global AUC (P=0.09). Bitches exposed to ADAPTIL trended towards being more active compared with placebo (P=0.09; figure 5 and table 4).

No differences between groups were observed regarding the daily activity or respectively the daily resting time recorded by the device, at any specific time (D-1 before parturition, while giving birth, and on D7, D14 and D21 postpartum). However, at two days before parturition, bitches exposed to ADAPTIL were significantly more active for an additional hour on average than the placebo group (P=0.07), with a mean active duration of 705.3 minutes (11 hours and 45 minutes) versus 641.7 minutes (10 hours and 41 minutes; see table 5) based on the FitBark readings. On that day (D-2), bitches exposed to the placebo rested more. This difference in activity level was also reported to be close to significance on D0 (P=0.11).

Discussion

The tested treatment in this study consisted of an analogue of the dog-appeasing pheromone naturally released by bitches during lactation within the first three to four days after giving birth and up to two to five days after weaning.[33] Since bitches were assessed from whelping time to three weeks postpartum, they were all exposed to their own natural pheromone. However, the quantities of pheromone released by the ADAPTIL diffuser during the study are much more concentrated (5 per cent) than natural secretions and added markedly to the natural secretions, leading to important differences between bitches exposed to ADAPTIL and their own secretions, compared with bitches exposed to their own secretions only (placebo group). Moreover, no data so far are available in the literature on the variability of natural dog-appeasing pheromone release between bitches, breed, age or interindividual variations. Therefore, only the group under the treatment was exposed to the product in a controlled fashion. This study was the first to assess the use of ADAPTIL in the bitches' environment while they physiologically release this pheromone, to explore the benefit of exposing puppies and their mothers to greater quantities of appeasing pheromone.

All litters and bitches tolerated ADAPTIL treatment well, with no reported health problems or serious behavioural trouble during the trial. One methodological limitation of the study was the duration of the video surveillance, which might not be representative of the full behaviour of the dam. The decision to set the video observation to around one hour on average for four specific times during the postpartum period was guided by the objective to compare two treatments and not to describe maternal behaviour. In addition, although the authors tried to standardise the time during the day for the video recording, the final decision was made by the breeder and/or the caregiver, and was therefore

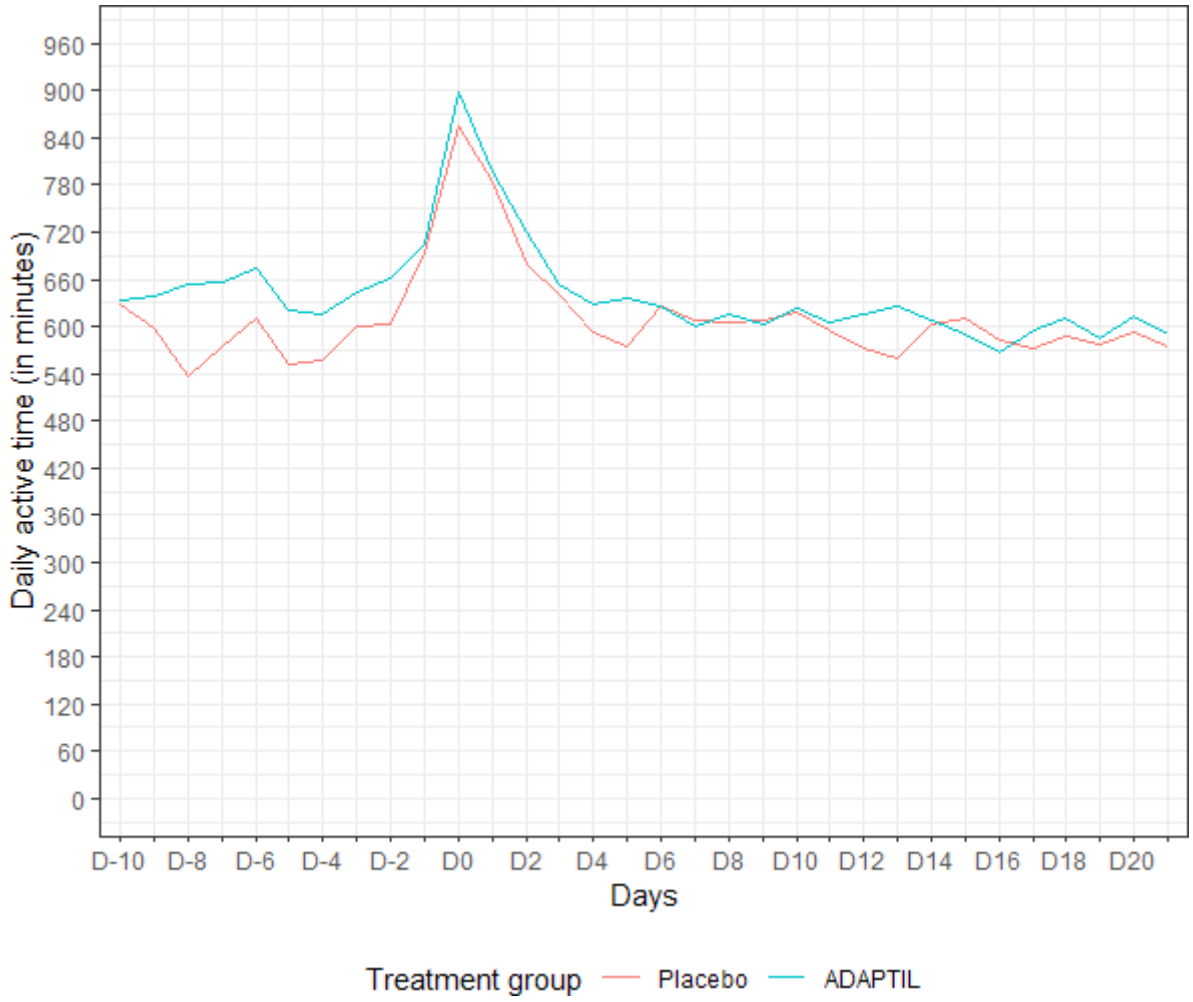

Figure 5 Evolution of the mean activity time according to treatment and time (Days).

heterogeneous. However, this heterogeneity was more evident as the bitch progressed in the postpartum period. Independently of daytime, in the video of W0, the majority of bitches of both treatment groups were in close contact with their puppies. Then, from W1 the majority of bitches spent less time with the puppies. The progression in the puppies' development was inversely related to the interest and the interaction by the bitch. By W2–W3, few bitches spent more than 70 per cent of the video observation time in contact with their puppies. Further, despite the rigour of the inclusion criteria, the routine of each kennel was not changed during the trial. Therefore, the frequency and duration of exits of bitches from the maternity area most likely also had an impact on the intensity of maternal–puppies dyad observed in the video. The number of different breeds as well as the characteristics of maternal style specific to each breed might have also influenced the data. Another factor to consider is environmental variation, which could influence the time spent in the whelping box. In the clinical trial, all maternities had a source of heating. The temperature of the whelping box most likely has a direct influence on the time that the bitch spends in the whelping box. The use of a heating source was at the discretion of the caregiver and/or breeders with good experience on kennel management, so the authors believed that it was well regulated according to the wellbeing of the bitch and puppies, but it remained an uncontrolled factor. These singularities were not taken into consideration in the evaluation of the results. Regardless, all activities in the maternal behaviour repertoire decrease over time as the puppies become more mature, as described in the literature.[12–15 42–45] Behaviours such as licking the puppies, essential to stimulate the puppies and maternal bonding around parturition, become less frequent with time. After three weeks, the puppies do not need the stimulation of the mother for physiological elimination (urine and faeces), and around this time the bitch seems to be more focused on cleaning the environment, looking carefully where

Table 4 Global AUC for the active time recorded by the FitBark device, according to treatment (n=39)

Parameter	Statistics	Placebo (n=21)	ADAPTIL (n=20)	Total (n=41)	P value *t*
Global AUC for the active parameter	n (missing)	20 (1)	19 (1)	39 (2)	
	Mean (sd)	16,510.0 (3551.4)	18,522.0 (3690.2)	17,490.2 (3714.2)	0.09
	[Minimum; maximum]	[9420.0; 21,198.0]	[6920.5; 22,970.0]	[6920.5; 22,970.0]	

Table 5 Distribution of the daily activity time (in minutes) recorded by the FitBark device, according to treatment

Treatment	Statistics	D-2	D-1	D0	D1	D7	D14	D21
Placebo (n=21)	n	15	18	19	19	20	20	19
	Mean (sd)	641.7 (111.5)	742.1 (153.3)	904.0 (171.0)	823.8 (149.6)	660.9 (95.1)	657.7 (154.0)	627.2 (123.7)
	[Minimum; maximum]	[441.0; 877.0]	[449.0; 1047.0]	[465.0; 1229.0]	[468.0; 1054.0]	[405.0; 797.0]	[430.0; 1065.0]	[390.0; 809.0]
ADAPTIL (n=20)	n	18	18	19	19	18	18	19
	Mean (sd)	705.3 (123.5)	748.5 (154.7)	966.6 (111.1)	852.8 (129.5)	669.3 (72.2)	670.4 (96.1)	673.1 (100.6)
	[Minimum; maximum]	[528.0; 961.0]	[488.0; 1042.0]	[772.0; 1171.0]	[521.0; 1080.0]	[495.0; 807.0]	[522.0; 876.0]	[493.0; 915.0]

D, day.

the puppies urinate and defecate and licking the area. While the behaviour of licking the floor after puppies' elimination was not part of the repertoire observed, this shift in focus was also reflected by a decrease in oronasal interaction over time. In relation to nursing, the decrease in time dedicated to this activity was similar to all interactions observed during the study. However, as the puppies are more mature, contact time was almost totally committed to nursing (W1–W3), while at W0 the bitch might stay in close contact with the puppies without nursing them. Indeed, as observed by Grant,[44] puppies most likely become more efficient and eager to nurse with time.

Although the demonstrated maternal care showed a steady and slow decline over time and no difference was observed in the video recording between both groups, the perception of the caregiver of the dogs was not the same. For breeders, based on the VAS results, females treated with ADAPTIL were more often in contact with their puppies and showed more attention in general. The same was observed for nursing time. While no significant differences were detected through the video in the proportion of time for nursing between groups, for bitches exposed to ADAPTIL the decline in the activity was less pronounced than for the bitches in the placebo group (figure 1). This slight difference might have been more noticeable for the breeders since the duration of the video only reflected on average 4 per cent of daily time and the caregiver had longer time of contact with the bitch and the puppies.

Another difference was the nursing postures between groups, with bitches under ADAPTIL nursing more often in lying-down position. It is believed that the lying-down position might reflect a more relaxed state of the dog. The position at nursing has been associated with the quality of maternal care in one study,[15] where the more challenging nursing posture was retrospectively observed to have a positive effect on the selection of puppies to become guide dog.

While no difference was observed in the proportional time of all activities measured as part of maternal behaviour, it is possible that the sample time for the video was not long enough to highlight any difference. Nevertheless, the caregivers more easily perceived the difference in the expression of maternal behaviour based on the VAS evaluation since they spent longer and more frequently with the animals. The VAS is a simple and valid instrument commonly used in human medicine to quantify subjective parameters,[39] such as the quality of maternal behaviour expressed through the attention and relationship of the bitch and their puppies. In the view of breeders/caregivers, a better adapted maternal behaviour, essential for the survival of the newborn at parturition,[46] was detected for longer time during the postpartum period of bitches under ADAPTIL. In addition, while poor maternal behaviour postwhelping can significantly affect pup mortality,[47] the mortality rate was very low for all recruited bitches (4.2 per cent). Moreover, although some bitches did not spend a lot of time with the puppies, total lack of maternal behaviour or mismothering resulting in neglect of puppies at the time of birth was not observed during the trial.

The use of telemetric monitoring of movement behaviours of dogs is a new tool for research since it is possible to monitor the animal remotely. Therefore, the data allow better understanding of the profile of activity of dogs on a daily basis without human interference. During the peripartum period, it is an even more interesting device, since the bitch normally can be overprotecting the puppies and the presence of human beings might interfere with the full demonstration of maternal behaviour. The profile of bitches' activity in maternity, to the authors' knowledge, is the first one described in the literature using a telemetric device. As expected, the levels of activity were higher around parturition, and then returned roughly to the levels before the whelping time. The difference in activity time at two days before parturition in favour of the bitches under ADAPTIL is hard to explain with no physiological evidence to support any statement. The common changes observed around parturition are to spend more time in the whelping area,[24] seek seclusion[24 28] or search excessively for human attention.[25 26 48]

Based on the results, exposure to ADAPTIL has a direct effect on improving maternal care in the bitch based on the breeders' perception. Although maternal behaviour is instinctive, it can be negatively influenced by external factors such as anaesthetic drugs, pain or excessive human interference[17] in a dynamic situation most likely associated to overstress. Therefore, by reducing the impact of stress, the bitches treated with ADAPTIL were more able to fully express their

maternal style. The absence of stress assessment using a physiological marker such as cortisol, in addition to behavioural assessments, could be seen as a limitation of the present study. However, only a marginal clinical meaning of baseline cortisol levels was demonstrated, with higher levels favouring bitches with high scores in maternal behaviour.[15] As stated before, the objective of the current study was to evaluate the possibility of modulating maternal behaviour. Therefore, another thought might be that ADAPTIL slightly increased maternal vigilance, as expressed by bitches under ADAPTIL being perceived by their caretakers as being more attentive. In this view, the fact that bitches under ADAPTIL were observed to nurse more often in a lying-down position could be seen as a possible way to stay closer to their puppies. The difference in the data from the FitBark collars, where bitches under ADAPTIL trended towards being more active, still needs further evaluation.

The perception of bitches' maternal behaviour through VAS is a qualitative assessment, which could be influenced by the placebo effect. Some breeders could be expecting to see greater effects due to the exposure to the test product, especially those from bitches qualified as nervous in terms of their general temperament. In this study, more 'anxious' bitches were in the ADAPTIL group (n=9) compared with the placebo group (n=5). However, this information (calm versus nervous) collected for all bitches relates to their general temperament, not necessarily their behaviour in relation to their maternal role. History data were also collected about their maternal attitude with their previous litters. All multiparous bitches were qualified by their breeders as having displayed an 'appropriate' or even 'excellent' maternal role in the past, suggesting that those breeders did not expect a particular improvement, at least no more than breeders of bitches qualified as calm.

Nonetheless, in this study, ADAPTIL modulated some aspects of the maternal behaviour, and in particular extended the period of greater attention of the bitch during the postpartum period. Bitches under ADAPTIL were more willing to stay with the puppies longer than returning to their normal activity before maternity. This first study assessing the effect of the dog-appeasing pheromone during maternity raises another question: once bitches are able to fulfil all their potential for maternal care and express it for longer as assessed during the first three weeks after whelping, could this prolongation of maternal behaviour also influence weaning time and the future cognitive behaviour of puppies? This avenue of inquiry could provide the basis for future clinical trials.

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Competing interests Ceva Santé Animale, which employs AB and TB, funded this study. NRS is currently involved in research projects sponsored by Ceva Santé Animale, under the supervision of AF and CM.

Data availability statement Data are available upon reasonable request.

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/vetrec-2019-105603).

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