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A triple blind placebo-controlled investigation into the assessment of the effect of Dog Appeasing Pheromone (DAP) on anxiety related behaviour of problem dogs in the veterinary clinic

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Abstract

The behaviour and emotional state of 15 dogs, known to be fearful of the veterinary clinic was evaluated during a standardised 5 min waiting room procedure and standardised 2 min consultation room procedure prior to a sham clinical examination, in the presence of Dog Appeasing Pheromone and placebo. Subjects acted as their own controls and were semi-randomly allocated into treatment groups to control for order effects. A triple blinding procedure was used in order to remove bias from the assessment of video recordings of the dogs, with two naïve independent raters used to analyse the video recordings of the behaviour of dogs during the test procedures. The raters showed good, and similar, agreement in their evaluation of both the specific behaviour of the dogs and their putative emotional state (relaxed, aroused and anxious). The results suggested that the use of DAP in the clinic was associated with greater relaxation of the dogs but there was no effect on aggressive behaviour during the clinical examination.

Keywords: Aggression; Anxiety; Biometry; Dog; Emotion; Pheromone

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1. Introduction

Fear and anxiety among dogs visiting the veterinary clinic and the associated problems this can cause is a well recognised and potentially significant welfare problem (Landberg et al., 2003). One study of the behaviour of dogs entering a veterinary clinic (Stanford, 1981) recorded that 60% appeared anxious, 18% expressed a fear biting attitude, 5% exhibited active defence tendencies and only 17% appeared unfrightened and friendly. The veterinary clinic frequently represents a novel environment or may be associated with a range of previous aversive experiences, which, together with the process of restraint during clinical examination or the perceived inability to escape from a potentially threatening situation, may provide specific cues for aggression (Archer, 1976). Fear, anxiety and frustration may also result in preparatory arousal and lower aggression thresholds further (Panksepp, 1998); as a result, some dogs can be extremely difficult and dangerous to handle in the clinic setting. The problem may limit the level of examination possible of an animal and the quality of treatment provided as a result, further increasing the welfare significance of the problem for the dog.

Different methods of control are adopted in practice to cope with this problem in the short term, such as muzzles and sedation; but these are not without their own risks and potential complications (Beaver, 2001; Mertens, 2002). These interventions do not help the dog overcome its underlying fear or anxiety of the clinic and may actually reinforce the behaviour in the longer term (Mertens, 2002). Whilst sensitive handling and careful management in the clinic can help to reduce the risk of the problem, specific behaviour therapy which seeks to eliminate the negative emotional associations with the clinic are often indicated in the dog's long term interest (Nielson, 2002). These generally involve sham visits to the premises at which no handling is undertaken initially and the animal is rewarded for relaxed behaviour. As the level of problematic arousal reduces with repeat exposure so the animal may be subjected to similarly rewarded sham examination procedures to desensitise it to these too. To successfully undertake such a programme requires a commitment from both the veterinary staff of the clinic and owner over a significant time period (possibly several months). Progress can be seriously compromised if the animal becomes ill in the interim and requires essential veterinary attention in the clinic (Nielson, 2002). In reality, few are able or willing to make this commitment and so there is a need to explore the potential for other, simpler, techniques which help improve the animal's perception of the veterinary clinic.

Dog Appeasing Pheromone (DAP—Ceva Santé Animale) is a synthetic mixture of fatty acids, based on those that have been identified from the secretions of sebaceous glands in the intermammary sulcus of bitches shortly after parturition (Pageat and Gaultier, 2003). These secretions are apparent 3–4 days after parturition and normally persist until 2–5 days after weaning (Pageat and Gaultier, 2003). The synthetic analogue is available as several products which allow the formulation to be delivered into the environment by differing means. For example, a spray formulation is often used to treat specific articles in the environment whilst delivery via a plug-in heated diffuser (similar to that used for some air fresheners) allows more general perfusion of the aerial environment. It has been suggested from a range of trials that DAP has calming effects in dogs in a range of stressful situations including those relating to separation from the owner (Pageat and Gaultier, 2002), firework

noises (Sheppard and Mills, 2003), travel in the car (Gaultier and Pageat, 2003) and the shelter environment (Tod et al., 2004). Although none of the aforementioned studies meet the strict scientific demands of experimenter blinding, they nonetheless provide a reasonable basis for considering the potential value of DAP to dogs who appear stressed in the clinic setting.

Therefore, the aim of this study was to evaluate the effect of DAP on the behaviour and putative emotional state of problematic dogs in the veterinary practice.

2. Materials and methods

The study was of a semi-randomised, fully-blinded, placebo-controlled, within subjects, cross-over design, as described below.

2.1. Recruitment and allocation of subjects to treatment group

Owners of dogs expressing concern about their dog's aggressive behaviour during consultations prior to the start of the study were invited to participate. All dogs had previously shown signs of fear or anxiety and some had shown aggression during examination procedures. Additional owners were recruited as a result of coverage by local media. Owners were offered the opportunity to receive assistance in modifying their dog's behaviour via a desensitisation programme at the end of the study.

Owners received a written description of the proposed procedure and an advice sheet on how to train their dog to be muzzled before the start of the study. All owners provided informed consent and were required to visit the clinic on two occasions 3 weeks apart (Fig. 1). On these days, they were asked to arrive at the clinic at individual time slots from 10.00 to 14.00 h. Owners were randomly allocated to one of two treatment groups within the constraint of their availability. Thus semi-randomisation of the allocation of subjects to treatment group and test time was achieved due to owner availability.

2.2. Experimental environment

The experimental environment consisted of the waiting and consulting room area perfused with either placebo or pheromone (verum) treatments. Both treatments, were visually identical, odourless to humans and administered via identical electrically heated plug-in diffusers. The verum treatment consisted of a mineral oil carrier (98%) and fatty acid mixture (2%), whilst the placebo consisted of just the carrier (100% mineral oil). These were provided by Ceva Santé Animale free of charge and without restrictions within the aims of the study. The device was plugged in to the waiting room and consulting room of the clinic 1 week before any assessments were made in order to give time for the treatment to perfuse throughout the experimental environment. Neither the field investigator (CH) nor principal investigator (DM), knew the true identity of the device being used at any given time.

In the waiting room (approximately, 9 m $\times$ 2.75 m), the treatment device was plugged into a socket close to the corner of the room adjacent to the waiting room chairs that were to

Schedule of test procedure.
Experimenters involved in data collection and analysis were blind to the formulation (placebo or verum) used at the time.

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Plug in device in waiting room and consulting room	Test 1. Group A. (PLACEBO)	Washout Week	Plug in device in waiting room and consulting room	Test 2. Group A. (VERUM) Test 1. Group B. (VERUM)	Washout Week	Plug in device in waiting room and consulting room	Test 2. Group B. (PLACEBO)

Fig. 1. Schedule of test procedure. Experimenters involved in data collection and analysis were blind to the formulation (placebo or verum) used at the time.

be used by the trial subject's owner. A camera (Panasonic VHS NV-S20 Palmcorder) was positioned so as to be able to record the subjects once they were seated (Fig. 2a).

In the consulting room (3 m × 2.7 m), the treatment device was placed close to the corner of the room near the examination table. Digital video cameras (JVC 700 digital cameras) were positioned so as to be able to record the subjects wherever they were in the room (Fig. 2b).

In order to give the experimenters more control over the experimental environment whilst at the same time making use of a real clinic setting, the evaluation of subjects was undertaken on a Sunday, when the clinic was normally closed.

2.3. Treatment procedures

Subjects acted as their own controls by visiting the clinic on two occasions, once in the presence of placebo, and once with verum (cross-over within subjects design). Placebo and verum diffusers were identified simply by code and used in the clinic in a different order for each of the two, approximately equally sized (8 and 7) treatment groups to control for order effects. A 2 week ''washout'' period during which animals were not taken to and so exposed to DAP at the surgery was provided between treatments for each group (Fig. 1).

Owners were provided with written instructions upon their arrival at the clinic, explaining what would happen that day and how they should behave towards their dog. They were asked to try to remain relaxed and interact with their dog as little as possible in order to maintain everyone's safety. All dogs were videoed during the test period in the two test areas. First, in the waiting room they were filmed for a 5 min period, with their owner seated on a designated chair. After 1 min, an unfamiliar dog and handler entered the room,

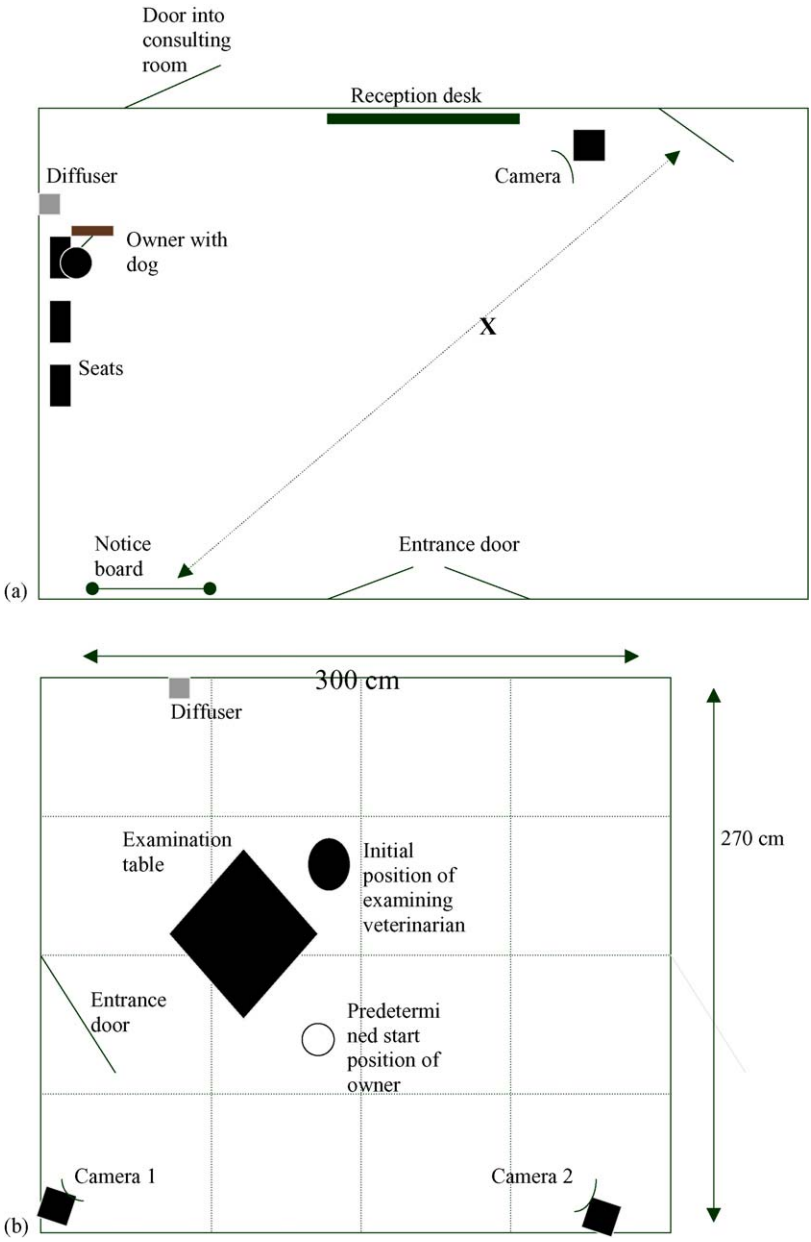

Fig. 2. (a) Waiting room layout and (b) consulting room layout.

walked past the test dog to the nearby notice board where they stayed for 10 s and then left through the door from which they had initially entered (route X in Fig. 2a). This process was managed so that it took 1 min. At the end of the 5 min period, the owner and dog were called into the consulting room, where the second set of observations were made.

In the consulting room, the dog was released from its lead and the subject was filmed for a further 2 min period. Following this, the dog underwent an ordered, non-invasive, but increasingly intrusive veterinary examination, as detailed below.

1. Dog stroked gently from head to base of tail three times.
2. Hand placed over inner right thigh pulse point for 30 s.
3. Stethoscope placed against each side of the chest for 30 s.
4. Gentle abdominal palpation undertaken for 30 s.
5. Paws lifted and examined for 5 s in the order of right then left hind limb then right and left fore limb.
6. Right then left ear flaps lifted for 5 s each.
7. Rectal thermometer placed in position for 30 s.
8. Eyes examined directly for 5 s each.

If the dog responded aggressively, including a threat to bite, the examination was terminated.

Table 1
Behavioural and emotional assessments used in the analysis of the dog's behaviour in the waiting and consulting rooms

	Definition
Mutually exclusive behaviour states	
Sit	Dog with hind quarters on the floor but forelimbs extended so that chest is not in contact with the floor
Stand	Dog standing on all four limbs
Lie	Dog with chest in contact with the floor
Mutually exclusive emotional states	
Relaxed	Calm with no visual evidence of tension in the body
Aroused	Tense and alert, but no visual evidence of anxiety
Anxious	Tension accompanied by licking, yawning, crying, agitation or observable fearful postures
Response to unfamiliar dog (waiting room)	
Approach dog	The subject moves towards the unfamiliar animal without any signs of aggression
Avoid dog	The subject moves away from the unfamiliar animal
Ignore dog	The subject neither approaches nor avoids the unfamiliar animal
Aggressive to dog	The subject barks, growls, snaps or lunges towards the unfamiliar animal
Location (consulting room)	
Door	Adjacent to the door (section 1c in Fig. 2b)
Owner	Adjacent to the owner
Edge	Majority of body within one of the peripheral blocks, but not adjacent to the door
Centre	Majority of body within one of the six non-peripheral blocks but not adjacent to the owner
Veterinarian	Approached veterinarian (recorded as a behavioural event)

Response to the passage of an unfamiliar dog was recorded in the waiting room and location of the dog was recorded in the consulting room where it was off lead.

2.4. Evaluation of behaviour and emotional state

An ethogram was drawn up of mutually exclusive behaviours to be assessed from the video tape recordings of the dogs in each of the experimental environments (Table 1). However, because a given emotional state may be expressed in a variety of differing behavioural ways, it was recognised *a priori* that measurement of specific behaviours may not reveal significant differences between individuals especially when a small sample was used. Therefore, it was decided to include an emotional assessment of the dogs to facilitate an holistic evaluation of the dogs' behaviour, which might act as a more sensitive measure of the effect of the treatment. This was achieved by profiling the animals by reference to three predetermined mutually exclusive terms relating to emotional arousal (relaxed, aroused and anxious). Reference definitions are given in Table 1. Table 1 also includes details of the location of the dog in the 2 min pre-examination period in the consulting room.

In order to maximise the objective assessment of data and increase the validity of the terms used to describe emotional arousal in the test dogs prior to their examination a triple blind procedure was used. This required the owners of the dogs, the field investigator recording the data onto video tape (CH), and those evaluating the tapes (MG, DR) all to be naïve about which treatment was being used at any given time. The use of independent raters, naïve of the field work, ensured that there was no bias in the evaluation of the tapes as a result of experience gained in the field during the course of the study. The raters independently evaluated the tapes in a non-predefined order using continuous focal sampling whilst blind to the treatment details.

To score each stage of the examination procedure conducted in the consulting room, a check list of behaviours and signs associated with anxiety, fear and aggression based on the description of Shepherd (2002) was prepared for reference. For each procedure, the dog was observed for: only signs of relaxation (score 1), lip/nose licking and other potential appeasement signs (score 2), signs of tension without a growl (score 3), signs of tension with a growl (score 4), attempts to escape (score 5), snapping, biting or other overtly aggressive behaviour (score 6). If the dog began to struggle so that it became difficult to restrain on the examination table or it became aggressive the examination was stopped and the dog was scored 6 for all subsequent parts of the examination procedure. A record was also made of the number of stages of the examination procedure which were completed for each individual (score 1–8 from the list above).

2.5. Statistical analysis

The duration of the various behaviour and emotional states recorded by each of the independent raters was converted into a proportion of the total observation time prior to any analysis. Frequency data were unchanged. All statistical analyses were conducted using Minitab 12.1 and 13.0 (Minitab Ltd.).

The agreement between the two raters for the states was assessed using Spearman's rank correlation coefficient (Martin and Bateson, 1993) since the data were not normally distributed and a Kappa coefficient was not considered to be appropriate (Maclure and Willett, 1987).

A Wilcoxon matched pairs test was used on the average of the combined data to assess the significance of any difference in the frequency and duration of each behaviour and emotional state of the dogs under the two treatment conditions.

A Wilcoxon matched pairs test was also used to assess differences in the individual stage and total examination scores of the dogs in the two treatment conditions as well as the number of stages of the examination procedure completed by subjects under each treatment.

Experimenters were deblinded about treatment identity following statistical analysis using treatment code.

3. Results

3.1. Subjects

Fifteen dogs were enrolled onto the study from 45 initial contacts. All were reported to have exhibited signs of fear within the veterinary clinic environment for over 1 year. Details of the subjects are given in Table 2.

3.2. Agreement between experimenters

Spearman rank correlation revealed an above acceptable level of agreement (>0.7, Martin and Bateson, 1993) between the two raters for all behavioural states. Across all observations of behaviour in the waiting room a correlation of 0.91 for measures of duration and 0.87 for measures of frequency was evident ($p < 0.001$ in both cases). In the consulting room, correlations of 0.80 were found for both sets of measures of behavioural state ($p < 0.001$ in both cases). Correlation of the data relating to the emotional state of the dogs was generally similar being 0.93 for durations and 0.85 for frequency measures in the

Table 2
Subject details

Dog ID	Group allocation	Sex	Age (years)	Breed
1	A	M(N)	7	German Shepherd
2	A	M	2	Yorkshire Terrier
3	A	F(N)	10	Cross Breed
4	A	F	2	German Shepherd
5	A	M(N)	4	Retriever
6	A	M	5	Border Collie
7	A	M	3	Yorkshire Terrier
8	A	F(N)	5	Staffordshire Bull Terrier
9	B	M	2	Staffordshire Bull Terrier
10	B	M(N)	10	Bichon Frise
11	B	M	9	Bichon Frise
12	B	M	2	Cocker Spaniel
13	B	M	7	German Shepherd
14	B	M(N)	7	German Shepherd
15	B	M	7	Bearded Collie

M: Male, F: female, N: neutered.

Table 3
Spearman rank correlation coefficients for each apparent emotional state of dogs in each test environment recorded by two independent raters

	Duration	Frequency
Waiting room; emotional state		
Relaxed	0.80	0.83
Aroused	0.92	0.74
Anxious	0.97	0.93
Consulting room; emotional state		
Relaxed	0.89	0.89
Aroused	0.87	0.84
Anxious	0.91	0.86

All values $p < 0.001$.

waiting room, whilst in the consulting room it was 0.87 for durations and 0.86 for frequency measures ($p < 0.001$ in all cases). Correlations for both measures of each specific emotional state are given in Table 3. Inter observer correlations for all other measures were also acceptable and highly significant.

3.3. Effect of treatment

3.3.1. Waiting room

There were no significant differences in the specific categories of recorded behaviour of the dogs under the two treatment conditions whilst the owners were in the waiting room. However, there were significant differences in the duration ($W = 10$, $p = 0.045$) of the anxious state recorded and both the frequency ($W = 78$, $p = 0.003$) and duration ($W = 91$, $p = 0.002$) of the relaxed state, with the dogs appearing more relaxed and less anxious when DAP was used (Table 4).

3.3.2. Consulting room

There were no significant differences in the specific categories of recorded behaviour of the dogs under the two treatment conditions during their 2 min exploration of the consulting room. However, during the DAP treatment sessions dogs visited the edge of the room significantly more frequently ($W = 71$, $p = 0.013$). There were also significant differences in the duration of the anxious state ($W = 16$, $p = 0.024$) and frequency of expression of the relaxed state ($W = 45$, $p = 0.009$). Exposure to DAP was associated with the reduced measure of anxiety and increased measure of relaxation (Table 4).

There was no significant difference in the overall scores or stage of examination achieved in the two treatments.

4. Discussion

Recently, there has been growing recognition of the potential value of holistic assessments of the behaviour of animals, such as those relating to underlying emotional

Table 4
Comparison of summary measures of behaviour and apparent emotional state of dogs recorded in waiting room and consulting room with and without DAP

	Frequency		Duration (%)	
	Placebo	DAP	Placebo	DAP
Waiting room				
Behaviour				
Sit	3.3	3.6	42.0	36.3
Stand	7.1	7.0	49.7	54.7
Lie	1.0	1.2	8.3	9.0
Approach dog	1.4	1.7		
Avoid dog	0.1	0.4		
Ignore dog	0.3	0.1		
Aggressive to dog	0.4	0.6		
Emotional state				
Relaxed	1.0a	2.5a	6.64b	19.82b
Aroused	5.5	4.5	21.13	18.41
Anxious	7.5	7.5	65.70c	55.70c
Consulting room				
Behaviour				
Sit	0.5	0.5	9.7	9.0
Stand	4.7	4.5	88.6	87.5
Lie	0.1	0.2	1.7	3.5
Position				
Door	2.5	1.8	24.7	16.7
Owner	1.8	2.0	18.3	22.5
Edge	2.9	3.9	34.5d	41.3d
Centre	2.6	2.7	22.5	19.5
Approached veterinarian	0.2	0.3		
Emotional state				
Relaxed	0.0e	2.5e	0.00	18.33
Aroused	1.5	2.0	14.58	15.00
Anxious	6.00	3.5	72.50f	40.83f

Values presented are the population median derived from the average scores for each dog recorded by the two raters. Values with the same letters are significantly different from each other (Wilcoxon matched pairs test, $p < 0.05$).

state (e.g. Wemelsfelder et al., 2000, 2001). This approach has been highlighted particularly by those working in the field of animal welfare, where the ultimate goal is often to develop scientifically rigorous approaches to the assessment of a psychological state which might not be amenable to direct measure. A similar problem is faced by those working in the field of veterinary behavioural medicine, where the aim is often to alleviate an inferred emotional state which gives rise to problematic behaviours (Casey, 2002). However, whilst it might be possible to infer emotional state from indirect measures of behaviour and/ or physiology, a given state may be expressed in a variety of behavioural ways. This means that simple behavioural measures may not be very sensitive measures of these emotional states. Individuals have different general coping styles associated with their temperament (Koolhaas et al., 1999), and the specific behaviours shown at any given

time may vary with the environmental appraisal made by the animal at the given time. For example, when anxious, animals tend to inhibit ongoing activities (Gray, 1987); some may choose to withdraw to a point of perceived safety whilst others may remain where they are and become more vigilant. Therefore, since a range of behaviours may be associated with a single emotional state and different individuals may have different behavioural styles, quantification of specific behaviours alone may not be the most appropriate or efficient method of assessing emotional state. This is particularly relevant when one wishes to minimise the use of animals in research. With smaller sample sizes, the variability in specific measures of behaviour between subjects may hide significant differences between individuals, when real differences in less specific (but potentially more important) measures, such as emotional state, may exist. In this situation, and so long as the measures can be shown to be reliable (i.e. repeatable) it may be preferable to use such holistic judgements. Reliability should not be confused with validity (the extent to which the measurement reflects the phenomenon of interest (Martin and Bateson, 1993)). The reliability of the measure suggests that a change is occurring at some level, whilst the validity of the measure helps to identify at what level this change is occurring. Therefore, the issues of reliability and validity will be discussed separately.

The measures of behaviour and emotional state used in this study appeared good and to have similar levels of reliability as revealed by the level of agreement between the two independent raters. The finding that there were no significant differences in behaviour, but that the dogs were consistently rated as being more relaxed and less anxious in the presence of DAP is in accordance with our expectations based on the argument above that simple behaviour measures are a potentially less sensitive and inappropriate measure of psychological changes within subjects in some circumstances. This is further reinforced by the finding that the nature of these changes are consistent with the expected psychological change given the results of previous studies of the application of DAP in practice (Pageat and Gaultier, 2002, 2003; Gaultier and Pageat, 2003; Sheppard and Mills, 2003; Tod et al., 2004). Accordingly, we conclude that if just behaviour measures are used in this scale of study, there is a danger of a Type II statistical error. This is a more consistent interpretation of the evidence than the alternative which would be to infer that the apparent change in emotional state being a type I error. However, we recognise that this is a possibility, albeit an unlikely one in our opinion. The next question to consider is whether these holistic measures are indeed valid.

Valid measures should be as unbiased as possible, and to this end the triple blind was an important control in this study. The use of independent raters who had not been involved previously in the study meant that the tapes could be analysed without systematic bias as a result of knowledge and experience of the field tests. This was particularly important as the field worker (CH) noticed that a characteristic breath odour, which is frequently associated with distressed dogs, appeared to be absent under one of the treatment conditions. It should also be noted that several owners commented that their dog appeared more relaxed in one treatment condition than the other. These factors, together with a recognition of which recording related to which treatment, could have led to inadvertent bias, should the tapes have been evaluated by this researcher. We believe the terms used have at least face validity as they were chosen following discussion between the raters and principal investigator who are all experienced veterinarians with a special interest in companion animal behaviour problems.

Thus, whilst we cannot strictly conclude that the DAP directly reduced the anxiety of the dogs in the clinic, we believe we can suggest that it appeared to produce changes consistent with a reduction in anxiety in the dogs as perceived by the authors. These changes could be as a result of direct effects of DAP on the dog and/or changes in owner behaviour.

If the anxiety of the dogs was reduced, it remains to be explained why there was no apparent difference in the response of the dogs to the clinical examination procedure during the two treatment conditions. The touching of an individual is a potentially increased level of perceived threat (Archer, 1976) and so in this condition, the aversiveness of the environment might have been more intense for the dogs and so over-ridden any mild anxiolysis induced previously. We cannot, therefore, conclude that DAP helps to reduce aggression in the veterinary clinic on the basis of this study. However, longer term studies are warranted to investigate this further since there may be a cumulative effect with repeat exposure. In support of this, it is worth noting that several owners, who were subsequently identified as having been exposed to DAP on their first assessment, commented that on both occasions their dog was not as anxious or aggressive as they would normally expect. Sample sizes were too small to realistically assess if there was a difference in the magnitude of effect between the group exposed to DAP first and that which had placebo first.

Finally, we need to explain why dogs under DAP treatment tended to spend more time at the periphery of the consulting room. Whilst this result could be anomalous, there is also a viable explanation for this effect. The diffuser used to deliver DAP and its carrier continuously heats a small amount of the mixture for effective volatilisation. This tends to condense on the cooler surfaces within the treated areas. We, therefore, hypothesise that the wall surfaces had a higher concentration of the active constituent which was detectable by and attractive to the dogs.

5. Conclusion

Initial exposure to Dog Appeasing Pheromone in the veterinary clinic appears to help reduce the signs of anxiety associated with the less aversive aspects of a visit to the veterinary clinic. However, there is no evidence that, on a single exposure, it reduces overt expressions of aggression during the clinical examination of dogs predisposed to this behaviour. Further longer term studies are warranted in order to determine if repeat exposure has a consistent or cumulative effect on anxiety, which might help to reduce the risks posed by distressed animals in the clinic.

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