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Evaluation of a plant-based food supplement to control flea populations in dogs: A prospective double-blind randomized study

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ABSTRACT

A prospective double-blind randomized placebo-controlled study evaluated the tolerance and efficacy of the biological plant-based food supplement Bioticks® (extracts of thyme, rosemary, melissa, fenugreek, absinthe and lemongrass) as a flea-control product. Twelve dogs were used as placebo controls (group A). Ten dogs under similar housing conditions received the same food daily but supplemented with Bioticks® (group B). Flea counts were performed on D0 and 14, then 1, 2, 3, 4 and 5 months after the beginning of the study. No flea treatment was given or environmental modifications made during the 6 months prior to beginning and throughout the duration of the study. Efficacy was calculated according to Abbott's formula. No adverse event was recorded. At inclusion, dogs in groups A and B hosted a mean ± standard deviation of 7.9 ± 3.3 and 9.5 ± 3.6 fleas, respectively. The mean flea population in group A steadily increased until 4 months after D0 (21.5 ± 4.9 fleas/dog). Meanwhile, the mean flea population in group B dogs remained stable for the first month but then steadily decreased to reach an average of 3.1 ± 1.7 fleas/dog at D0 + 5 months. The percentage efficacy in the treated group as compared to the non-treated group was 33%, 51%, 71%, 80% and 82% at 1, 2, 3, 4 and 5 months, respectively.

Bioticks® was shown to be safe and effectively limited the flea population in dogs with a moderate flea infestation in conditions that were highly favourable to flea development. This is the first study to evaluate a plant-based product as an oral supplement for flea control.

1. Introduction

The cat flea (*Ctenocephalides felis felis*) is the most common ectoparasite of cats and dogs and the most prevalent flea species in France (Franc and Cadiergues, 1998). Its presence may cause pruritus which can be severe in cases of large infestation or flea allergy dermatitis. It is also a vector for several diseases agents of veterinary and human public health importance such as those causing dipylidiosis (Rust, 2017), rickettsioses (Perez-Osorio et al., 2008; Bitam et al., 2010; McElroy et al., 2010), bartonellosis (Mogollon-Pasapera et al., 2009; Breitschwerdt et al., 2010), plague (McElroy et al., 2010; Eisen and Gage, 2012), filariosis caused by *Achantocheinonema reconditum* (Napoli et al., 2014) and flea-allergy dermatitis (Blagburn and Dryden, 2009; Dryden, 2009). Over the last few years, several new active ingredients have been developed and marketed to control a variety of arthropod pests, often combining two or more molecules to broaden the spectrum of activity (Rust, 2017).

Veterinary products for flea control that are given directly to animals have different modes of application: surface (shampoos, spot-on applications, collars, sprays, powders, dips) or systemic (tablets, spot-on applications, injectables). Their active ingredients are designed to break the flea cycle by killing adults (adulticides) and/or larvae (larvicides) and/or eggs (ovicides) or prevent the larvae from developing (growth-hormone analogues or insect growth regulators). They can also have a repellent effect aimed to prevent fleas from biting. Other veterinary products focus on environmental treatment (sprays, powders, flea traps) (Pfister and Armstrong, 2016). A growing demand for non-chemical, biological or "natural" flea products has been noted but this has not resulted in the development of successful strategies (Rust, 2017).

Flavonoids are plant metabolites with multiple health effects on plants and humans (Panche et al., 2016). Some of them have

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demonstrated insecticidal properties at different life stages of various insect species (Treutter, 2005; Palma-Tenango et al., 2017). However, no studies have been conducted to assess the *in vivo* efficacy of plant extracts as a way to control flea populations.

The purpose of this study was to assess the clinical efficacy and tolerance of a plant-based food supplement as a biological flea control approach in dogs naturally infested by fleas. The study was designed as double-blind randomized placebo-controlled study.

2. Material and methods

2.1. Animals and environment

Twenty-two adult hunting dogs, of three different breeds (Bruno du Jura, Bleu de Gascogne and Griffon), in good general health, belonging to the same owner and living on the same site in groups of one to five dogs in seven semi-open kennels were included in the study.

The written consent of the dogs' owner and approval from the Toulouse veterinary school (Université de Toulouse, ENVT) Ethical Committee were obtained prior to beginning the study.

The inclusion criteria were animals in good general health as confirmed by a general physical examination by a licensed veterinarian and the absence of clinical signs such as gastrointestinal signs, lethargy, obvious lameness, respiratory signs or cutaneous signs other than signs related to flea infestation (mild alopecia and scaling were allowed). The animals should not have received any antiparasitic treatment over the past six months. At least five live fleas should be present on each animal at the time of inclusion.

The 22 dogs were allocated to groups A and B at random (simple randomization, Research Randomizer (Version 4.0)). The two groups were housed in separate kennels and were never in direct or indirect contact with dogs of the other group. There were 12 dogs in group A and 10 dogs in group B. The mean age of dogs was 5.4 years for group A and 3.6 years for group B. All the dogs in group B were males while group A comprised seven male dogs and five females. The mean body weight was similar in both groups (24 kg for group A and 25.3 kg for group B). The environmental, housing conditions and level of care remained the same in both groups (semi open kennels on clay courts) throughout the study. No cleaning was done or insecticidal application carried out on the premises before or during the entire duration of the study. Water was freely accessible. All dogs received a standardized, exclusively dry grain-free base feed (A complet/chien/grain free, Sauvale Production, Chateau-Gonthier sur Mayenne, France). The amount of feed (420 g per dog and per day) was based on the manufacturer's recommendation for very active dogs (more than 2 h of exercise daily). No antiparasitic drug of any type was allowed for the duration of the study.

2.2. Active ingredient

The active ingredient was composed of biological extracts from several plants including: 25 (w/w) % thyme (*Thymus V.)*, 20 (w/w) % rosemary (*Rosmarinus O.*), 37 (w/w) % melissa *(Melissa O.),* 15 (w/w)% absinthe (*Artemisia absinthium* L.), 1 (w/w)% lemongrass (*Cymbopogon citratus*) and fenugreek (*Trigonella foenum G.*) (Bioticks®, Biodevas Laboratoires Savigné L'Evêque, France; proprietary data). It was incorporated into the diet by the food manufacturer [1 (w/w) %] prior to shipment to the animals' owner (3.5 mL for an individual daily food intake of 420 g). The supplemented feed was visually identical to the non-supplemented feed. The bags (12 kg each) were only distinguishable by the letter "A" or "B" on the packaging. The bags were stored in an appropriate location, under identical conditions of temperature and humidity, in a dry clean place, below 25 °C.

All dogs in the same group received the same feed throughout the entire duration of the study, i.e. 150 days. Group A dogs received the neutral non-supplemented feed while group B dogs received the feed supplemented with Bioticks®. The feed was given to the animals once daily by the owner. Neither the owner nor the investigators were aware of the nature of the feed. Unblinding occurred after the conclusion of the study.

2.3. Assessment

2.3.1. Evaluation of the flea population

Flea counts were performed by applying the method recommended by the European Medicines Agency (EMA) for carnivores in field studies (EMEA/CVMP/EWP/005/2000-Rev.3, 2016). The flea population on each dog was evaluated by visual counting on five different zones: dorsal line, base of tail, right flank, left flank and inguinal region. The counting time for each zone was limited to 1 min and counting was performed by spreading the fur apart with two hands until the entire zone had been assessed. Seven counts were performed for each animal: the first count before beginning the study (D0), the second count 14 days later (D14), and the 5 others 1, 2, 3, 4 and 5 months after beginning the study (D30, D60, D90, D120 and D150, respectively). The study was conducted in the South of France during the summer and fall of 2018 (closure of the hunting season).

2.3.2. Clinical evaluation

The immediate tolerance was evaluated by the animals' owner. Signs of intolerance included reluctance to eat the feed, vomiting, diarrhoea, decreased activity levels and any other obvious clinical signs. The short and medium-term tolerance was evaluated during the follow-up visits and consisted of a general and dermatological clinical examination.

2.3.3. Dermatological evaluation

Dermatological evaluations were conducted, all by the same investigator, on D0, D14, D30, D60, D90, D120 and D150.

Six parameters were evaluated on each animal, (surface area affected, pruritus, erythema, scaling, presence of secondary lesions (excoriations and crusts) and lichenification) and graded on a 0–3 severity scale [0: absence; 1: mild 2: moderate; 3: severe] (Viaud et al., 2012).

2.3.4. Results analysis

Flea count reduction was calculated at each time point *t* using the arithmetic mean of flea counts according to the Abbott formula: flea count reduction (day *t*) (%) = 100 x (mean flea count group A day *t* –mean flea count group B day *t*)/mean flea count group A day *t* (Abbott, 1987). Flea counts for both groups were compared using a *t*-test for two independent samples with a significance threshold of 5%. Calculations were performed using the XLSTAT (Addinsoft – *2018.5.52447*) software.

3. Results

3.1. Animal population

Twenty-one of the 22 dogs, completed the study as one dog was removed from group B on D60 when the owner moved it from the kennels for reasons unrelated to the study.

3.2. Tolerance

No adverse reaction after feeding was reported by the owner. There was never any leftover food after feeding throughout the study. No abnormalities were detected during any of the clinical examinations at any time.

Table 1
Flea counts on dogs from groups A and B (SD: standard deviation, NS: non-significant difference, S: significant difference).

		D0	D14	D30	D60	D90	D120	D150
Group A	Mean	7.9	9.8	14.5	17.8	20.8	21.5	17.1
	SD	3.3	3.6	4.6	9.1	7.7	4.9	5.1
Group B	Mean	9.5	10.1	9.7	8.8	6.0	4.3	3.1
	SD	3.6	2.6	3.1	3.3	3.1	1.7	1.7
% of efficacy		/	−2.7%	33.1%	50.8%	71.1%	79.8%	81.8%
Test t p value (p = 0.05)		0.296	0.846	0.011	0.011	< 0.0001	< 0.0001	< 0.0001
		NS	*NS*	*S*	*S*	*S*	*S*	*S*

3.3. Dermatological examinations

The dermatological examinations revealed an overall good skin and coat quality, despite scaling graded at 1/3 at the beginning and during the study for some dogs (two dogs in group A and two dogs in group B on D0, D14, D30; three dogs in group A and one dog in group B on D60; four dogs in group A and one dog in group B on D90 and four dogs in group A on D120 and D150).

Seven dogs (all in group A) exhibited moderate to severe pruritus from D60 or D90 to D150.

3.4. Flea populations

At inclusion, dogs harboured between 5 and 15 fleas (group A mean 7.9 ± 3.3 fleas/dog and group B mean 9.5 ± 3.6 fleas/dog).

The mean flea population in dogs from group A (feed non-supplemented) steadily increased until 4 months after D0 (21.5 ± 4.9 fleas/dog) and then decreased on D150 (17.1 ± 5.1 fleas/dog). Meanwhile, the mean flea population in dogs from group B (feed supplemented with Bioticks®) remained stable for the first month of the study but then progressively and steadily decreased to reach an average of 3.1 ± 1.7 fleas/dog on D150 (Table 1, Fig. 1).

The percentages of efficacy in group B dogs were 33% on D30, 51% on D60, 71% on D90, 80% on D120 and 82% on D150.

Statistical analysis did not show any significant difference at D0. The mean flea counts in the two groups between D30 and D150 were significantly different ($p < 0.05$).

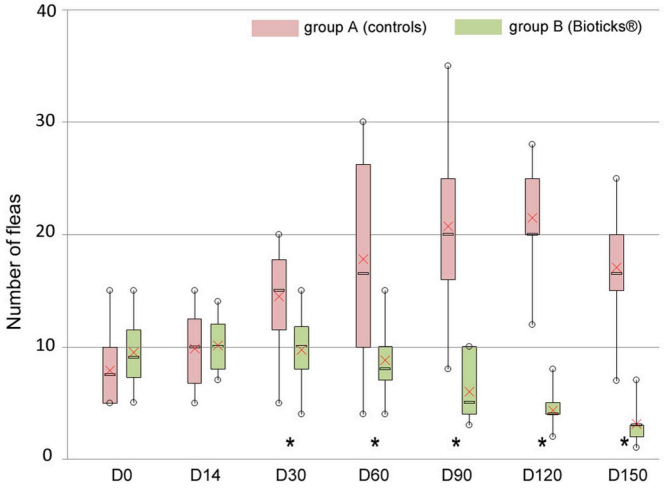

Fig. 1. Comparison of the distribution of the number of fleas per animal depending on the type of feed received (group A non-supplemented feed in pink; group B Bioticks® in green) and time (D0 = beginning of study). The horizontal line in the box indicates the median value, the borders of the box correspond to the 25th and 75th percentiles and the whiskers indicate the lowest and highest results. The cross (x) inside the box indicates the mean value. The star (*) shows a statistical difference between the two groups ($p = 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

In the present study, the orally supplemented flavonoid, terpenes and sulphur heterosides-rich Bioticks® was able to significantly reduce the flea population in the treated group of dogs.

The Bioticks® preparation which was evaluated in the present study only contains biological hydroalcoholic extracts of thyme, rosemary, melissa, fenugreek, absinthe and lemongrass, especially antioxidant flavonoids, terpenes and sulphur heterosides (Biodevas Laboratoires, proprietary data).

Flavonoids are secondary plant metabolites, derivatives of 2-phenyl-benzyl-γ-pyrone. They have a diverse chemical structure and participate in plant protection against biotic (herbivores, pathogens) and abiotic stresses (UV radiation, heat). Because of their antioxidative properties, they also maintain a redox state in cells (Mierziak et al., 2014). Flavonoids play an important role in protecting plants against feeding insects and herbivores (Harborne and Williams, 2000). By affecting enzymatic activity and preventing the growth of larvae of different insect species, some *in vitro* studies have shown that certain types of flavonoids can target various agricultural pests through ovicidal effects or by altering oviposition and fecundity, increasing adult mortality, causing weight reduction, and decreasing the emergence of adults (Palma-Tenango et al., 2017). A significant number of insect species have been shown to be sensitive to flavonoids in feeding tests (Treutter, 2005). *In vitro* studies have indicated that a number of flavonoids exhibit anti-cholinesterase activity (Panche et al., 2016). However, a significant difference seems to exist between the biological properties of flavonoids *in vitro* and their bioactivity *in vivo* and bioavailability and biotransformation are limiting factors for biological activities in humans. The degree of absorption depends on several factors, including the individual flavonoid subtype (Viskupičová et al., 2008).

Although the dietary supplement Bioticks® is not a veterinary medicinal product and does not require such regulatory evaluation, we elected to evaluate it according to the strict requirements of the EMA for veterinary medicines (EMEA/CVMP/EWP/005/2000-Rev.3, 2016). At the end of the study, its parasitological performance attained 80% (D120 and D150) in the feed-supplemented group, and led to low levels of infestations in treated dogs. The difference in counts between treated and untreated animals from D30 was statistically significant at a level of 5%. The performance is indeed below the efficacy of conventional veterinary products (Rust, 2017), which should be over 95% (EMEA/CVMP/EWP/005/2000-Rev.3, 2016).

The initial level of infestation (5–10 fleas per dog) was considered as medium ([0–5[fleas per dog = low; [5–10[fleas per dog = medium; [10–20[fleas per dog = high; > 20 fleas per dog = very high). This level of infestation on animals living in an environment favourable to flea development (clay courts, external and internal temperature between 20 and 30 °C) is likely to increase within a few weeks to become high to very high. This occurred in the control group. It is also likely to cause, in some animals, cutaneous lesions (hair loss, erythema) associated with various degrees of pruritus. Pruritus, along with scaling was principally observed in control dogs. At D150, control dogs haboured fewer fleas than at D120. This was likely due to the decreasing

temperatures (end of fall), slowing down the flea life cycle.

Four weeks were necessary before the first effects of the tested formulation became apparent. This might be due to the fact that the active molecules need to be incorporated into the skin secretion products (sebum) to exert their repellent activities. We also speculate that the active ingredients, once in the blood stream and therefore ingested by the adult fleas during the blood meal, exert an antifeeding effect (Koul et al., 2008) by modifying the blood's flavour. The amount of blood ingested is therefore reduced, leading to fewer eggs being laid and ultimately a decreased efficiency of the life cycle. Considering the characteristics of the flea life cycle (eggs and all immature stages in the environment), this would explain the slow efficacy compared to conventional adulticidal products commonly given to pets (Rust, 2017). Nevertheless, Bioticks® acted more rapidly than lufenuron, an insect growth regulator given alone to dogs living in infested households, which took between two to three months (Smith et al., 1996; Cadiergues et al., 1999), to achieve full efficacy, albeit at an ultimately higher ceiling than that reported for Bioticks in the present study.

The ingredients used in the Bioticks preparation are certified to meet human food safety standards. No clinical abnormalities were detected throughout the time course of the present study. Nevertheless, urinary, hematological and biochemical assessment during and at the end of the study would have been relevant.

The advantages and limitations of this “non-conventional” product may place it in a particular context of use. Indeed, its composition based solely on plant extracts makes it a product likely to satisfy owners seeking to reduce the use of “chemical” products and searching for “natural” and easy methods of flea control in dogs in the long term. On the other hand, its slow mode of action and a lower percentage reduction of flea populations than usually required for adulticidal drugs seem relatively incompatible with the current expectations of most owners who want products acting very quickly and efficiently. Additionally, these limitations cannot support its sole use in flea allergic dogs, as in order to deliver the best possible control of flea allergy, the antiparasitic protocol should combine a very fast flea adulticide activity with a prolonged efficacy. Thus, this product could be part of an integrated flea control approach, administered in combination with an adulticide given as a spot treatment initially, and then Bioticks® would be used alone over the long term. Else, it could be envisaged as a sole treatment when the initial flea burden is low, in the absence of flea allergic dogs, in order to prevent or limit re-infestations and achieve sustainable ectoparasite control in pets and in canine communities (shelters, hounds, pet stores …). With respect to both of these scenarios, occasional ‘booster’ treatments with an adulticide could also be considered, reducing reliance upon adulticidal products. These two treatment schemes could provide opportunities for further work. In addition, it would be relevant to test the efficacy of these plant extracts on other pests, such as ticks, and also in other pet species, e.g. cats.

5. Conclusion

This double-blind randomized placebo-controlled study conducted according to the current regulatory guidelines, shows that treatment of flea-infested dogs with the plant-based Bioticks feed supplement for five consecutive months resulted in a reduced flea burden, in the absence of environmental cleaning or insecticidal measures. No adverse event or signs of intolerance to the product were recorded. The results suggest that this natural adjunct product (initially combined with an adulticidal product) would be a very attractive proposition to owners and practitioners seeking natural alternatives.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The study was partly supported by Laboratoires BIODEVAS, Savigné l’Evêque, France.

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