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EVALUATION OF ACTIVITY AGAINST TICKS AND FLEAS ON
DOGS AND CATS AND HUMANS OF AN ELECTRONIC
ULTRASOUND EMISSION DEVICE (BATTERY-POWERED)
CALLED

ZEROBUGS™ and ZEROBUGS™ PLUS (+)

Medical Microbiology and Infectious Diseases Laboratory
School of Biosciences and Veterinary Medicine
University of Camerino
PROF. VINCENZO CUTERI



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1. INTRODUCTION

The fleas and ticks infestation in pet animals is one of the most common external parasites, also thanks to the considerable increase in canine and feline population. Equally frequent are reports in humans, which share with animals, home environments, gardens and, often, various objects.

The control of external parasites infestations generally is based on the combination of several strategies that include both the use of insecticides on animals and into the environment. Today in the market there are many active drugs available against fleas and ticks. They may be applied on the skin or administered orally.

To choose the pesticide to use on the animal, must be taken into account several characteristics such as speed, duration and spectrum of action, the compatibility with any other topical and / or systemic treatments, any side-effects, the age the animal and the route of administration.

Another extremely important factor in the choice of the pesticide is the ease of application (compliance) by the owners, and in the case of products for topical use, the frequency with which the animal is washed. Because these products are localized in the upper layers of the skin, their effectiveness is affected by the application of shampoo or the use of copious amounts of water. In addition, many of the products, are provided with residual action of short duration, for which they must be applied with even monthly frequency. The use of products administered orally is obviously not influenced by the baths or by frequent shampooing, but the duration of action is usually short and may occur, even if rarely, side effects in the gastrointestinal tract such as vomiting and diarrhea or there could be interactions with other drug treatments, which can increase the side effects.

Finally, it is important to remember that the most pesticides drugs cannot be administered in dogs, before 8-14 weeks of age.

Diseases spread by fleas and ticks are transmitted when these insects feed on the blood of a host. Fleas and ticks are externally parasitic not only to dogs and cats, but also humans and many small mammals. Different species of fleas and ticks are vectors of specific viruses, bacteria or protozoan parasites. These infections are often host and/or carrier specific.

1. Parasitic Dermatitis

Parasitic dermatitis is an allergic reaction that is caused by a pet's hypersensitivity to substances in flea saliva. Itchy, inflamed skin and papules will appear on the skin where fleas are concentrated. Eventually the irritation may cause hair loss and infection. These symptoms can exist long after flea infestation has been eliminated and may require treatments of antihistamines and antibiotics. Parasitic dermatitis is usually caused by fleabites but can sometimes be triggered by tick bites.



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2. Lyme Disease

Lyme disease is caused by a bacterium that is transmitted by the bite of a tick. Lyme disease is considered the most common tick-borne disease in the United States. Symptoms include a sudden onset of lameness that is occasionally accompanied by fever, anorexia and lethargy. Lyme vaccinations in conjunction with flea and tick preventatives are recommended for all dogs, specifically those in endemic areas or those that are often outdoors.

3. Bartonella

Bartonella strains are bacterial parasites that are transmitted through flea or tick bites. *Bartonella* can infect humans, dogs, cats and rodents. *Bartonella* invades red blood cells and uses the cell's membrane as protection while multiplying. *Bartonella* can cause multiple ailments depending upon the strain existent in the host. It is responsible for "Cat-scratch disease" in humans.

4. Ehrlichiosis

Ehrlichiosis is a bacterial infection transmitted through tick bites. *Ehrlichia* infect and destroy the white blood cells in the body of the host. Infection results in lethargy, weight loss, anemia and enlarged lymph nodes and spleen.

5. Rickettsiosis

Rickettsiae are bacteria that can be transmitted by flea or tick bites. Multiple strains of *Rickettsia* exist that can cause different ailments. Rickettsiae ailments include typhus, Rocky Mountain spotted fever, flea-borne spotted fever and tick bite fever. Diagnosis and treatment are dependent upon the strain of rickettsia and the associated illness.

6. Meningoencephalitis

Meningoencephalitis is an inflammatory disease that can be caused by numerous tick-borne viruses. Meningoencephalitis infects the brain and spinal cord, and their surrounding membranes. The result of infection is a loss of nervous system function. Fever, pain, convulsions and paralysis are symptoms of infection. Meningoencephalitis has a rapid onset and can be fatal. Diagnosis can be made by cerebrospinal fluid analysis.

7. Tapeworms

Tapeworms are parasitic flatworms that can be transmitted by fleas that are ingested during grooming. Tapeworms exist in the digestive tract and shed reproductive segments of their body called proglottids. The proglottids are passed in the feces of the host and are visible to the naked eye. Proglottids are often the only noticeable indication of a tapeworm infestation.



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For the diseases listed above, which are only some of those transmitted by fleas and ticks, there are very few vaccines, so the best recommended prevention is only to avoid or limit the presence of fleas and ticks in our animals.

The product, tested in this study, defined below "*ZEROBUGS™ and ZEROBUGS™ PLUS (+)*", is marketed as a "*device capable of acting on the nervous system of ticks and fleas*".

With this aim, the Savifin Srl, located in Via Volta, 34 - 20825 Barlassina (MB), has requested me, Professor Vincenzo Cuteri, Chair of Microbiology and Epidemiology, and Chair of Infectious Diseases and Head of the Laboratory of Medical Microbiology and Infectious Diseases, School of Biosciences and Veterinary Medicine, University of Camerino, to verify the effectiveness of ultrasound emitted by "*ZEROBUGS™ and ZEROBUGS™ PLUS (+)*" in reducing the number of parasites present, or not allow the increase, on infested dogs.

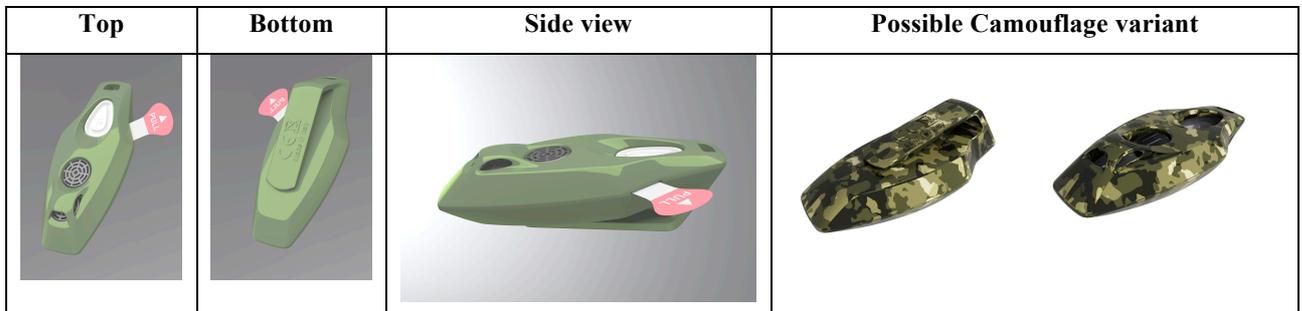
2. QUALITY AND QUANTITATIVE DESCRIPTION OF “ZEROBUGS™ and ZEROBUGS™ PLUS (+)”

- The product is individually wrapped and is identified as pictured:

ZEROBUGS™



ZEROBUGS™ PLUS (+)



3. PROPERTIES AND CHARACTERISTICS OF “ZEROBUGS™ and ZEROBUGS™ PLUS (+)”

The following is the manufacturer's Technical Data Sheet:

ULTRASOUND)))
tech

ZeroBugs™

ULTRASOUND REPELLENT TO COUNTER TICKS AND FLEAS ON HOUSEHOLD PETS (DOGS AND CATS) AND HUMANS

TECHNICAL CHARACTERISTICS AND FEATURES

The **ZeroBugs™** device emits a series of ultrasound pulses, a strong disturbance that affects the tick and flea nervous system and forces them to quit the host while, at the same time, preventing a new attack.

The device also acts as a form of non-toxic, environmentally-friendly prevention without using potentially harmful chemicals.

ZeroBugs™ operates by emitting mechanical sound (ultrasound) waves that are completely safe for household pets and humans — even for wearers of pacemaker or other devices.

The device is particularly suitable for children, for older pets weakened by disease, young cubs, nursing females and, in particular, pets that live in the home environment.

It should be used all year round but particularly during the parasite season (March – October) as a form of prevention and pest control.

OPERATING MODE

The device automatically starts up when the red tab is pulled out, the red light flashing five times.

Press the special button once to check the charge: the unit flashes five times, confirming that it is operating properly.

The device covers a radius of 1.5 metres of open space and can protect large pets and even people.

The non-replaceable battery lasts approximately 10 months (an entire season).

WEIGHT: 10 gr

DIMENSIONS: 42x29x12 mm

MATERIAL: ATOXIC ABS AND HYPOALLERGENIC THERMOPLASTIC RUBBER

COLOUR: all types of colours, to be defined



Rev. 30/06/2016

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REGISTERED OFFICE: VIA VOLTA 34
20825 BARLASSINA (MB) ITALY
VAT: 02238390963 Tax ID number 08482330157
EEE REGISTRATION NUMBER IT1603000009213
BATTERY PRODUCER REGISTRATION NUMBER
IT16030P00004018

ULTRASOUND)))
tech

ZeroBugs™ Plus (or ZeroBugs™+) (REGISTERED MODEL)
ULTRASOUND REPELLENT TO COUNTER TICKS AND FLEAS ON PEOPLE

TECHNICAL CHARACTERISTICS AND FEATURES

The **ZeroBugs™ Plus** device emits a series of ultrasound pulses, a strong disturbance that affects the tick and flea nervous system and forces them to quit the person while, at the same time, preventing a new attack.

The device also acts as a form of non-toxic, environmentally-friendly prevention without using potentially harmful chemicals.

ZeroBugs™ Plus operates by emitting mechanical sound (ultrasound) waves that are completely safe for household pets and humans — even for wearers of pacemaker or other devices.

Use is particularly indicated during the parasite season (March-October).

It is ideal for all those who love physical activity, sports and spending time in the great outdoors, in contact with nature.

It is recommended when hunting, fishing trekking and for all those activities that involve walking or spending time in parasite-infested areas.

OPERATING MODE

The device automatically starts up when the red tab is pulled out, the red light flashing five times.

For a more rational use of the device, it can be turned off by pressing the button once which will cause a single red flash. The device has an auto-off function that will automatically turn the unit off after 24 hours of constant operation. To turn it back on, just press the button.

Press the special button once to check the charge: the unit flashes five times, confirming that it is operating properly.

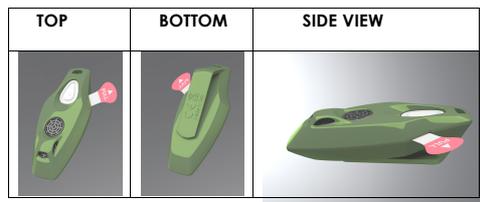
The non-replaceable battery lasts not less than 2,400 hours.

WEIGHT: 20 gr

DIMENSIONS: 62x28x20 mm

MATERIAL: ATOXIC ABS AND HYPOALLERGENIC THERMOPLASTIC RUBBER

COLOUR: all types of colours, to be defined



Possible Camouflage variant



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08682330157 EEE REGISTRATION
NUMBER IT1603000009213 BATTERY
PRODUCER REGISTRATION NUMBER
IT16030P00004018

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4. EVALUATION OF FLEAS AND TICKS CONTROL

The aim of the work was to evaluate the effectiveness of an ultrasonic device (**ZEROBUGS™**) in controlling the infestation of fleas and ticks, of various genus and species, in dogs. In particular, the study was aimed at assessing the ability to reduce the number of fleas and ticks or not to allow the increase, and to evaluate the effectiveness of up to 30 days after application.

All work was carried out at the municipal kennel of Camerino (MC). Inside the structure, which housed about 340 animals, 45 dogs, of different race, gender and ages, to be included in the study were selected. The inclusion criteria were that the dog had not been treated, in the previous month, with external spot-on pesticides or, in the previous three months, with collars containing active principles against ticks and fleas and that the minimum number of fleas or ticks counted on the dog's body (according to the procedure described below) were at least 4. Dogs with fur or dark skin or dogs with coat too thick, in which the count of the number of parasites could be difficult or not real, were excluded from the study.

For the parasites counting, each operator must carefully observe the subject for a standard period of 5 minutes: during this time the operator had to lift and move the fur until to well see the skin and count the number of parasites. The count was made in the rear train, and in the ventral area of the dog (where is usually easier to find the parasites as the hair is absent, or more rarely): in particular in the perineal area, in the back of the thigh, abdomen and groin, and back in the croup near the tail.

For each subject, a questionnaire including information about the personal data and the physical status of the dog and the presence of any symptoms or injury in any way attributable directly or indirectly to the action of pests was filled in.

The same operator for each sub-unit always carried out all counts.

The first day of the study, the 45 selected dogs were numbered, identified by coloured collar, and divided into 3 groups of 15 units each one (two control groups and treatment group); the three groups were, far as possible, consistent with each other, relatively to the total parasitic load.

The three groups was so constituted:

Group 1 - n. 15 subjects treated with the ultrasound device **activated**

Group 2 - n. 15 subjects treated with a pesticide spot-on based on Fipronil and Lufenuron; the subjects were still applied the ultrasound device, **but not activated**.

Group 3 - n. 15 subjects to which the **not activated** ultrasound device was applied.

Since all dogs had the device, and the operator being unable to distinguish whether activated or not, and therefore to know at which group they belonged, the count was carried out blindly and without any outside influence (study "blinded").

The subjects included in the trial were kept in separate enclosures for the whole duration of the study, when possible in a single box, or with a number of other similar dogs.



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Follow-up.

The first control was carried out after 3 days, from the beginning of the study, while the following two checks were made at 2 and 4 weeks after the beginning of the study, to verify the effectiveness over a month.

During each control the counting of the number of parasites was carried out in all subjects included in the study, always following the same procedure already used.

Also a short questionnaire was compiled to record any improvements or worsening of symptoms, or the possible occurrence of side effects related to the use of the device or the spot-on pesticide.



ZEROBUGS™ device effectiveness evaluation in control of flea / ticks in dogs

Date/..... /2016

Observe for 5 minutes and count the total number of parasites in the perineal area, back in the thigh, and near the tail, in the abdomen and groin.

Date/..... /2016 (Beginning of treatment T0)

- **Subject number**
- **Group number**
- **Number of parasite (T0)**
- **Dog**
 - Race: Short hair..... long hair
 - Size: Big Medium Small
 - Gender: M F Year of birth:
- **Presence of skin lesions related to the action of parasites (short description):**
.....
.....
.....
.....



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5. RESULTS AND CONCLUSIONS

The entire experiment was carried out into a municipal sanitary dog shelters with an high level of infestation and with a large number of animals (about 340) closely related and maintained into open space boxes. This means that ticks were always present into the box and with a constant possibility of re-infestation and also with the opportunity for ticks to cross from one box to the next.

The test was carried out for 30 days, and during this period animals had the opportunity to be infested again.

The results obtained 30 days after the product activation, demonstrate the efficacy of "**ZEROBUGS™**" in not increase the concentration of parasite in all the subjects included into the test and to reduce the load in some animals.

This mean that, despite the time of year with the highest prevalence of ticks, and an highly infested environment, the animals included into the experiment, in some cases (8 out of 20 subjects, 40%) did not increase the number of ticks, and into the others (12 out of the 20 subjects, 60%) the number of ticks decreased significantly.

In the **group 2**, control animals with "**ZEROBUGS™**" **not activated** and pharmacologically treated, at T1 a drastic reduction of the number of parasites was recorded, and this situation remained stable for the entire duration of the test. This was an expected result, since the drugs used have the ability to kill in a short time all ticks and to remain on the skin, for different time, by continuing the effect. In this group **ZEROBUGS™** was not activated, so the registered activity can be attributed only to the drugs.

In the **group 3**, control animals with "**ZEROBUGS™**" **not activated and not pharmacologically treated**, the number of parasites remained stable in few subjects, but increased considerably in others. In this group, because of the high-infested environment, the animals, without any protection (no ultrasound, no drugs) had the possibility to have continuous contact with ticks. For this reason, in some cases (1 out of 5 subjects, 20%) the number of ticks did not increase, and in the remaining (4 out of the 5 subjects, 80%) the number of ticks increased significantly.

Finally, in the **group 1**, animals treated with "**ZEROBUGS™**" **activated**, the number of parasites remained stable in 4 out of 15 animals (27%), while in 11 animals (73%) a considerable reduction was observed. In no case an increase of parasites concentration was recorded.

"**ZEROBUGS™**" showed to be able to prevent parasites, however present into the animals detention place, are able to attack the host. The advice, for better use of the ultrasound device, is to treat animals with pharmacologically active product and then turn on the device in order to avoid re-infestation.



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This, from a veterinary point of view is a very important sentence. Our aim is to safeguard the health of animals. When an animal have a tick infestation, always he runs the risk of taking several infectious diseases, as shown in the introduction of this report. So, when an animal is infested we have the duty to advice, as first treatment, a drug able in few hours to kill the highest number of ticks and then to apply the device to prevent further infestation. For animals that are kept at home or in the garden, with an environment presumably with a low infestation rate, the application of the ultrasound device may be able to eliminate ticks from the animal and to prevent those new ticks may infest again the animal.

Operators throughout the test recorded no hearing impairment.

The results obtained through the application of “**ZEROBUGS™ and ZEROBUGS™ PLUS (+)**” confirm the efficacy of the product in not to increase the concentration of parasites and to reduce its concentration in some subjects. Therefore, the product can be used, without any side effect, in subjects which have been demonstrated a tick/fleas infestation in order to avoid an exacerbation of the situation and in healthy subjects in order to prevent infestation.

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