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Leishmaniasis prevention: what should be known before recommending a topical product against sand fly bites in dogs?

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The paper covers the topical ectoparasiticides currently available in Italy useful for protection of dogs against sand fly bites and for prevention of *Leishmania* transmission. The information included provides veterinary practitioners operating in the field of prevention of canine leishmaniasis with scientific information and guidance relative to: i) active principles and modes of action of these ectoparasiticides, ii) criteria for efficacy evaluation, iii) available products, iv) their application, and v) potential toxicity and adverse effects.

Key words - Prevention, leishmaniasis, dog, ectoparasiticides, pyrethroids.

INTRODUCTION

In recent years, considerable progress has been made in the development of ectoparasiticides for dogs. This has led to an increase in the number of products available for the treatment against haematophagous arthropods such as fleas, ticks, mosquitoes and sand flies.[1]

This paper will examine the topical ectoparasiticides currently available in Italy for the prevention of canine leishmaniasis (CanL). It is important to remember that in order to obtain the registration of the veterinary drug regulatory authorities these products must have a documented capacity of reducing the risk of bites from vector phlebotomine sand flies and therefore, indirectly, of reducing the risk of transmission of *Leishmania,* which could possibly lead to the development of infection and/or disease. In particular, the paper will focus on existing data on the main vector for leishmaniasis in Italy, *Phlebotomus perniciosus*.[2]

The paper is structured in the form of questions that could be asked to veterinarians considering the prevention of CanL (Box 1), with the corresponding answers; it is divided into five sections: (i) Active principles and mechanisms of action; (ii) Evaluation of efficacy; (iii) Products available in Italy; (iv) Modalities of application; (v) Toxicity and adverse effects. Each section is divided into

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a series of questions whose answer is based on the data available in scientific publications, integrated with the information contained in the 'summary of product characteristics' of the European Medicine Agency (EMA), in the notes of the Italian Ministry of Health (MinSal) published in the Italian Official Gazette concerning authorization to market in Italy and, finally, in the package inserts of each product.

BOX 1
***Frequently Asked Questions* to veterinarians on the use of topical products useful for the prevention of canine leishmaniasis**

1. Active principles and mechanisms of action

a) Which active principles are currently used in the prevention of sand fly bites, the vector of CanL?
b) How do pyrethroids act against insects?
c) What is the action of pyrethroids against sand flies?
d) How are pyrethroids useful in the prevention of sand fly bites?
e) How do pyrethroids spread on the dog's skin?
f) Following application of the product is the action against sand flies immediate?

2. Evaluation of efficacy

a) Which studies should be carried out to prove the protective efficacy of pyrethroids against sand fly bites?
b) Which field trials should be carried out to prove the efficacy of these protective measures against the spread of CanL?
c) Can field trials alone be sufficient to prove the protective efficacy of pyrethroids against sand fly bites?

3. Products available in Italy

a) Which are the products currently available in Italy for which the protective efficacy against sand fly bites and/or the reduction of the *Leishmania* sand fly-transmission risk has been studied?
b) In addition to experimental laboratory studies, have field tests been carried out to assess the efficacy of these products in the prevention and spread of leishmaniasis in general?

4. Modality of application

a) What are the doses, the modality and frequency of application?
b) Are weight and age limits reported?
c) Should the application be continued regularly throughout the year?

5. Toxicity and adverse effects

a) Are pyrethroids contained in the ectoparasiticides used in the prevention of sand fly bites toxic?
b) Following application of these products on the skin, which adverse effects have been reported in the dog?

1. ACTIVE PRINCIPLES AND MECHANISMS OF ACTION

a) Which active principles are currently used in the prevention of sand fly bites, the vector of CanL?

The active principles for topical use with a proven action against sand flies, vectors of the causative agent of CanL, *Leishmania infantum*, are limited to synthetic pyrethroids,[3] substances chemically similar to the pyrethrins present in natural pyrethrum, derived from a flower of the family of Asteraceae. Pyrethrins have long been known for their insecticidal activity, but they consist of photolabile molecules that rapidly degrade in the environment. In contrast, synthetic pyrethroids tend to be stable when exposed to sunlight, while maintaining excellent insecticidal activity. In particular, the so-called

The active principles of topical use products with a proven action against sand flies are limited to synthetic pyrethroids.

"second generation pyrethroids" make up a group of highly stable chemical compounds which are endowed with a protracted residual activity (permanence of an unaltered chemical activity at the site of delivery or application). These make up a large family of molecules with an insecticidal activity that is from 10 to 1,000 times greater compared to pyrethrins and first generation pyrethroids. Among the second-generation pyrethroids, permethrin, deltamethrin and flumethrin find their major field of application both as ectoparasiticides and as protectors against the biting and feeding of haematophagous arthropods, including phlebotomine sand flies (Table 1).

It should be noted that synthetic pyrethroids are over-the-counter products on sale without the need of a veterinary prescription.

b) How do pyrethroids act against insects?

Pyrethrum and its synthetic derivatives are neurotoxic and act mainly by contact; penetration mainly occurs through the natural openings in the cuticle of insects. The exact site of action of pyrethroids is not yet fully known, however their action, although surely involving different neurotransmission pathways, is mainly targeted on the sodium channels of cell membranes of the nervous system of arthropods. Following neuronal hyperstimulation the insect rapidly develops convulsive episodes, paralysis and death. However, in case of exposure to sub-lethal doses, the insect can recover com-

Table 1 - Trade name, formulation and active principles of ectoparasiticides for topical use available in Italy with proven efficacy against the bites of phlebotomine sand flies, vectors of leishmaniasis, and/or useful in reducing the risk of transmission of *Leishmania* by phlebotomine sand flies in treated dogs [information collected from the 'summary of product characteristics' (EMA, MinSal) and/or from the package insert]

Trade name	Formulation	Active principles (concentration)*	
		Pyrethroids	Other principles
Advantix®	Spot-on	Permethrin (500 mg)	Imidacloprid (100 mg)
Effitix®/*Fipratix*®	"	Permethrin (40:60 cis:trans; 545 mg)†	Fipronil (61 mg)†
Exspot®	"	Permethrin (40:60 cis:trans; 65% p/p, 715 mg)	-
Frontline Tri-Act®	"	Permethrin (504.8 mg)	Fipronil (67.6 mg)
Vectra 3D®	"	Permethrin (397 mg)	Dinotefuran (54 mg); Pyriproxifen (4.84 mg)
Scalibor®	Collar	Deltamethrin (15,3-15,8 mg)†	-
Seresto®	"	Flumethrin (29 mg)†	Imidacloprid (64.29 mg)†
Duowin®	Spray	Permethrin (18.80 mg)	Pyriproxifen (0.20 mg)

* The concentration is intended per 1 mL of product, with the exclusion of collars, where it is expressed per linear cm.
† Calculated concentration.

pletely; this is indicative of the fact that the mechanism of action is reversible and that the insect is capable of metabolizing small amounts of the insecticide.
Synthetic pyrethrum derivatives act at much lower concentrations compared to those typical of obsolete insecticides, such as organophosphates or carbamates. Their toxic action against insects is therefore extremely high, however it is not possible to speak of an exclusive selectivity against these invertebrates. If on one hand there is indeed a tendency to consider many pyrethroids almost devoid of toxicity to humans and

Synthetic pyrethroids have a neurotoxic action by contact; the toxic action is mosIty against insects, but not exclusively.

other mammals, incidents of systemic poisoning have in fact been described both in humans and in the veterinary sector.[4] Cats, in particular, may suffer from severe pyrethroid toxicosis, e.g. from the accidental application of topical dog formulations.[5-7] In addition, pyrethroids are highly toxic to fish and aquatic organisms in general, and are therefore not recommended for use in inland waters, i.e. rivers, lakes and seas near the coast. For this reason, the package insert of products containing pyrethroids recommend that dogs should not be allowed to enter watercourses for 48 hours or longer after treatment. For more information on the toxicity of pyrethroids, see points 5a and 5b.
Episodes of resistance to pyrethrum and to some of the most common pyrethroids have long been known. They mainly concern domestic flies and insects of agricultural interest; recently, however, in Africa, the use of pyrethroids in the fight against malaria vectors has led to the selection of resistant populations of *Anopheles*.[8] The most important mechanism of resistance seems to be linked to the selection of *kdr* genes (*knock-down resistance*), which can make the action-site on the nerve fibre inaccessible to the insecticide.

c) What is the action of pyrethroids against sand flies?

To answer this question it is necessary to recall the main characteristics of synthetic pyrethroids (see Box 2 for a bilingual glossary of mechanisms of action and related effects):

- Pyrethroids are lipophilic, so they bind to the lipid fraction of the dog's skin and hair.
- Their volatility is low and hence once applied on the dog's skin they persist for a long period of time. This characteristic differentiates them from typically volatile substances (such as diethyltoluamide or picaridine) that act from a distance and for which the term "repellent" is appropriately used.
- They have an excito-repellent effect; in view of this, phlebotomine sand fly females in search of a blood meal on a dog treated with pyrethroids are irritated by

Synthetic pyrethroids have an excito-repellent effect which is expressed during the biting of phlebomine sand fly.

BOX 2
Terms used in defining the mechanism of action of anti-vector products and related effects

English term	Italian term	Meaning
Repellent	Repellente propriamente detto	Action at a distance on vector chemoreceptors that interferes with their approach and landing on the treated host. The action is not toxic for the vector, only disruptive
Excito-repellent	Eccitante-repellente	Dose-dependent toxic action on the nervous system of the vectors following contact with the product, which interferes with the normal movements necessary for taking a blood meal on the treated host
Anti-feeding	Anti-puntura	The ultimate effect, resulting from the specific action of a repellent proper or of an excito-repellent agent
Landing and probing	Posarsi e fase iniziale della puntura	These terms are used to discern between the specific repellent or excito-repellent action of a substance on the treated host. The landing of a vector is an objective parameter that can also be assessed on animal hosts, while the initial stage of the biting can only be felt and recorded by human volunteers
Knock-down	Abbattente	Paralysing action on the nervous system of the vectors which results in their temporary or permanent knock-down
Insecticidal	Insetticida	Toxic action capable of causing death of an insect within a standard time of 24 hours

the simple contact with the animal and move away without biting (anti-biting protective effect). With regard to sand flies, it is important to recall that only the females are haematophagous, and that the insertion of the biting apparatus, followed by the sucking of blood, requires in fact very little time (3-4 minutes at the most), especially if compared, for instance, to that of dog ticks (5-7 days). Once completed the meal, females may then mate with one of the males which has in the meantime arrived, and then abandon the host definitively.

d) How are pyrethroids useful in the prevention of sand fly bites?

In summary, it can be said that:

- As demonstrated by various laboratory tests, compared to an untreated dog, the irritant effect of pyrethroids on sand flies on the skin of a treated dog can prevent the biting of over 90% of sand flies trying to have a blood meal, (Table 2). High levels of protection against biting, which is potentially associated with the transmission of *Leishmania* (protection that can be defined as "individual"), are maintained for periods of variable duration depending on the composition and formulation of the product. It should be remembered that not all phlebotomine sand flies are infected: the average infection rate in natural populations of *P. perniciosus* in areas with leishmaniasis is of around 1%.[9]
- The insecticidal action of the pyrethroid is expressed through the more or less prolonged contact between the vector and the treated skin. This means that even the few sand flies that have been able to feed are in most cases destined to die within 24 hours. In fact, as shown in laboratory tests (Table 2), the insecticidal action can affect up to 98% of the sand flies that have taken a blood meal on a treated dog, and also in this case the mortality rate is maintained more or less over time depending on the composition and formulation of the product. It is important to note that should the treated dog be infected or ill, and has therefore a good chance of being an infecting reservoir for the vector, the insecticidal effect conferred by the pyrethroid al-

Laboratory tests show that the excito-repellent action of pyrethroids on the skin protects against bites with an efficacy of more than 90%, while the insecticidal action can affect up to 98% of the phlebotomine sand flies that complete the blood meal.

Table 2 - Bibliographic references of laboratory and field experimental studies carried out to ascertain the anti-biting and insecticidal action against colonized phlebotomine sand flies and to assess the efficacy of each product in preventing leishmaniasis

Product	Bibliographic references	
	Laboratory studies	Field trials§
Advantix®	31	43-44
Effitix®/Fipratix®	32	*
Exspot®	33-34	45
Frontline Tri-Act®	35	46
Vectra 3D®	36	*
Scalibor®	37-39	47-53
Seresto®	*	54-56
Duowin®	40-42	*

§ Field trials are not unique to the *P. perniciosus* species; * No studies are reported in national or international scientific publications.

lows to block the transmission of the disease, as the eventually infected sand flies will die before completion of the parasite development cycle (which occurs in about 6-7 days). This is defined as "mass" or "community" protection, as it can interfere with the transmission of *Leishmania* in an endemic area.

e) How do pyrethroids spread on the dog's skin?

To the authors' knowledge, the mechanism of distribution of pyrethroids on the dog's skin has not been specifically reported and reasonably varies with the type of formulation as well as with the excipients and/or other active substances present in the different products.

Being lipophilic, pyrethroids typically diffuse by "migrating" by continuity through the superficial lipid fraction.[1,10] Experimental studies in dogs have shown a good distribution in the *stratum corneum* and hair coat of dogs treated with permethrin spot-on formulations; however, within 2-4 weeks of application, the concentration of permethrin seems to decrease faster in the hind limbs compared to the skin of the animal's back.[1,10]

With regard to collars, the active substance is apparently gradually released into the lipid component of the *stratum corneum* thanks to the continuous friction of the synthetic band with the skin and hair coat.

f) Following application of the product is the action against sand flies immediate?

Knowledge of the time necessary for pyrethroids to exert their protective action is important in order to correctly plan for their use as the sand fly season approaches or for when a dog is to be taken from a region where sand flies are absent to one where they are present. To the authors' knowledge, no specific studies are available indicating the time required by pyrethroids, in their various formulations, to reach the adequate concentrations necessary to perform their anti-biting action.

In general, it is recommended to apply spot-on formulation pyrethroids at least 2 days before exposure to sand flies.[11,12] Experimental studies have shown that following application of some spot-on formulations, permethrin is distributed quickly and homogeneously in the *stratum corneum* and hair coat of the dog within a 24-hour period.[10]

As for collars containing pyrethroids, their application is generally recommended at least 1-2 weeks before exposure to sand flies.[11,12] In addition, to ensure good distribution of pyrethroids over the skin, the collar should be worn continuously; intermittent collar application is therefore not recommended, e.g. removing the collar in the morning and reapplying it at sunset.

Knowledge of the time interval required for the different pyrethroid formulations to have a protective effect on the skin is important for the correct planning of product use.

For pyrethroids in spray formulation, although not specifically reported, they can be assumed to provide immediate effect, at least limitedly to the sites of application.

2. EVALUATION OF EFFICACY

a) Which studies should be carried out to prove the protective efficacy of pyrethroids against sand fly bites?

The protective efficacy of pyrethroids against the transmission of *Leishmania* should be evaluated through successive steps, referred to as Phase 1 (laboratory trials) and Phase 2 (clinical field trials). The latter can be performed only after having evaluated the following parameters, obtained in Phase 1 trials using colonized phlebotomine sand flies:

- dose of active substance necessary for anti-biting protective effect;
- degree of biting protection conferred by the formulation;

- insecticidal action;
- duration of the anti-biting protection and of the insecticidal effect conferred by the formulation.

The efficacy of pyrethroids against the transmission of *Leishmania* must be evaluated through laboratory studies and field clinical trials against the species of vectors present (in Italy: *P. perniciosus*).

Considering that both the anti-biting effect and the insecticidal action of a product containing pyrethroids may vary with the species of phlebotomine sand fly, these parameters must be assessed against the vector species present in the endemic area where the field trials are to be carried out (as already mentioned, in Italy, the main vector in all endemic areas is *P. perniciosus*).

The above procedures usually follow the guidelines for the evaluation of the efficacy of parasiticides in dogs and cats and of repellents for human use.[13,14,15] As for the EMA guidelines, which the European pharmaceutical industry must comply with when registering a new product, the wording for all target Diptera insects (including phlebotomine sand flies, even if not specifically mentioned) states: "In the period indicated by the manufacturer, the overall ectoparasiticide efficacy when treating infestations in household pets must be in the range of 80-100%, and preferably higher than 90%".

b) Which field trials should be carried aout to demonstrate the efficacy of these protective measures against the spread of CanL?

No guidelines are present for the experimental design of a field trial. Generally, these studies are conducted in areas with a high incidence of CanL, as in this case large sample sizes are not necessary to achieve statistical significance of results. The significance of the results gives a measure of the difference in the incidence rate of *Leishmania* infection between treated and untreated dogs (control). The subjects of the study may be either household dogs or a population of kennel dogs. The difficulty of these trials is mainly due to two factors: i) the starting population must necessarily not be infected by *Leishmania*; this complicates the enrollment and implies the use of multiple diagnostic tests in order to exclude the infection with reasonable certainty; ii) the confirmation of infections in the "treated" and in the " control" groups can be assessed only several months after the end of exposure to the natural transmission of *Leishmania*.

c) Can field trials alone be sufficient to prove the protective efficacy of pyrethroids against sand fly bites?

Field observation alone of the protective action of a pyrethroid against CanL is of limited value, as the most important variables are not considered, i.e. the degree and duration of protection from bites of vectors belonging to the species responsible for the endemic transmission. Without such information, it becomes impossible to plan the duration of treatment throughout the entire period of vector activity. These data can only be obtained in the laboratory, using colonies of phlebotomine sand flies bred in an insectarium and experimental dogs.

3. PRODUCTS AVAILABLE IN ITALY

a) Which are the products currently available in Italy for which the protective action against sand fly bites and/or the reduction of the *Leishmania* sand fly transmission risk has been studied?

Currently, products are available in three different formulations: (i) spot-on (Advantix®, Effitix®/Fipratix®, Exspot®, Frontline Tri-Act®, Vectra 3D®); (ii) collar (Scalibor®, Seresto®); and (iii) spray solution (Duowin®) (Table 1). All products, with the exception of Scalibor® and Exspot®, contain other active principles, in addition to pyrethroids, with varying efficacy against other insects (i.e. fleas) or mites (i.e. ticks).

In Italy, products containing synthetic pyrethroids are available in three different formulations: spot-on, collar and spray.

An additional product is available, Activyl® tick plus spot-on, based on permethrin and other active principles, whose protective efficacy against the bites of *P. perniciosus* has been studied in the laboratory;[16] however, this indication is not included in the EMA 'summary of product characteristics'.

b) In addition to experimental laboratory studies, have field trials been carried out to assess the efficacy of these products in the prevention and spread of leishmaniasis in general?

Field trials have shown that the application of such preventive measures leads to a significant decrease in the incidence of both canine and human leishmaniasis.

Table 2 reports the experimental laboratory and field trials that have been undertaken to: (i) ascertain the insecticidal and anti-biting action against phlebotomine sand flies, and (ii) evaluate the efficacy of each product in preventing leishmaniasis.

Unfortunately, bibliographic research shows that for some of the products listed in the table, even if already on the market, no published data on laboratory and/or field tri-

als are available on national or international scientific journals. There is also variability in the protocols used in the field trials of the various products.

4. MODALITY OF APPLICATION

a) What are the doses, the modality and frequency of application?

Table 3 summarises the information on the recommended dose of pyrethroids per unit of body weight of the dog, the duration of the anti-biting action for sand flies, the possible reduction of action following contact with water and the maximum recommended frequency of application, where specified in the 'summary of product characteristics' (EMA; MinSI) and/or package insert.

The dosage of pyrethroids in different formulations varies with the weight of the dog; it depends on the minimum recommended dose and on the concentration of pyrethroids in the product and must obviously be complied with to ensure efficacy.

With regard to the modality of application, spot-on products must be applied directly on the skin surface, separating the hair coat, on one or more points, depending on the size of the dog and on the instructions in the package insert. In dogs with long, dense hair, the correct application of spray products can be complex due to the difficulty in reaching the surface of the skin. However, as suggested in the package insert, the hair should be completely moistened with a variable number of vaporizations depending on the size of the dog, then massaged, left to air dry and finally brushed.

The optimal frequency of application of the different products is based on the duration of the anti-biting action against phlebotomine sand flies. Since pyrethroids

Table 3 - Summary of the information and/or recommendations collected from the 'summary of product characteristics' (EMA, MinSI) and/or from the package insert

Product	Active principle (minimum recommended dose)	Duration of anti-biting action (P. perniciosus)	Age limit (weeks)	Weight of subject to be treated (Kg)	Possible reduction of efficacy following contact with water (1)	Maximum frequency of application (2)
Advantix®	Permethrin (50 mg/Kg)	3 weeks	not < 7	not < 1.5	Prolonged/intensive exposure to water may reduce the duration of action. Shampoo at least 2 weeks post-application	1 week
Effitix® *Fipratix®*	Permethrin (60 mg/Kg)	4 weeks	not < 12	not < 1.5	Shampooing or soaking in water may reduce the duration of action	4 weeks
Exspot®	Permethrin (49.3-53.6 mg/Kg)*	4 weeks	not < 2	N.A.	Bathing and sponging can reduce the duration of action depending on the amount of water in contact with the skin. Avoid contact with water in the first 12 hours	3 weeks
Frontline Tri-Act®	Permethrin (50.5 mg/Kg)*	3 weeks	not < 8	not < 2	Frequent bathing and washing can reduce the duration of the action. Avoid contact with water within 48 hours after treatment	4 weeks
Vectra 3D®	Permethrin (46.6 mg/Kg)	4 weeks	not < 7	not < 1.5	Frequent bathing and shampoos or bathing within 48 hours of treatment may reduce the duration of action	N.A.
Scalibor®	N.A.	5 months**	not < 7	N.A.	Occasional contact with water will not result in reduced efficacy. Dogs should not enter in water for the first five days after application of the collar***	N.A.
Seresto®	N.A.	N.A.	not < 7	N.A.	Intense and prolonged exposure to water and abundant washings with shampooing can reduce the duration of action	N.A.
Duowin®	N.A.	2 weeks in adult dogs, 1 week in puppies	not < 3 months	N.A.	Exposure to water immediately after trattamento riduce l'azione (consigliata treatment reduces the action (repetition of the treatment is recommended)	N.A.

(1) Information not related to anti-phlebotomine sandly activity; (2) for precautionary reasons; N.A. not available; * calculated dose; **suggested by the company; ***precaution to prevent the environmental pollution of waters.

are distributed primarily in the *stratum corneum* (point 1e), the eventual presence of skin diseases characterised by excessive desquamation, the intensity of grooming, contact with water and its duration as well as the frequency of baths carried out for therapeutic purposes and the type of shampoo used could affect the persistence of pyrethroids on the skin and hence their duration of action.

For many products (Table 3), in the product insert there is only a generic assertion referring to a reduction in activity duration following frequent washings and it is suggested not to wash or wet the dog especially in the first days after application. However, it is worth noting that, to the authors' knowledge, the possible reduction of sand fly anti-biting action resulting from contact with water or the use of shampoo has not been specifically studied. If the dog needs to be washed, or if it gets wet frequently, a higher frequency of application of pyrethroids compared to what is indicated in the package insert is often usually suggested (despite this being an improper use). Reducing the interval between treatments is in fact recommended for some products both in the package insert and in publications.[17] The package insert usually also specifies the maximum recommended frequency of application (Table 3).

Information is provided on application modality, dose, duration of action and maximum recommended application frequency during the phlebotomine sand fly season.

For prevention purposes the application of a single ectoparasiticide containing pyrethroids is normally considered to be appropriate. However, based on the results of a recent survey, it has been reported that the concomitant use of a spot-on formulation containing permethrin and of a collar containing deltamethrin might significantly reduce the risk of seropositivity for *Leishmania* compared to the use of a single product.[18]

b) Are weight and age limits reported?

For some products, weight and age limits ranging from 1.5-2 kg and 2-12 weeks are reported. These limits are generally due to the absence of experimental safety data in dogs below the above limits (Table 3).

c) Should the application be continued regularly throughout the year?

The treatment should be continued throughout the phlebotomine sand fly activity period and not for the entire calendar year. A recent survey carried out over 3 years throughout the Mediterranean basin has established that the beginning of the phlebotomine sand fly activity period, that is the first emergence of adults from the larval stages that remained dormant during the winter, is highly correlated with the latitude (i.e. the further South you are, the more adults emerge sooner during the year). The end of the sand fly activity period is instead less associated with this geographical variable and probably depends on the peaks of population density reached in a given season, together with the average seasonal temperatures.[19]

In Italy, dividing the peninsula into North, Centre and South, it can be estimated that the " phlebotomine sand fly season" runs, respectively, in the periods of June-September, May-October and April-November, with annual variations of about fifteen days.

Obviously, the number of treatments depends on the duration of the efficacy declared in the product specifications rather than on a fixed timetable protocol. It is also worth emphasising that the protocol must be the same whether the product is used on a healthy dog or on an infected or sick dog.

5. TOXICITY AND ADVERSE EVENTS

a) Are pyrethroids contained in the ectoparasiticides used in the prevention of sand fly bites toxic?

In humans, the percutaneous absorption of pyrethroids is very low, under 2%, which is followed by a first pass intradermal metabolism, that reduces its systemic spread, and then by a rapid liver metabolism, with excretion of non-toxic metabolites primarily through the urine.[1,20] It is still worth recommending to owners to read in the package insert the precautions that must be taken when handling the product, especially by those who apply it. In about 10% of cases of pyrethroid contact with the skin, irritation and itching has been reported and occasionally also allergic reactions with contact eczema. In addition, paresthesia events due to the direct action of even very low doses of pyrethroids on sensory nerve endings have been described. The paresthesia usually occurs 0.5-2 hours after exposure, it peaks after 6 hours and then generally spontaneously disappears within 24 hours.[6,20] Necrosis following direct application on the human skin of products not intended for use in the dog, such as anti-mosquito sprays, has also been reported.[21]

The inhalation of pyrethroids, usually due to occupational exposure, causes nasal and deep respiratory tract irritation.[20] In addition, cases of systemic intoxication have been documented, again for problems linked to occupational exposure or following accidental or intentional ingestion of products usually not intended for veterinary use, with severe neurological signs, such as seizures and coma.[4,22] In some cases the intoxications had a fatal outcome.[20]

In the veterinary sector, in the dog, pyrethroids contained in ectoparasiticides used for the prevention of phlebotomine sand fly bites apparently do not have a neurotoxic effect when applied to the skin, mainly due to the practically absent dermal absorption and the rapid hepatic metabolism, with subsequent excretion of conjugated metabolites in the urine and stools.[1,10]

In the cat, instead, pyrethroids are extremely toxic due to several problems in the liver metabolism of these substances that are not yet fully clarified, in particular the absence of the enzyme glucuronosyltransferase.[1,23] Cats become intoxicated mainly due to exposure to products registered for the dog following accidental contact or ingestion; consequently, contact with treated dogs should be avoided, at least while the dog's hair coat is still moist, as suggested in the package insert of spot-on product formulations.

In humans, and in the dog, pyrethroids for topical use rarely have adverse effects, while in the cat they are very toxic and potentially fatal.

In the cat, pyrethroid intoxication can be fatal and clinical signs, which generally begin a few minutes to 24 hours after exposure, include sialorrhea, mydriasis, tremors, hyperesthesia, ataxia and seizures.[7,23,24] It should be remembered that in all mammals, including humans, the toxicity of pyrethroids is greater in young subjects.[6,24] It is also interesting to remember that there seems to be no direct correlation between the amount of pyrethroids to which the cat has been exposed to and the severity of the clinical signs, thus supporting the existence of an individual sensitivity.[24,25]

For more information on the clinical signs, therapy and prognosis of pyrethroid poisoning in cats, please refer to veterinary toxicology and pharmacology textbooks and to specific publications.[5,6,7,24,25,26,27,28,29]

b) Following application of these products on the skin, which adverse effects have been reported in the dog?

As reported in the package inserts, products containing pyrethroids may cause itching, usually localized, erythema and partial alopecia at the application site or in friction areas in the case of collars. The cause of these reactions, which are usually transient and self-limiting, is presumably due to contact reactions, both irritative and due to hypersensitivity, even if not clearly documented in the dog. In the case of contact irritation, it is reasonable to assume that the severity of the reaction is dose-dependent. In case of intense and generalized itching it is advisable to remove the collar, if considered associated with the reaction, and, in any case, to wash the dog with warm water and a non-irritating and degreasing shampoo, given the lipophilicity of pyrethroids. It is not recommended to use either cold water, as the low temperature favours the action of pyrethroids on the sodium channels, or water that is too hot, as it increases percutaneous absorption.[6,24]

Transient and self-limiting episodes of paresthesia, although difficult to document in the dog, may justify some occasionally reported clinical signs, such as nervousness, hyperexcitability and involuntary limb movements.[25] Recently, sporadic cases of pemphigus foliaceus, the pathogenesis of which remains to be determined, have also been described in dogs treated with spot-on products containing pyrethroids in combination with other active substances.[30]

In the dog, the package insert of several products reports the onset, albeit rare, of GI problems, such as vomiting, diarrhoea and scialorrhoea, or behavioural and neurological problems, including restlessness, aggression, rolling movements, lethargy, tremors, ataxia and paresis. Similar problems are reported following the accidental ingestion of pyrethroids. For more information on clinical signs and treatment of pyrethroid intoxication, please refer to veterinary toxicology and pharmacology textbooks and to specific publications.[6,25,27,28]

To reduce the risk of adverse effects it is advisable to carefully read on the package insert the precautions for use, special warnings, contraindications and all the information concerning the use in specific categories of patients, such as in pregnant and lactating females or in subjects with general health problems.

CONCLUSIONS

This paper is an update on the CanL prevention measures published in the past by members of the Gruppo di Studio sulla Leishmaniosi Canina (GSLC) (Canine Leishmaniasis Working Group, CLWG):

1. Leishmaniosi canina: linee guida su diagnosi, stadiazione, terapia, monitoraggio e prevenzione. Parte III: Prevenzione.[57] Maroli. M., Gradoni L., Oliva G., Castagnaro M., Crotti A, Lubas G, Paltrinieri S, Roura X, Zatelli A, Zini E. Veterinaria, Year 23, n. 4, August 2009
2. Guidelines for prevention of leishmaniasis in dogs.[3] Maroli. M, Gradoni L, Oliva G, Castagnaro M, Crotti A, Lubas G, Paltrinieri S, Roura X, Zini E, Zatelli A. Journal of the American Veterinary Medical Association Vol 236, No. 11, 2010

The paper, structured in the form of questions and answers, aims to facilitate the work of veterinary practitioners when choosing ectoparasiticides for CanL prevention. The initial questions focus on the mechanisms of action of synthetic pyrethroids, i.e. the only mole-

cules with proven action against phlebotomine sand flies, vectors of *Leishmania infantum*, the agent of CanL. In particular, the review focuses on *P. perniciosus*, the most common phlebotomine sand fly species present in Italy. Subsequent questions focus on the criteria for evaluating the efficacy of pyrethroids in preventing CanL. It is recalled that in order to obtain registration, products must have a documented capacity to reduce the risk of biting from vector sand flies and therefore, indirectly, to reduce the risk of transmission of *Leishmania,* which could consequently lead to the possible development of infection and/or disease (leishmaniasis).
The main characteristics of ectoparasiticides available in Italy that have been studied to be effective against sand fly bites and/or in reducing the risk of transmission of *Leishmania* are then schematically reported in tables. Knowledge of the composition and of the application modalities of the different ectoparasiticides may help the veterinarian to choose those products considered suitable for each patient and to recommend their correct use to the owner, which is essential to guarantee their efficacy.
Finally, information is provided on metabolism, potential toxicity and adverse effects of pyrethroids.

NOTE

The information collected in this article is the result of a thorough review of scientific publications and of the official documentation available, conducted by the authors according to criteria of objectivity and impartiality.
Any omissions found in the article are to be considered entirely unintentional. Furthermore, the authors are not to be held liable for any reason whatsoever for the summary of the information and/or recommendations collected from the 'summary of product characteristics' of the products mentioned, which have been taken from documents of EMA, from the Italian Ministry of Health and from package inserts. The authors are also not responsible for any errors or negative consequences resulting from the use of the information contained in this article.
References to products available in Italy have been indicated in italics and with the addition of ®; this method may not accurately represent the official registration rights of the products.

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NOTE OF THE AUTHORS

Alessandra Fondati, Luigi Gradoni and Michele Maroli contributed equally to the drafting of this article.

KEY POINTS

- The paper, structured in the form of questions and answers, aims to facilitate the work of veterinary practitioners when choosing topical ectoparasiticides for the prevention of canine leishmaniasis.
- The article illustrates the mechanisms of action of synthetic pyrethroids, the only molecules with proven action against phlebotomine sand flies, vectors of *Leishmania*.
- An overview is made of the criteria for evaluating the efficacy of pyrethroids in preventing canine leishmaniasis.
- The main characteristics of products available in Italy that have been studied to be effective against sand fly bites and/or in reducing the risk of *Leishmania* transmission by phlebotomine sand flies are shown schematically in tables.
- Information is provided on the metabolism, potential toxicity and adverse effects of pyrethroids.

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