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World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for studies evaluating the efficacy of parasiticides in reducing the risk of vector-borne pathogen transmission in dogs and cats

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ABSTRACT

These guidelines are intended to provide an in-depth review of current knowledge and assist the planning and implementation of studies for evaluating the efficacy of parasiticides in reducing transmission of vector-borne pathogens (VBPs) to dogs and cats. At present, the prevention of VBP transmission in companion animals is generally achieved through the administration of products that can repel or rapidly kill arthropods, thus preventing or interrupting feeding before transmission occurs. The present guidelines complement existing guidelines, which focus on efficacy assessment of parasiticides for the treatment, prevention and control of flea and tick infestations, but also give guidance for studies focused on other vectors (i.e. mosquitoes and phlebotomine sand flies). The efficacy of parasiticides in reducing VBP transmission can be evaluated through laboratory or field studies. As such, the present guidelines provide recommendations for these studies, representing a tool for researchers, pharmaceutical companies and authorities involved in the research, development and registration of products with claims for reducing VBP transmission in dogs and cats, respecting the overall principles of the 3Rs (replacement, reduction and refinement). Gaps in our current understanding of VBP transmission times are herein highlighted and the need for further basic research on related topics is briefly discussed.

1. Introduction

Ticks, fleas, phlebotomine sand flies, mosquitoes, lice and, to a lesser extent, other arthropods (e.g., black flies, biting midges, triatomine bugs and keds) may cause direct damage (e.g., skin injuries, blood loss and allergic reactions) to their hosts, and can also inoculate neurotoxins and transmit a range of pathogens, including bacteria, protozoa, viruses and helminths. Vector-borne diseases (VBDs) threaten the health and

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welfare of dogs and cats globally (Beugnet, 2013), with many vector-borne pathogens (VBPs), including *Bartonella* spp., *Dirofilaria* spp., *Leishmania* spp. and *Rickettsia* spp., being of zoonotic concern (Colwell et al., 2011; Dantas-Torres et al., 2012; Kilpatrick and Randolph, 2012; Otranto et al., 2013).

The existing World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats (Marchiondo et al., 2013) are not focused on VBP transmission, prevention or treatment. Consequently, the present W.A.A.V.P. guidelines are intended to provide researchers, pharmaceutical companies and authorities with recommendations for studies evaluating the efficacy of parasiticides in reducing the risk of VBP transmission in dogs and cats. Moreover, the existing guidelines do not include some insects of veterinary significance (e.g., mosquitoes and phlebotomine sand flies), which are vectors of pathogens to dogs and cats. In principle, the transmission of pathogens can be reduced either by repelling (i.e. repellent effect), disrupting feeding or by rapidly killing arthropod vectors (i.e. killing effect) before pathogen transmission occurs. There is a need to harmonize studies evaluating efficacy of parasiticides in reducing the risk of VBP transmission to dogs and cats, as indicated by the recently drafted European Medicines Agency (EMA) guidelines for veterinary medicinal products for the prevention of transmission of VBDs in dogs and cats (EMA, 2018).

Traditionally, W.A.A.V.P. guidelines have targeted eukaryotic pathogenic organisms falling within the classical definition of 'parasite'. In addition to 'classical' vector-borne parasites (e.g., *Dirofilaria* spp., *Leishmania* spp. and *Babesia* spp.), the present guidelines also encompass vector-borne bacteria (e.g., *Anaplasma* spp., *Borrelia* spp., *Ehrlichia* spp. and *Rickettsia* spp.), which may be transmitted to dogs and cats by ticks or fleas (Table 1). Conversely, vector-borne viruses (e.g., tick-borne encephalitis virus) are not considered within these guidelines due to the limited hazard they pose, at present, to the health of dogs and cats (Reperant et al., 2016). In this context, the present guidelines provide recommendations for studies evaluating the efficacy of parasiticides in reducing VBP transmission in dogs and cats. They will represent a tool for researchers, pharmaceutical companies and authorities involved in the research, development and registration of products with claims for reducing VBP transmission in dogs and cats and concurrently respecting the overall principles of the 3Rs (replacement, reduction and refinement).

2. Aspects that may affect VBP transmission

While scientific knowledge on the biology of VBPs and their vectors has increased in recent decades (Schorderet-Weber et al., 2017; Otranto, 2018), there is still a need to translate much of this knowledge into effective prevention tools and strategies for preventing VBP transmission. Indeed, several aspects of the vector-pathogen-host interaction do influence the efficacy of parasiticides in reducing VBP transmission in dogs and cats. Therefore, relevant aspects, like route of transmission, feeding behaviour and duration of the vector's feeding and pathogen transmission rates and times, migration paths and incubation time of pathogens in their vectors, must be considered when designing efficacy studies.

Regarding routes of transmission, some pathogens are inoculated (e.g., *Babesia spp*., *Leishmania* spp. and *Rickettsia* spp.) during blood feeding (Schorderet-Weber et al., 2017), whereas others (e.g., *Dirofilaria immitis*) are deposited onto the skin and penetrate actively through the bite wound (Venco et al., 2011). In contrast, other pathogens are transmitted through ingestion of vectors (e.g., *Hepatozoon canis* and *Dipylidium caninum*) or paratenic hosts (e.g., *Hepatozoon americanum*) (Baneth, 2011). Additionally, the transmission by dog bites (e.g., *Babesia gibsoni*), vertical (e.g., *H. canis* and *Leishmania infantum*), venereal (e.g. *L. infantum*) and blood transfusion transmission (e.g., *Anaplasma phagocytophilum*, *Anaplasma platys*, *Babesia* spp., *Ehrlichia canis* and *L. infantum*) have also been demonstrated for a range of VBPs of dogs and cats.

Feeding behaviour of vectors and pathogen transmission times are governed by a number of variables related to the vectors, the pathogens and the susceptible vertebrate hosts (de la Fuente et al., 2017; Otranto, 2018). Overall, the duration of the blood-feeding period is shorter in insects (e.g., sand flies, mosquitoes and black flies) as compared to ixodid ticks (Petrić et al., 2012; Bates, 2017). Arthropod vectors may be attracted by different host stimuli and use different strategies to locate and approach the host. Accordingly, some may land (e.g., flying insects), while others crawl (e.g., ticks) or jump (e.g., fleas) on the host. For instance, mosquitoes and other flying insects (e.g., sand flies) are highly mobile and, once landed on the host, they feed rapidly, thus having a short-lasting contact with their hosts (Gingrich and Williams, 2005; Faraji et al., 2014). With regard to fleas, many cat fleas (*Ctenocephalides felis*) will begin feeding within minutes of arriving on their host, with the majority of them completing the blood meal within 1 h (h) of arrival (Cadiergues et al., 2000). Hard ticks (Ixodidae), however, are slow feeders and may feed for several hours or days, thus having a relatively long-lasting contact with their hosts (Sonenshine and Roe, 1993; Dantas-Torres et al., 2012). Consequently, transmission times of bacterial and parasitic pathogens are usually shorter with insect vectors (from seconds to minutes) as compared to ticks (from a few hours to several days).

Pathogen transmission is influenced by several factors, including pathogen pre-activation times (Kidd and Breitschwerdt, 2003; Otranto, 2018). In fact, some tick-borne pathogens require a pre activation period, resulting in a longer transmission time. An example is represented by *Borrelia burgdorferi* spirochetes causing Lyme disease, which are usually transmitted by infected ixodid ticks within 24–48 h following their attachment to the host (Hajdušek et al., 2013). Although the determinants affecting pathogen pre-activation times are not completely understood, the ingestion of blood is reputed to play a role in this process (Socolovschi et al., 2009). For instance, interrupted feeding may reduce the transmission time of *Rickettsia rickettsii* by *Amblyomma aureolatum* from more than 10 h to 10 min (min) (Saraiva et al., 2014) and *Babesia canis* by male *Dermacentor reticulatus* from 48 h to 8 h (Varloud et al., 2018). In addition to the speed of transmission of VBPs by ticks, establishment of infections may be dependent on a minimum inoculation dose for bacterial pathogens like *A. phagocytophilum* (Fourie et al., 2019). While the transmission times and their influencing factors may be considered in the design of laboratory studies, they cannot be easily assessed and controlled under field conditions. In any case, all factors potentially affecting VBP transmission should be considered when designing efficacy studies.

3. Parasiticides and their role in preventing VBP transmission

Theoretically, the transmission of VBPs can be prevented either by completely impeding feeding by arthropods or by ensuring that arthropod vectors are killed before pathogens are transmitted to the hosts. The efficacy of a parasiticide against a given arthropod vector does not imply a significant reduction of the risk of VBP transmission *per se* and therefore specific studies are required by authorities in order to pursue label claims in this respect. As a result of the lack of specific guidelines, until now the approval of a parasiticide as efficacious in reducing the risk of VBP transmission has been handled on a case-by-case basis by regulatory authorities and respective registrant companies (Bobey, 2015).

A number of pyrethroids (e.g., permethrin, deltamethrin and flumethrin) with repellent and anti-feeding effects have been proven efficacious to reduce the risk of transmission of VBPs (e.g., *B. canis*, *E. canis* and *L. infantum*) in both laboratory and field studies (Jongejan et al., 2011, 2015, 2016; Fourie et al., 2013b, c; Brianti et al., 2014). Other chemical compounds, by virtue of their fast killing effect, may also reduce the risk of VBP transmission in dogs and cats. For example,

Table 1
Pathogens causing vector-borne diseases in dogs and cats, the disease they cause, their arthropod vectors, transmission times. The desired features of a product to prevent pathogen transmission to dogs and cats are described, but do not replace the need for specific VBP prevention studies. N/A indicates that no literature is available.

Pathogen	Disease	Vector	Minimum reported transmission time [a]	Theoretical desired effect of a parasiticide for preventing transmission [b]	Supporting reference for transmission time studies
Acanthocheilonema reconditum	Non-pathogenic	Fleas (*Ctenocephalides felis*) and lice (*Heterodoxus spiniger*)	Immediate	Repellent or fast killing effect (all fleas and lice killed as soon as possible after coming in contact with the host)	N/A
Anaplasma phagocytophilum	Canine granulocytic anaplasmosis	Ticks (*Ixodes* spp.)	Within hours (establishment after 48 h, to dogs)	Repellent or fast killing effect (all ticks killed in <48 h)	Fourie et al. (2019)
Anaplasma platys	Canine cyclic thrombocytopenia	Unknown (ticks are suspected vectors)	Unknown	Unknown	N/A
Babesia canis	Canine babesiosis	Ticks (*Dermacentor reticulatus*)	36 h (8 h in interrupted feeding experiment) (to dogs)	Repellent or fast killing effect (all ticks killed in <36 h)	Varloud et al. (2018)
Babesia conradae	Canine babesiosis	Unknown (ticks are suspected vectors)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	N/A
Babesia felis	Feline babesiosis	Unknown (ticks are suspected vectors)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	N/A
Babesia gibsoni	Canine babesiosis	Ticks (*Haemaphysalis longicornis*)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	N/A
Babesia rossi	Canine babesiosis	Ticks (*Haemaphysalis elliptica*)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	N/A
Babesia vogeli	Canine babesiosis	Ticks (*Rhipicephalus sanguineus sensu lato*)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	N/A
Babesia vulpes	Canine babesiosis	Unknown (ticks are suspected vectors)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	N/A
Bartonella henselae	Feline bartonellosis	Fleas (*C. felis*)	Immediate via contact with flea faeces (but after fleas feed and digest the blood)	Repellent or fast killing effect (all fleas killed as soon as possible after coming in contact with the host)	Bouhsira et al. (2013a)
Borrelia burgdorferi	Canine Lyme disease	Ticks (*Ixodes* spp.)	24 h (to hamsters)	Repellent or fast killing effect (all ticks killed in <24 h)	Piesman et al. (1987)
Cytauxozoon felis	Feline cytauxzoonosis	Ticks (*Amblyomma americanum*)	48 h (to cats)	Repellent or fast killing effect (all ticks killed in <48 h)	Thomas et al. (2018)
Dipylidium caninum	Canine dipylidiosis	Fleas (*C. ides felis*, *Ctenocephalides canis*) and lice (*Trichodectes canis*, *Felicola subrostratus*)	Immediate after the ingestion (via ingestion of the arthropod vector)	Repellent or fast killing effect (all fleas and lice killed as soon as possible after coming in contact with the host); parasiticides with additional larvicial and ovicidal effiacy are more effective in reducing the overall risk transmission	N/A
Dirofilaria immitis	Canine and feline heartworm disease	Mosquitoes (several genera and species)	Immediate	Repellent (all mosquitoes killed as soon as possible after coming in contact with the host)	N/A
Dirofilaria repens	Canine and feline subcutaneous dirofilariosis	Mosquitoes (several genera and species)	Immediate	Repellent (all female mosquitoes killed as soon as possible after coming in contact with the host)	N/A
Ehrlichia canis	Canine monocytic ehrlichiosis	Ticks (*Rh. sanguineus s.l.*)	within hours (3 h) (to dogs)	Repellent or fast killing effect (all ticks killed in <3 h)	Fourie et al. (2013a)
Hepatozoon canis	Canine hepatozoonosis	Ticks (*Rh. sanguineus* s.l., *Rhipicephalus turanicus*, *Amblyomma ovale*)	Immediate (via ingestion of infected ticks)	Repellent or fast killing effect (all ticks killed as soon as possible after coming in contact with the host) which result in the reduction of tick population	N/A
Leishmania infantum	Canine and feline leishmaniosis	Sand flies (several genera and species)	Immediate	Repellent or fast killing effect (all female sand flies killed as soon as possible after coming in contact with the host)	
Rangelia vitalii	Canine rangeliosis	Ticks (*Amblyomma aureolatum*)	Unknown	Repellent or fast killing effect (all ticks killed in <36 h); based on minimal transmission time for *B. canis*	Soares et al (2018)
Rickettsia conorii	Canine spotted fever rickettsiosis or canine Mediterranean spotted fever	Ticks (*Rh. sanguineus* s.l., *Rh. turanicus*)	Unknown	Repellent or fast killing effect (all ticks killed in <10 h); based on minimal transmission time for *R. rickettsii*	N/A
Rickettsia felis	Feline flea-borne rickettsiosis	Fleas (*C. felis*)	Immediate	Repellent or fast killing effect (all fleas killed as soon as possible after coming in contact with the host)	Wedincamp and Foil (2002)

(*continued on next page*)

Table 1 (*continued*)

Pathogen	Disease	Vector	Minimum reported transmission time [a]	Theoretical desired effect of a parasiticide for preventing transmission [b]	Supporting reference for transmission time studies
Rickettsia rickettsii	Canine spotted fever rickettsiosis or canine Rocky Mountain spotted fever	Ticks (several genera and species)	10 h (10 min in an interrupted feeding experiment with *Am. aureolatum*) (to dogs)	Repellent or fast killing effect (all ticks killed in <10 h)	Saraiva et al. (2014)

[a] The transmission time is defined here as the minimum period required from blood feeding initiation by the arthropod vectors to actual pathogen transmission to the vertebrate host. In all studies with ticks, transmission times are shortened if ticks are initially placed on a host to start feeding and then removed from this host and placed on a second host (=interrupted feeding experiments). Moreover, the transmission efficiency by ticks increases with time (the longer the feeding period, the higher is the proportion of animals infected) as well as the establishment of infections in dogs, being dependent on the minimum inoculation dose. Unless otherwise stated, pathogen transmission occurs by bite. Within this column, 'immediate' means within few minutes.

[b] Based on data currently available from studies conducted with dogs or cats. In some cases, transmission times using other animal models may be shorter than those using dogs or cats (e.g., *A. phagocytophilum* needs 48 h to establish infection in dogs, but only 24 h in mice). When the specific transmission time is unknown, theoretical desired effects of parasiticides are inferred from data obtained with phylogenetically close organisms (e.g., desired effects of parasiticides against *Babesia* spp. transmission are inferred from data obtained with *B. canis*).

isoxazolines (e.g., afoxolaner, fluralaner, lotilaner and sarolaner) might be indicated for the prevention of the transmission of several tick-borne (e.g., *A. phagocytophilum, B. canis and Bo. burgdorferi*) and flea-borne pathogens (e.g., *Acanthocheilonema reconditum and D. caninum*) (Beugnet et al., 2014; Taenzler et al., 2015; Baker et al., 2016; Honsberger et al., 2016; Geurden et al., 2017; Gopinath et al., 2018).

Insecticides and acaricides are available as topical (i.e. spot-on, spray or collar) or oral (i.e. chewables or hard tablets) formulations. These products may act by killing (i.e. insecticidal and acaricidal products, formulated for topical or oral applications) or repelling (i.e. repellent products, formulated for topical application only) arthropods. Therefore, these products can prevent arthropod feeding or interrupt the feeding process in its early phases, ultimately reducing the risk of VBP transmission to dogs and cats (Otranto and Wall, 2008) and potentially reducing the ingestion of vectors while these animals are scavenging and grooming (Baneth, 2011). Some active ingredients like pyrethroids have the unique feature of possessing dual killing (insecticidal, acaricidal, or both) and repelling effects, whose efficacy may vary depending on the active ingredient, formulation and target arthropod (Beugnet and Franc, 2012). On the other hand, the combination of several active ingredients with different modes of action within the same formulation enables certain products to attain a broad spectrum of activity targeting several arthropods (e.g., fleas, ticks, mites, lice, mosquitoes and sand flies).

Once administered to the host, parasiticides can act before the target arthropods start feeding, as soon as they begin to feed or following a certain lag time from the initiation of feeding. This depends mainly on the intrinsic properties and mode of action (i.e. acting via contact, ingestion, or both) of each parasiticide, and is independent (or less dependent) on the route of administration (i.e. topical versus oral). For instance, some products display a pronounced volatility (e.g., diethyltoluamide - DEET, which is not available for use in dogs and cats), enabling the repellency of certain arthropod vectors prior to (e.g., mosquitoes and sand flies) or soon after (e.g., ticks and fleas) their contact with the host (Bissinger and Roe, 2010). Other products display a lower volatility (e.g., pyrethroids, which are available for use in dogs and cats) and require the contact between the arthropods and the host, only after which arthropods are repelled (Bissinger and Roe, 2010). The killing effect can be achieved either through external contact (i.e. the arthropod dies after coming into contact with the parasiticide on the fur or the skin of the treated host) or ingestion (i.e. the arthropod dies after ingesting the parasiticide, with the blood of the treated host). Therefore, depending on the active ingredient(s) present in the formulation, a product may prevent attachment (i.e. repellent effect), disrupt an already existing contact between the arthropod and the host (also referred to as expellent effect), cause the close to or immediate death of the arthropod (killing effect), or interfere with egg fertility and subsequent development of the arthropod (i.e. growth inhibition) (Halos et al., 2012).

The mode of action of different active compounds should be evaluated regarding their impact on the biology, ecology and parasitic behaviour of each arthropod and VBP. Parasiticides licensed for preventing or treating ectoparasite infestations on dogs and cats belong to several chemical families, namely formamidines (e.g., amitraz), neonicotinoids (e.g., dinotefuran and imidacloprid), phenylpyrazoles (e.g., fipronil), indoxacarb (oxadiazine), macrocyclic lactones (MLs) of the spinosyn group (e.g., spinosad) and MLs of the avermectin/milbemycin group (e.g., selamectin), and synthetic pyrethroids (e.g., deltamethrin, flumethrin and permethrin) (Beugnet and Franc, 2012). Recently, isoxazoline class molecules have been successfully introduced as parasiticides for dogs and cats (Pfister and Armstrong, 2016; Weber and Selzer, 2016; Wright, 2018). When administered to dogs, these molecules display a fairly rapid onset of action against existing infestations (i.e. curative effect), starting from 4 h and reaching >90 % of killing effect in about 12 h, with the highest efficacy in killing ticks displayed within 24 h (Beugnet et al., 2014; Pfister and Armstrong, 2016; Geurden et al., 2017; Murphy et al., 2017). Fluralaner and afoxolaner were found also to kill up to 100 % of *Aedes* (e.g., *Aedes aegypti*), *Anopheles* (e.g., *Anopheles gambiae*) and *Culex* (e.g., *Culex pipiens*) mosquitoes, and *Lutzomyia* (e.g., *Lutzomyia longipalpis*) and *Phlebotomus* (e.g., *Phlebotomus argentipes*) sand flies, 24 h after artificial feeding on blood containing different concentrations of these compounds (Miglianico et al., 2018).

Isoxazolines in oral formulations were successfully used in dogs for the prevention of the transmission of *B. canis* by *D. reticulatus* (afoxolaner, Beugnet et al., 2014; fluralaner, Taenzler et al., 2015; lotilaner, Cavalleri et al., 2017; sarolaner, Geurden et al., 2017) as well as of *Bo. burgdorferi* and *A. phagocytophilum* by *Ixodes scapularis* (sarolaner, Honsberger et al., 2016). Considering the toxicity of most synthetic pyrethroids to cats, the efficacy of an isoxazoline (e.g., sarolaner in association with selamectin) has been recently proposed for preventing feline VBDs (Otranto and Little, 2017). A fast-killing spot-on combination of fipronil, amitraz and (S)-methoprene was demonstrated to inhibit the transmission of *B. canis* by *De. reticulatus* (Jongejan et al., 2011) and of *Bo. burgdorferi* and *A. phagocytophilum* by *I. scapularis* (McCall et al., 2011); however, this combination is no longer commercialized. The efficacy of any product in blocking VBP transmission is however also dependent on the pathogens' transmission times; quickly transmitted pathogens such as *E. canis* (transmitted by *Rh. sanguineus* sensu lato (s.l.) in about 3 h, in *in vitro* conditions) (Fourie et al., 2013a) are better targeted by synthetic pyrethroids than isoxazolines (Jongejan et al., 2016). In fact, pyrethroids have been proven efficacious in preventing the transmission of rapidly transmitted VBPs, such as *E. canis* (Jongejan et al., 2011; Fourie et al., 2013a, c; Stanneck and Fourie, 2013) and *L. infantum* (Brianti et al., 2014), as well as slower transmitted VBPs such as *Bo. burgdorferi*, *A. phagocytophilum* (Blagburn et al., 2004) and *B. canis*

(Jongejan et al., 2011; Fourie et al., 2013b; Navarro et al., 2015), under laboratory or field conditions.

The efficacy requirements for a parasiticide to be registered may vary according to national and regional regulatory authorities. For instance, to be licensed in Europe, a parasiticide should have efficacies of >95 % within 24 h and >90 % within 48 h post-treatment against existing flea and tick infestations, respectively (i.e. curative or immediate efficacy). Likewise, a parasiticide should have efficacies of >95 % within 24 h and >90 % within 48 h post subsequent infestations by fleas and ticks, respectively, to assess the persistent efficacy (Marchiondo et al., 2013; EMA, 2016a; Pfister and Armstrong, 2016). In the USA, the product should have an efficacy of >90 % against both fleas and ticks (Pfister and Armstrong, 2016). However, thus far neither the evaluation time points nor the required efficacy thresholds for such time points have been validated for their relevance in the reduction of the VBP transmission risk. In any case, studies on the speed of kill (SOK) and persistent efficacy may indicate the potential of reducing the risk of VBP transmission. Accordingly, products with a rapid onset of action and sustained SOK throughout the treatment duration have the potential to reduce this risk (Dantas-Torres and Otranto, 2016; Schorderet-Weber et al., 2017), as demonstrated by a number of studies in dogs and cats (Stanneck et al., 2012; Tielemans et al., 2014; Fourie et al., 2015; Geurden et al., 2017).

While laboratory studies are important for assessing the efficacy of a product in reducing the risk of VBP transmission, a spectrum of potential confounding factors (e.g., interrupted feeding and co-infection with multiple pathogens) can occur under field conditions. As such, field studies are beneficial to confirm the data obtained in the laboratory. Even if study data generally confirm the efficacy of repellents and fast-killing parasiticides in reducing the risk of VBP transmission, none of the available products can ever ensure a total protection. Therefore, integrated control strategies are advocated by the scientific community for VBP prevention (e.g., association of ectoparasiticides and MLs for the prevention of heartworm disease in dogs and cats).

4. Evaluation of efficacy in reducing VBP transmission

The label claim 'reduction of the risk of infection with < name of VBP > via transmission by < name of the vector> 'needs to be requested from the regulatory authority [e.g., the Food and Drug Administration (FDA) in the USA, the European Medicines Agency in the European Union (EMA) and the Ministry of Agriculture, Livestock, and Food Supply (MAPA) in Brazil], as an extension of an existing label claim, and should be based on laboratory and/or field studies. While experimental studies are considered mandatory by the FDA and EMA, field studies are generally acknowledged as 'supportive'. However, regulatory agencies may issue a preventive indication based on well-designed field studies, which are conducted according to the Good Clinical Practice (GCP) guidelines (VICH, 2000).

For all studies involving the use of animals, utmost care must be given to the welfare of any animals used and all procedures must be approved by regulatory authorities and an animal ethics committee (EMA, 2016b). The life cycle and pathogenicity of the VBP of interest should be also considered to avoid unnecessary suffering of infected animals, which should be treated or humanely euthanized after use, based on the recommendation of the study director/investigator and approval of an animal ethics committee.

The principle of the 3Rs (replacement, reduction and refinement) should be taken into account when clinical studies are designed and conducted. The use of animal-free *in vitro* methods can help to better understand some phenomena such as vector-VBP relationships or feeding behaviour of vectors prior to the use of animals, thus enabling the comprehensive set-up of meaningful animal studies. Suitable study conditions (e.g., care of animal and study procedures) should be used to avoid pain, suffering or distress of the animals as much as possible and to minimize the number of animals used (EMA, 2016b).

4.1. *In vitro studies: artificial feeding systems*

Artificial feeding systems have been increasingly used to infect arthropods with pathogens, assess transmission times of pathogens (Fourie et al., 2013a, 2019) and to test the efficacy of systemic parasiticides (Kroeber and Guerin, 2007) as well as the efficacy of parasiticides in preventing VBP transmission. However, such *in vitro* efficacy data are usually regarded only as 'supportive' by authorities. Different artificial feeding systems have been implemented for fleas (*C. felis*) (Bouhsira et al., 2013a, b), mosquitoes (Luo, 2014), sand flies (Ghosh, 1994) and some tick species (e.g., *Rh. sanguineus* s.l.) (Fourie et al., 2013a). To assess the efficacy and SOK, parasiticides are solubilized in a blood medium and fed to the arthropods through a silicone membrane (i.e. Parafilm® or Nescofilm) or skin derived from 1 to 5 day-old chicks (Ghosh, 1994; Bouhsira et al., 2013a, b; Luo, 2014). Exposure time will vary according to arthropod vectors.

The successful implementation of these systems is assessed based on the feeding rates of arthropods, which in the case of ticks, is also conditioned by the size of these parasites' mouthparts (e.g., artificial feeders have thus far yielded higher feeding rates in *Ixodes* spp. than in *Rhipicephalus* spp.) (Fourie et al., 2013a, 2019). One limitation of artificial feeders is the lack of innate and adaptive immune responses from the host, which may play a role in arthropod feeding and VBP transmission (Cantillo et al., 2014; Kamhawi et al., 2014; Shaw et al., 2016; de la Fuente et al., 2017). Therefore, results obtained from studies using artificial feeders should be interpreted cautiously. Indeed, a recent study showed that while the presence of *A. phagocytophilum* deoxyribonucleic acid (DNA) could be detected in blood samples taken from artificial feeders on which infected *Ixodes ricinus* ticks had fed for only 6 h, the establishment of infection in dogs required 48 h to occur under experimental conditions (Fourie et al., 2019); hence, the transmission to dogs was assumed to be dependent on a minimum inoculation dose (Fourie et al., 2019).

Artificial feeding systems are of limited use for testing compounds acting only by contact following topical administration on dogs and cats. Moreover, further research is needed to validate the use of these systems in studies evaluating the efficacy of parasiticides in the prevention of VBP transmission.

4.2. *In vivo studies*

In vivo studies to demonstrate efficacy of parasiticides in reducing VBP transmission in dogs and cats can be conducted under both laboratory and/or field conditions. In both cases, studies should follow GCP guidelines (VICH, 2000) if the results are intended to be submitted to a regulatory authority. The study design must be adequate for the intended purpose and may be different from studies solely evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats (Marchiondo et al., 2013).

Laboratory studies should include an untreated control group and be designed to test final formulations as close to the minimum recommended dose as possible using the approved commercial product, if available. Field studies usually involve privately owned pets, which makes the use of a negative control group less feasible, except if the disease is not highly pathogenic and is relatively easy to treat. While in other geographic regions field studies still frequently include a negative control (Coura-Vital et al., 2018), there is currently a growing concern in using untreated control groups in studies conducted in the EU. When the inclusion of a negative control group is not feasible, the investigational product should be compared to an established treatment (i.e. non-inferiority clinical studies), available for the targeted label claim. Products should be used at the registered/intended dose and administered according to label recommendations.

The set-up of studies to evaluate the prevention of VBP transmission differs from the classical designs for the evaluation of efficacy against the arthropods themselves. As an example, in laboratory studies

evaluating the efficacy against tick-borne pathogen transmission, the design might foresee tick counts and removals only several days after the artificial infestation (Cavalleri et al., 2017), to ensure sufficient time for transmission to occur in the control group. On the other hand, in studies evaluating the efficacy of parasiticides for the treatment, prevention and control of tick infestations on dogs and cats, the tick count and removal is usually done at 48 h, or at earlier time-points (e.g., 6, 12 and 24 h) in the case of SOK studies (Marchiondo et al., 2013).

The duration and frequency of follow-ups of infestations and monitoring of animals and the diagnosis of infection depend on the pathogens and models under investigation (see section '5. Specific study designs' for details). The transmission of pathogens to hosts can be detected by several means, varying according to the target pathogen, including cytology, serology, molecular biology (e.g., polymerase chain reaction (PCR)) and, to a much lesser extent, by culturing (Bouhsira et al., 2013a, b; Fourie et al., 2013a, 2019). Usually several diagnostic methods are used in combination, including serology (to assess the presence of antibodies against the pathogens or even pathogen antigens), PCR (to detect the pathogen's DNA), cytology, histology and culture (to detect the pathogen itself). Animals should be followed-up for a sufficiently long period, which usually corresponds to the prepatent period of each pathogen, to allow detection of the target pathogen by one or more diagnostic tests. Relevant clinical parameters for the respective VBD should also be considered to assess the patency of infections and to allow for rescue treatments in a timely manner. Study protocols should define the identified 'rescue treatments' and the specific laboratory or clinical criteria that trigger their use (for details, see '5. Specific study designs').

While the level of exposure to the vectors may be pre-determined in laboratory studies, this is unfeasible in field studies, where the number of arthropods infesting and/or biting each individual animal as well as the pathogen load in the arthropods is unpredictable. As such, generating baseline prevalence data is imperative to estimate the risk of infection with the target pathogen(s) in the study area.

4.2.1. Laboratory studies

Laboratory studies require an untreated control group. Whenever possible, a pre-inclusion testing of a slightly larger number of animals should be done to evaluate their attractiveness to the parasitic arthropods being used. This will allow the randomization of animals by group allocation according the respective pre-inclusion counts and exclusion of the surplus animals with lowest attractiveness. Each laboratory study design may vary according to the vectors under investigation (e.g., flying versus non-flying insects), VBPs (e.g., transmission time and prepatent period) and investigational product (e.g., mode of action and formulation). Moreover, the study design will also depend on the intended claim (e.g., repellent versus killing efficacy).

In laboratory studies, animals should be challenged with infective arthropods at least at the beginning and the end of the claim duration. For instance, for a 1-month claim, animals need to be challenged within the first 2 days post-treatment and not earlier than day 28 post-treatment. Arthropods used in experimental studies must be infected by the pathogen of interest and, animals should be followed up beyond the actual challenge time points for a period that is equal or longer than the prepatent period of the infection caused by the pathogen (e.g., at least 5 months for *Di. immitis*), to allow its detection by one or more diagnostic tests. Usually, it is advisable to combine different diagnostic methods in order to increase the probability of detection of pathogens with both short (e.g., *Babesia* spp. and *A. phagocytophilum*) and long (e.g., *Dirofilaria* spp. and *E. canis*) prepatent periods (Otranto et al., 2010a).

4.2.1.1. Preparation of arthropods to be used in laboratory studies. Laboratory studies evaluating the efficacy of parasiticides for the prevention of VBP transmission depend on the successful establishment of laboratory colonies of infected arthropod vectors. As an alternative, populations of naturally infected arthropods can also be used (e.g., *I. scapularis* naturally infected with *Bo. burgdorferi*), provided that the level of infection in the vector population is sufficiently high to allow experimental challenge. Moreover, it is fundamental to properly confirm the identity of the target arthropods that will be used in the studies, either based on morphology or genetics, which is particularly recommended for some species groups (e.g., *Rh. sanguineus* s.l.) (Nava et al., 2018).

The successful infection of ticks and fleas with a specific VBP depends on their feeding on either an infected animal or an artificial feeding system containing infected blood (either blood from a naturally infected animal or originally non-infected blood mixed with cultured pathogens). The isolates used to experimentally infect the arthropods should belong to a known strain, but if not, the isolates should ideally be genotyped. If an infected animal is used as a source of infection for the arthropods, the animal should be a naïve, purposed-bred dog or cat, though other animals may be used as a source of infection, such as infected sheep as a source of *A. phagocytophilum* for *I. ricinus* (Fourie et al., 2019). The presence of co-infections should be excluded. Depending on the infectivity, pathogenicity and immunogenicity of the VBP of interest, special measures (e.g., administration of corticoids or splenectomy) may be required to enhance pathogen multiplication in the animal, thus facilitating the infection of the arthropod vector.

The species and life stage of arthropods to be used should be based on vector competence and the biology of the VBP. For example, one should consider whether the pathogen is passed transovarially and/or transstadially in the specific vector. Since the successful infection of any vector depends on their acquisition of pathogens during feeding on an infected host, special attention should be given to the timing of infestation, which should coincide with the expected period of parasitaemia/bacteraemia. This is especially relevant for ticks, which need to feed up to repletion on a parasitaemic/bacteraemic animal, which, depending on the tick species and developmental stage, may take from a few to several days. Moreover, it is important to consider that three-host ticks feed only once during each developmental stage (i.e. larva, nymph and adult). Therefore, it is important to consider the pre-moulting and moulting periods (from larva to nymph and from nymph to adult), while preparing infected ticks to be used in future challenges.

Successful infection of the arthropods with the VBP of interest should be confirmed by dissection followed by microscopic examination or by molecular techniques (e.g., PCR) on a representative number of individuals. The minimal sample size to be tested should be calculated using expected infection rates in the vectors based on data obtained from previous field and/or laboratory studies. It is imperative to confirm that arthropods are infected by the pathogen of interest and also to assess the presence of other related pathogen species, which might be also present in the arthropods. If co-infections are confirmed, the arthropods may still be used, provided that diagnostic tools used to detect the infections in the experimentally challenged animals are able to distinguish between target and non-target VBPs. Highly specific serological tests, specific PCR protocols or generic PCR followed by DNA sequencing should be used for this purpose. For obvious reasons, arthropods ingesting an infected blood meal will immediately become DNA positive (due to carry-over of DNA), which, however, does not mean that the infection is successfully established in the arthropod vector. Thus, in the case of ticks, in which some VBPs may be passed transstadially or transovarially, testing should be done using subsequent developmental stages that will be used for the experimental transmission to ensure that the infection is actually established. When the same developmental stage of the arthropod will be used (e.g., female sand flies), testing should be done after a certain period of time to allow blood meal digestion and to ensure that the infection is established and the pathogen's infective forms have developed (e.g., metacyclic promastigotes of *Leishmania* spp. take usually 5–7 days to develop). Once the infection rate has been established, whenever feasible, vector competence should be validated prior to use in a pivotal study, by challenging naïve animals with a batch of infected vectors.

The infection rates in the vectors vary widely according to pathogen and vector species. For instance, a study showed that 10–60 % of the nymphs of *Amblyomma sculptum* (reported as *Amblyomma cajennense*) were infected with *R. rickettsii* after feeding as larvae on infected guinea pigs, whereas 80–100 % of the nymphs of *Am. aureolatum* and *Rh. sanguineus* s.l. were infected with the same pathogen (Labruna et al., 2008). Since vector challenge models aim at simulating natural exposures in a standardized and controlled manner in the laboratory, the infection rates in arthropods to be used in laboratory studies should generally reflect rates found in the field (Fourie et al., 2019). Therefore, it is not possible to define a precise minimal infection rate that would be required for laboratory studies. In theory, only one infected arthropod is necessary to infect a susceptible host. Thus, the most important thing is to demonstrate that most (if not all) untreated control animals become infected after experimental challenge, regardless of the infection rate detected in the infesting arthropods. Accordingly, the higher the infection rate that is detected in the infesting arthropods, the higher will be the chances to provide both treated and untreated animals with an actual "challenging'' situation. Therefore, the infection rates found in the arthropods used in laboratory studies should always be reported in the study dossier submitted to the regulatory authority.

4.2.2. Field studies

As for laboratory studies, specific care should be taken to mitigate the risk of adverse events and any clinical signs related to the specific infection in exposed animals. Pet owners involved in the study should be aware of the study objective and design, any potential adverse events and of the limited liability of the sponsor and of the investigator. In addition, they should provide their written consent for the inclusion of their animals in the study and should be given the possibility to consult the investigator at any time. A rescue treatment, if any is available, must be clearly specified in the protocol in cases of infection detected in study animals. Field studies must be performed according to GCP guidelines if the results are intended to be submitted to a regulatory authority.

Due to the risk of infection and disease development in privately-owned pets the use of an untreated group in field studies may not always be feasible. Whenever the inclusion of an untreated group is not possible, the inclusion of a treated control group (animals treated with an established treatment) is necessary. Under field conditions, the infection challenges are natural and baseline prevalence data should be generated prior to the study initiation. Knowing prevalence and, whenever possible, the incidence of the infection caused by the VBP under investigation, will allow the estimation of the sample size to be included in the study (see section '*4.4. Sample size and methods for calculating the efficacy*'). If treated and untreated groups are located in different areas (e.g., in pair matched-cluster randomized controlled studies) (Courtenay et al., 2019), baseline prevalence data are mandatory, also to guarantee that both groups are at similar levels of exposure to the risk of infection. The study design should consider several aspects that are inherent to the vector-pathogen system under study (e.g., vector seasonality pathogen transmission time and prepatent period) and intended product claim.

4.3. General recommendations for diagnosing VBP infections in dogs and cats

Various diagnostic methods are available to infer or to confirm VBP infection in dogs and cats. Each pathogen may be more reliably detected by a test or a group of tests at different times considering specific factors such as their prepatent periods and any possible effects of seasonality in the infection dynamics (e.g., seasonal and circadian variation of microfilaraemia of *Dirofilaria* spp.) (Ionică et al., 2017). For antibody testing, the time needed for seroconversion must be considered when defining the earliest meaningful testing time point. In both laboratory and field studies, animals should be tested before the inclusion, in order to assess their negativity at the beginning of the study and at following time-points. Hence, according to the peculiarities of specific pathogens and diagnostic tests used, the frequency of testing varies largely (for specific recommendations see Section 5). For tick-borne pathogens, a combination of tests aiming at different targets (e.g., pathogen DNA detection by PCR and pathogen antibody detection by serological test) should be used. The direct visualization of some pathogens (e.g., *Anaplasma* spp., *Babesia* spp. and *Ehrlichia* spp.) in peripheral blood smears may be extremely challenging due to the low parasitaemia, which is a frequent occurrence in chronically infected dogs. However, the level of parasitaemia is also dependent on individual host factors and whenever possible, cytological examinations of buffy coat, bone marrow and lymph node smears should be performed, particularly in field studies when mixed vector-borne infections may occur. When co-infections occur, a diagnosis based on clinical signs is challenging considering, for example, the similar alterations occurring in animals co-infected by tick-borne pathogens or *L. infantum*. In that case, different PCR protocols, various target genes and distinct clinical samples (e.g., bone marrow, lymph nodes, skin, buffy coat and blood) have been shown to be useful for the diagnosis of multiple VBPs, with different degrees of sensitivity and specificity among protocols (Otranto et al., 2009) and should be used according to the most recent literature. In addition, molecular tools have advanced the diagnosis of many VBPs overcoming difficulties in culturing some pathogens (e.g., *Bartonella* spp.), enhancing the sensitivity and specificity, accelerating the time between sample collection and test result, and allowing a rapid expedition of the appropriate chemotherapy. Where necropsy results are used for worm counts in laboratory studies (e.g., in experimental infections with *D. caninum* or *Di. immitis*) the correct timing post-infection must be observed (usually around 5–6 months for *Di. immitis* and 1–3 months for *D. caninum*). For recommendations on specific pathogens, see Section 5.

4.4. Sample size and methods for calculating the efficacy

Laboratory studies typically require a smaller sample size in comparison with field studies, in which the level of exposure to vectors and pathogens may vary widely. The number of animals in each group should be sufficient to demonstrate successful infection in the control animals and obtain a significant difference (typically $p < 0.05$) when compared to the treated group. In most laboratory studies, a minimum of 6–10 animals per group is sufficient. The number of animals included in field studies is usually higher than in laboratory studies. The calculation of the appropriate sample size for detecting a difference between two proportions requires data on the expected proportion in the treated and untreated groups (defined either theoretically or based on existing prevalence/incidence data), confidence level (typically 95 %) and desired power (usually 80 %). The sample size calculation method should be clearly reported in the study dossier. The number of animals per group should also account for potential losses (e.g., owner unwillingness to continue in the study, non-allowed concomitant treatments, death and collar loss in case of studies with collars). Though it is common to include 50–100 animals per group, a smaller number of animals might be accepted according to the minimum sample size calculation. Non-inferiority studies also require defining the non-inferiority limit (Wang and Chow, 2007). In this case, it should be usually assumed that the percentage of protection in both standard and experimental treatment groups is the same.

Different methods can be used to calculate the efficacy of parasiticides in preventing VBP transmission in laboratory and field studies. These methods may vary according to study design and the VBP of interest. In long-term studies, the efficacy calculation can consider the number of infected animals at a given time point, with animals not completing the study being not usually considered in the efficacy calculation. Nonetheless, results from animals removed during the course of the study, according to pre-defined post-inclusion removal criteria, can still be included in the statistical assessment (e.g., using the 'last observation carried forward' approach). Alternatively, the

incidence density rate (IDR) (also known as 'animal-time incidence rate') can be used, allowing the inclusion of data from animals that did not complete the study in the efficacy calculation (Otranto et al., 2010a). In either case, animals tested only once in long-term studies do not contribute to the efficacy calculation.

In laboratory studies, in which VBPs can be counted at necropsy (e.g., *D. caninum* and *Di. immitis*) at the end of the observation period, preventive efficacy is usually calculated using Abbott's formula based on parasite counts:

$$\text{Preventionefficacy}(\%) = \left(\frac{Mc - Mt}{Mc}\right)x100 \quad (1)$$

where *Mc* and *Mt* are the arithmetic mean of parasites found at necropsy in animals in the control and treated groups, respectively.

When VBPs cannot be precisely counted (e.g., in laboratory studies dealing with bacteria and protozoa) or when necropsy is not an option (e.g., in field studies), the preventive efficacy is usually calculated using the following formula based on number of infected animals or IDR:

$$\text{Preventionefficacy}(\%) = \left(\frac{Tc - Tt}{Tc}\right)x100 \quad (2)$$

where *Tc* and *Tt* are either the number of infected animals or the IDR in control and treated groups, respectively.

An alternative approach proposed to calculate the percentage of protection of parasiticides against VBP transmission is based on number of challenges until an animal is successfully infected in a laboratory study (Jongejan et al., 2015). According to the authors, this approach provides a better view of the reduction of risk infection provided by the treatment:

$$\text{Protection}(\%) = \left(\frac{IcC - IcT}{IcC}\right)x100 \quad (3)$$

where *IcC* is the number of infective tick challenges conducted in the control group that lead to positive infection and *IcT*, the number of infective tick challenges in the treated dogs that lead to infection.

In non-controlled studies, the efficacy cannot be calculated using the above-mentioned formulae. In this case, the authors should report the percentage of protection conferred by the product to treated animals providing supportive evidence to show that the product protected the animals from VBP transmission. For instance, an uncontrolled field study on heartworm prevention conducted in Brazil provided evidence by reporting prevalence data obtained from untreated animals living in the study area, at baseline and at the end of the study (Labarthe et al., 2015). Nonetheless, it is important to emphasize that this is supportive evidence and does not confirm the efficacy of the investigational products. Therefore, protection data from uncontrolled field studies cannot be used for registration purposes.

Differences in the calculated prevention efficacy between control and treatment groups should be shown to be statistically significant (typically $p < 0.05$) using a suitable statistical test. For non-inferiority studies, the protection in the experimental group should not be significantly less efficacious than the treatment standard.

5. Specific study designs

5.1. Prevention of flea-borne pathogen transmission

The efficacy of a parasiticide against fleas can be evaluated as early as 15 min post-infestation by collecting all on-host live, moribund and dead fleas from treated and untreated animals (Marchiondo et al., 2013). In the case of parasiticides acting topically through contact as well as parasiticides acting systemically, which require fleas to ingest the active compound(s) with the host's blood, the effects on the feeding depend on the nature, mechanism of action and SOK of the active ingredient(s). To quantify flea feeding, a real-time PCR assay can also be used to determine the amount of host DNA in the fleas collected from treated and untreated animals, allowing for appropriate comparative statistical analysis (Wang et al., 2012). Simple haemoglobin strips or simply viewing fleas under a light microscope can also provide qualitative information on flea blood feeding. The vast majority of fleas usually feed within 1 h of infestation and each flea usually takes several blood meals on the same host (Cadiergues et al., 2000). This implies the need for very rapid control of flea infestations to reduce the risk of pathogen transmission, especially in the light of limited available knowledge on transmission times of *Rickettsia felis* and *Bartonella henselae* (Eisen and Gage, 2012). Therefore, independent of the VBP species, the best approach to prevent flea-borne infections is minimizing the establishment of flea infestations on dogs and cats. Accordingly, the approach for preventing flea-borne pathogen transmission should aim at killing the majority (>95 %) of fleas as quickly as possible, ideally within 1 h of infestation. However, considering that fleas usually start laying eggs within 24 h from the initiation of their blood feeding (Akin, 1984), the 24 h time point has been considered as critical to demonstrate satisfactory efficacy (>95 % in Europe and >90 % in the USA) of parasiticide products against fleas in Europe (Marchiondo et al., 2013; EMA, 2016a). So far, no laboratory or field studies have been conducted for assessing the efficacy of parasiticides in preventing flea-borne transmission of *R. felis* in cats, but the design of such studies should generally follow the design adopted for *Ba. henselae* studies, as described in the following section.

5.1.1. Prevention of Bartonella henselae transmission

Bartonella henselae is a widespread vector-borne bacterium, usually associated with cats. It is the aetiological agent of 'cat scratch' disease in humans. Laboratory studies to assess the prevention of *Ba. henselae* transmission to cats have used fleas infected either via feeding on experimentally infected cats (Bradbury and Lappin, 2010; Lappin et al., 2013) or via artificial feeding systems (Bouhsira et al., 2015). The use of artificial feeding systems to infect fleas is more ethically acceptable and provides a more homogeneous source of infected fleas. Laboratory studies using fleas infected on cats need three groups (at least six cats per group): a group of experimentally infected *Ba. henselae* positive cats as source of infection, flanked by a group treated with the investigational product and an untreated group in a way that allows spreading of the flea infestation. The *Ba. henselae* positive cats are experimentally infested with fleas and the cats in all groups are followed up weekly or biweekly for the duration of the study (Bradbury and Lappin, 2010; Lappin et al., 2013). In laboratory studies using fleas infected via artificial feeding the two groups of animals needed are directly infested with the thus infected fleas (Bouhsira et al., 2015).

Field studies should include treated and untreated cats, with a higher number of cats per group (for sample size calculation, see Section 4.4) and a longer follow up (at least 1 year) than laboratory studies. The preventive efficacy in both laboratory and field studies is calculated using the formula (2) based on number of infected animals as described in Section 4.4.

For laboratory studies with *Ba. henselae* in cats, serology, blood culture and PCR are recommended, with testing being performed weekly or biweekly for 42–252 days, depending on the efficacy period of the investigational product (Bradbury and Lappin, 2010; Lappin et al., 2013; Bouhsira et al., 2015). Cats included in field studies targeting *Ba. henselae* should be tested monthly or every 2–3 months, for at least 1 year.

5.1.2. Prevention of Dipylidium caninum transmission

The control of *D. caninum* infection must primarily rely on regular deworming of dogs and cats with a product containing a cestocidal molecule (e.g., praziquantel) (CAPC, 2018; ESCCAP, 2017). The added preventive effect of parasiticides against the intermediate hosts of this tapeworm (primarily fleas but also lice) aids in the control as frequency of deworming may be insufficient. The parasiticide should rapidly kill

>95 % fleas to stop the development of the *D. caninum* cysticercoid into the infective stage (Pugh and Moorehouse, 1985), thus preventing *D. caninum* transmission, even if killed fleas are eventually ingested.

To obtain adult fleas to infest dogs and cats, flea larvae should be exposed *in vitro* to proglottids and eggs of *D. caninum* (Fourie et al., 2012, 2013e). Prior to each host infestation, the prevalence and mean intensity of infection with cysticercoids and degree of development of cysticercoid need to be determined by microscopic dissection of batches of the adult fleas (~100) (Fourie et al., 2012, 2013e; Gopinath et al., 2018).

Dogs and cats have to be infested with unfed infected fleas of mixed sex, with flea numbers ranging from 100 to 250 specimens, depending on the infection intensity determined; higher numbers of fleas should be used if infection intensity is relatively low. Infestations start on day 0 and continue usually weekly after treatment (e.g., on day 7 onwards), depending on the aspired duration of efficacy.

The efficacy evaluation is normally based either on necropsy counts of scolexes in the small intestine or macroscopic examination of faeces for shed proglottids. For necropsy counts, dogs and cats have to be euthanized at the end of the prepatent period (i.e. at least 4 weeks after the last flea infestation in cats and dogs) although necropsy examination for scolex detection should not be too delayed compared to the prepatent period, as self-cure may also occur (Craig et al., 1991). Scolexes of *D. caninum* are then counted allowing the efficacy calculation using the mean of adult worms in treated and untreated animals (see Section 4.4.). An alternative approach could be the daily examination of study animals (Beugnet et al., 2013; Gopinath et al., 2018) from the end of the prepatent period, to detect proglottids in freshly shed faeces or around the anal and perianal regions, in their fur or in their cages as described elsewhere (Fourie et al., 2012, 2013e; Beugnet et al., 2013). The reliability of recovering proglottids in the faeces is however affected by the inconsistent shedding of *D. caninum* proglottids in patent animals, which is not directly related to the degree of infection. A critical test, such as treatment with praziquantel and recovery of worms from faeces could be a valid alternative for evaluating the efficacy of the product. The preventive efficacy is then calculated using either the formula based on parasite counts (1) (i.e. when necropsy is feasible) or on infested animals (2) (i.e. when necropsy is unfeasible), as described in Section 4.4.

5.2. Prevention of tick-borne pathogen transmission

Tick repellency can be assessed *in vitro* and *in vivo* as described elsewhere (Marchiondo et al., 2013). Products that repel or rapidly kill ticks have the potential to reduce the risk of tick-borne pathogen transmission, with the transmission time of the specific pathogen tested largely determining the SOK needed to successfully prevent transmission. Nonetheless, transmission efficiency by ticks as well as the establishment of infections in dogs increases with time (i.e. the longer the feeding period, the higher is the probability of successful transmission), for some pathogens being apparently dependent on a minimum inoculation dose. The efficacy of an investigational product in preventing tick-borne pathogen transmission can be assessed under laboratory conditions by infesting treated and untreated animals (usually from 6 to 10 per group) with ticks infected by a given pathogen. The number and developmental stages of ticks may vary widely according to tick species, developmental stage, pathogen species and infection rate in ticks. Ticks can be applied directly on the animals or placed next to the animals in infestation crates or cages to mimic natural challenges. Ideally ticks should be left on the animals until completely engorged.

Following the first tick challenge, animals should be monitored daily for the appearance of clinical signs potentially related to the infecting pathogen. Rectal body temperature, mucous membrane colouration, capillary refill time and haematocrit are amongst the most common parameters to be checked. Tick-borne pathogens should be detected by cytology, blood culture, serology or PCR. In most cases, cytology and culture are not sensitive enough and PCR on blood samples is the preferred technique. Serology can be used whenever available, but cross-reactions between closely related pathogens are frequent and should be accounted for. In laboratory studies, testing of animals is usually performed before experimental challenge and then weekly or biweekly for around 2–3 months or longer, according to specific pathogens (Fourie et al., 2013d; Honsberger et al., 2016; Taenzler et al., 2016; Geurden et al., 2017). In field studies, considering the seasonality of tick infestation (Lorusso et al., 2010), the variable prepatent periods, the transient parasitaemia/bacteraemia and the uncertainty regarding the exact day (or days) of exposure to infected ticks, dogs and cats should be tested every 2–3 months, for at least 1 year (Otranto et al., 2010b; Dantas-Torres et al., 2013). While the number and timing of follow ups may be adapted to the needs of each study, animals should be tested at least before inclusion, at the end efficacy period of the investigational product and at the end of an observational period. The preventive efficacy in both laboratory and field studies targeting tick-borne pathogens is calculated using a formula based on number of infected animals (2) or number of challenges up to successful infection (3) as described in Section 4.4.

5.2.1. Anaplasma spp. Ehrlichia spp. and Rickettsia spp.

In laboratory studies, competent tick vectors should be used to transmit *Anaplasma* spp., *Ehrlichia* spp. and *Rickettsia* spp. to dogs and cats. The vector of *A. platys* is still unknown, and no laboratory studies have successfully achieved transmission of this bacterium by ticks, but field studies have suggested that tick repellents are efficacious in reducing the risk of *A. platys* transmission to dogs (Dantas-Torres et al., 2013). Transmission times are variable but might occur in less than 3 h (*E. canis*) to 10 h (*R. rickettsii*). Therefore, the preventive efficacy should be assessed at different time points, depending on the intended claim, being at least 3 h for *E. canis*, 10 h for *R. rickettsii* and 48 h for *A. phagocytophilum*, for instance.

Rickettsial pathogens infecting dogs and/or cats are found in mononuclear leukocytes (*E. canis*, *Ehrlichia chaffeensis*), granulocytes (*A. phagocytophilum*, *Ehrlichia ewingii*), platelets (*A. platys*), or endothelial cells of small blood vessels (*R. rickettsii*), appearing in circulating blood within a few weeks after transmission by ticks. As such, diagnosis may be confirmed by cytology of blood smears, which is usually recommended only in the acute phase due to the short-lasting bacteraemia; inclusion may be difficult to find even in the acute phase. Analogously, PCR on blood samples may result in false negative results in the chronic phase whereas it is highly sensitive in the acute phase, even considering that these organisms are not consistently present or detected in circulation. Serological tests, including indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assays (ELISA), are available to confirm exposure to those agents, although cross-reactions are common and should be considered. Because dogs are often seronegative during the initial clinical disease, serology should be repeated every 2–3 weeks. In addition, a four-fold increase in IgG titre or a single IgG titre >1024 on IFA are usually considered diagnostic. Culture of these organisms are not usually attempted.

5.2.2. Borrelia spp.

Borrelia burgdorferi is passed transstadially from a developmental tick stage to another. Nymphs and adults, experimentally infected as larvae and nymphs, respectively, may be used as a source of infection. Transmission of *Borrelia* spp. spirochetes by infected ixodid ticks occurs from 24 to 48 h following attachment to the host (Hajdušek et al., 2013) and therefore, the prevention efficacy should be assessed from 24 h. The diagnosis may be confirmed by serological tests, including whole-cell-based IFA or ELISA, which are commercially available for the diagnosis of *Bo. burgdorferi*. Since *Bo. burgdorferi* spirochetes enter the connective tissue upon tick inoculation, cytology of synovial fluid using dark-field microscopy is more effective than cytology of circulating blood and blood smear. Other diagnostic methods include culture isolation (though rather challenging and requiring relatively low density of organisms present in chronic infections), and molecular detection of

Bo. burgdorferi DNA in affected tissues.

5.2.3. *Babesia spp.*

Infective unfed or partially infected ticks (males and females but also nymphs) should be used for the experimental infection. For most of the *Babesia* spp. the transmission times are unknown but, considering data available for *B. canis* (transmitted about 36 h but only 8 h in interrupted feeding experiments) (Varloud et al., 2018), the preventive efficacy should be assessed from 24 h. The diagnosis may be performed by blood smear cytology from approximately 1–3 weeks, by molecular tests (both conventional and quantitative PCR are available) from 7 to 10 days and or by serology after 3–4 weeks. Blood samples should be collected for serum analysis at two, three, up to four or more weeks following exposure to infected ticks. Serology (IFA and ELISA assays are available) may not be useful in detecting acute infections and cross-reactions between different *Babesia* spp. may occur and should be considered in field studies, in areas where more than one *Babesia* spp. may circulate among dog and cat populations.

5.2.4. *Cytauxzoon spp. and Hepatozoon spp.*

In laboratory studies with *Cytauxzoon* spp. (typically *Cytauxzoon felis*), the transmission should be done by exposing cats to the bites of infected ticks (nymphs, males and/or females). Since transmission occurs within 48 h, the preventive efficacy should be assessed within 48 h after treatment or infestation. Diagnosis should be confirmed by identification of *Cytauxzoon* spp. inclusions in blood smears and/or fine needle aspirates from the liver, spleen and lymph nodes and the observation of schizont-infected myeloid cells confirms acute disease. Since the sensitivity of blood smears may be low, fine needle aspirates and cytology of liver, spleen, lymph nodes and lungs are indicated if blood smears are not diagnostic in a suspected case. Importantly, *Cytauxzoon* spp. inclusions in erythrocytes cannot be reliably distinguished from *Babesia* spp. inclusions in cats. Hence, PCR assays, which are more sensitive and specific than the cytological observation, should be employed to confirm the infection in both laboratory and field studies.

In laboratory studies with *Hepatozoon* spp., the transmission is performed by feeding dogs with infected nymphs, males and/or females. Considering that the transmission of *Hepatozoon* spp. is immediately following ingestion of ticks, reducing the tick infestation levels in the environmental is probably the only effective way to reduce the risk of infection in dogs (Dantas-Torres et al., 2013). The diagnosis of *H. canis* is achieved via blood smear cytology by detecting typical gamonts in mononuclear cells from 28 days post-infection, whereas that of *H. americanum* is also based on the finding of meronts in muscle biopsy samples (Baneth, 2011). Molecular tests (both conventional and quantitative PCR are available) on blood samples are the most sensitive methods for diagnosing *H. canis* even in low parasitaemic individuals.

5.3. *Prevention of pathogen transmission by flying insects*

A high repellent efficacy (typically inferred from the anti-feeding effect) of a parasiticide is the main desired feature for the prevention of the disease transmission of VBPs by flying insects. While a high killing efficacy cannot protect an individual animal from becoming infected, it may contribute to control the infection rate in the vector population, thus interrupting the propagation of a VBP, as demonstrated for *Di. immitis* (McCall et al., 2017b). Previous W.A.A.V.P. guidelines (Marchiondo et al., 2013) did not consider flying insects and the assessment of the repellent and killing efficacy for this group of arthropod vectors is briefly described below.

5.3.1. *Assessing the repellent and killing efficacy against flying insects*

Without specific guidelines such as those available for fleas and ticks (Marchiondo et al., 2013), laboratory studies conducted from 1997 to 2018 to assess the repellent and killing efficacies of topical formulations against sand flies (reviewed by Fondati et al., 2018) have progressively tended to reach a consensual, not yet definitive, protocol. Groups typically consist of 6–8 animals. The number of female sand flies (4–8 days old or older) used for each challenge ranges from 75 to 200, and a few males are included in the cages to promote biting. Exposure of dogs to sand flies typically lasts for 1–2 h (Fankhauser et al., 2015). Dogs may eventually be tested for natural attractiveness to sand flies, because of the wide individual variation detected in these animals (Bongiorno et al., 2013). In this case, allocation to treated and untreated groups is made by ranking dogs according to their individual pre-treatment sand fly engorgement test results (Bongiorno et al., 2013; Bouhsira et al., 2018), and the least attractive dogs are discarded. A properly sized cage should prevent accidental contacts between sand flies and treated dogs, which may occur in smaller cages. Ideally, a cage should be at least 100 cm in length, 75 cm in width and 90 cm in height if medium-size Beagle dogs are used. After exposure time, female sand flies should be carefully collected using mouth aspirators, avoiding unwanted damage of these delicate insects. This must be done by careful observation of the exposure cage for any visible flies, prior to and after removal of the dog. Additionally, compressed air can be blown into the dog's hair to help recover any remaining insects lodged in the fur. Infestations should start when the efficacy is supposed to start (day 1 or 2 or even later) after treatment (day 0) and continue after treatment (e.g., weekly or biweekly from day 7 onwards), depending on the aspired duration of efficacy.

Repellent efficacy against flying insects (e.g., mosquitoes and sand flies) is usually inferred from the anti-feeding effect produced by the investigational product. In practice, the repellent efficacy can be evaluated by exposing treated animals to the insects for 1–2 h, after which insects are counted and categorized as 'fed' or 'unfed'. Female sand flies or mosquitoes with red enlarged abdomens are considered as 'fed' (or 'engorged'), while those with no evidence of blood in their abdomen are considered as 'unfed'. Then, the repellent/anti-feeding efficacy (%) should be calculated based on feeding status, using the formula:

$$\text{Repellentefficacy}(\%) = \left(\frac{Cf - Tf}{Cf}\right) x100$$

where *Cf* and *Tf* are the arithmetic mean of fed females in control and treated groups, respectively.

The repellent efficacy calculated using arithmetic means from untreated and treated groups (EMA, 1994) may not be sufficient for the appraisal of persisting and homogeneous repellent effect on sand flies over time, as a result of the highly variable feeding data from control dogs. The recommended EMA formula can eventually be complemented by longitudinal sets of fed-to-unfed female ratios as described elsewhere (Bouhsira et al., 2018).

The insecticidal efficacy of flying insects should not only be assessed shortly after exposure to treated animals, but for up to 24–72 h following exposure (WHO, 2005), when insects are counted and categorized as 'live', 'moribund' or 'dead'. Insects are considered as 'live' when they exhibit normal behaviour and are capable of flying, 'moribund' when they are unable to move normally and are incapable of coordinated locomotion or flight upon external stimuli, and 'dead' when they are incapable of any behaviour and lacking any movement upon external stimuli. The killing efficacy (%) can be then calculated at different time points (e.g., 1, 2, 4 or even 20 h after exposure) (Killick-Kendrick et al., 1997; Paulin et al., 2018), using the formula as follows:

$$\text{Killingefficacy}(\%) = \left(\frac{Cl - Tl}{Cl}\right) x100$$

where *Cl* and *Tl* are the arithmetic mean of live females in control and treated groups, respectively.

5.3.2. *Prevention of Dirofilaria spp. transmission*

Though most of data about the prevention of *Dirofilaria* spp. refer to *Di. immitis*, they should be considered equally valid for *Dirofilaria repens*, which has a very similar biology in relation to the extrinsic incubation

period in the vector and prepatent period in the definitive host. Over the past 3 decades, dirofilariosis, especially heartworm disease prevention in both dogs and cats has essentially been based on the chemoprophylactic use of MLs (i.e. ivermectin, milbemycin oxime, moxidectin and selamectin) targeting the third- and fourth-stage migrating larvae (L3s and L4s) of *Di. immitis* (Bowman and Atkins, 2009).

However, repelling mosquitoes constitutes an important component in the control of *Di. immitis*, as proven in a laboratory study showing that repelling and killing mosquitoes prevented the transmission of this parasite to dogs (McCall et al., 2017a). The use of this 'double defence' strategy using a permethrin-containing product together with an ML was more efficacious than the monotherapy (with the same ML) in protecting dogs from becoming infected with a *Di. immitis* isolate with reduced susceptibility to the same ML (McCall et al., 2017a). This integrated prevention strategy is now also included in the Guidelines of the American Heartworm Society (AHS, 2018). However, environmental stressors not normally applied under laboratory conditions, like sunlight and bathing, may reduce the overall duration of efficacy of repellent products (Blagburn et al., 2015), potentially limiting the added repellency benefit of integrated strategies (American Heartworm Society, 2018). This, once again, emphasizes the need for field studies.

In order to demonstrate the efficacy of a parasiticide in preventing transmission of *Di. immitis* L3s, a study should employ infected female mosquitoes and assess the presence/absence of adult worms in exposed animals 5–6 months after the challenge (McCall et al., 2017a). Female mosquitoes can be infected *in vitro* by feeding on blood with microfilariae (McCall et al., 2017a). The estimated number of L3 transmitted to each dog should be calculated before each exposure by dissecting individual mosquitoes (at least three mosquitoes from each batch). Dogs or cats should be exposed to not less than 50 mosquitoes for 1 h, under sedation, within individual mosquito-proof containers. Using infected mosquitoes not older than 21 days is therefore advisable as, as in older mosquitoes the feeding behaviour is impaired and can therefore appear as disrupted (Gleave et al., 2016).

Calculating the efficacy of a product in preventing the transmission of *Di. immitis* requires dogs or cats to be humanely euthanized and necropsied from 5 to 6 months after the mosquito challenge (i.e. after worms reach pulmonary arteries but before they reach maximum size and increase risk of clinical signs). At necropsy, all (presumably adult) worms should be collected from the heart and associated pulmonary arteries, but also the pleural and peritoneal cavities, and enumerated. The preventive efficacy in laboratory studies involving experimental infection and necropsy is calculated using the formula (1) described in Section 4.4.

Field studies conducted so far assessing the efficacy in killing L3s and L4s of *Di. immitis* by means of MLs in dogs have adopted distinct designs (Boy et al., 2000; Genchi et al., 2002; Moraes-da-Silva et al., 2016). Animals positive to adult antigens 120 days post-infection should be removed from the study, as previous exposure cannot be ruled out (Carmichael et al., 2017). Treated and untreated groups should include a representative number of animals, which depends on the force of infection in the study area (see Section 4.4 for sample size calculation). Animals should be followed up for at least 1 year and testing should be performed at least before inclusion, on days 120, 180 and 360. Whenever feasible, animals can be followed up monthly or every 2–3 months from day 120 until the end of the observational period. For *Di. immitis*, the modified Knott test and serological tests targeting antigens of adult female worms can be used (Knott, 1939). However, these only show reliable results from 180 to 190 days after infection, when *Di. immitis* adults are formed and females are producing the first microfilariae. The preventive efficacy in field studies should be calculated using the formula (2) as described in Section 4.4.

5.3.3. Prevention of Leishmania spp. transmission

The assessment of the *L. infantum* prevention efficacy under laboratory conditions is not an easy task. Tests employing sand flies experimentally infected with *L. infantum* are usually impracticable, as under laboratory conditions only a small proportion of these vectors will survive after the first infectious blood meal, and an even smaller proportion will be willing to feed again (Aslan et al., 2016). Therefore, the only parameters that can be reliably calculated in laboratory studies are repellent and killing efficacies against sand flies (Bouhsira et al., 2018).

In the absence of a feasible laboratory model, field studies are pivotal to assess the efficacy of an investigational product in preventing *L. infantum* transmission in dogs and cats (Otranto and Dantas-Torres, 2013). Several field studies using topical formulations containing pyrethroids were performed from 2001 to 2016 (reviewed by Fondati et al., 2018). Studies should be conducted in areas where competent sand fly vectors are known to occur. Stringent inclusion criteria should be used to diminish the probability of including already infected dogs. The use of naïve purpose-bred dogs may overcome this problem (Papadopoulos et al., 2020), but this is not always feasible. In areas where *L. infantum* transmission is seasonal (e.g., in the Mediterranean region), this problem may also be overcome by including young dogs that were born during the winter season. Data on the prevalence and/or incidence of canine *Leishmania* infection in the study area is necessary for sample size calculation purposes (see Section 4.4). If the study is performed within a large territory (e.g., hilly to coastal level or rural to urban areas), control and treated dogs should be equally distributed over the different environmental conditions within the territory, to ensure that study animals are challenged with an equivalent level of *L. infantum* transmission risk. Randomization and allocation of animals to treated and control groups should be done by household, as treated and control animals should not be co-housed.

For *L. infantum*, dogs may become positive (e.g., cytology, culture, PCR and/or serology) 1–2 months after being bitten by infected sand flies, but the prepatent period and the time required for seroconversion may vary widely. Therefore, dogs and cats involved in field studies targeting *L. infantum* should be followed up for at least 1 year. The frequency of testing may be flexible, but animals should be assessed at least before inclusion, at the end efficacy period of the investigational product and at the end of an observational period. However, whenever feasible, it is advisable to test animals every 3–4 months in order to detect early positivity in exposed animals, though it may be more difficult in some field studies, particularly with cats (Brianti et al., 2017). The preventive efficacy in both laboratory and field studies is calculated using the formula (2) as described in Section 4.4. For field studies using a positive control group, a non-inferiority approach should be used. Considering the low sensitivity of cytology and culture, the combined use of serology and PCR is recommended. Serology should preferentially be performed using quantitative methods and PCR using reliable tissue samples (e.g., bone marrow, lymph nodes or conjunctival swabs). Blood may be the only convenient sample type to be used in large-scale field studies, but in this case the use of a highly sensitive real-time PCR assay validated for this type of sample is recommended. Cross-reactions with other *Leishmania* spp. (e.g., *Leishmania braziliensis* in Latin America) and other trypanosomatids are common and should be considered when interpreting serological results. In the same way, most PCR assays designed for targeting *L. infantum* will also amplify DNA from other *Leishmania* spp. (Dantas-Torres et al., 2017). Therefore, serological and PCR results should be interpreted with cautious in field studies, where different trypanosomatids may coexist.

6. Conclusions

The increasing importance and expanding distribution of VBDs in companion animals highlight the need for safe parasiticides with proven efficacy against VBP transmission. Efficacy against arthropods might not *per se* imply a reduction of the risk of VBP transmission by these arthropods. In fact, the current requirements for such claims (e.g., treatment and control of flea and tick infestations) (Marchiondo et al., 2013) do not allow any *a priori* conclusion in this sense. Therefore, even

products with proven efficacy against arthropod vectors need to be tested for their specific potential to reduce the risk of VBP transmission to achieve respective label claims. The present guidelines provide recommendations for studies evaluating the efficacy of parasiticides in reducing VBP transmission in dogs and cats. Considering the variability in VBP transmission patterns, strict efficacy threshold requirements in either laboratory or field studies cannot be requested by regulatory authorities. Nonetheless, products should display statistically significant efficacy in the overall prevention of VBP transmission in treated animals compared to untreated controls, or non-inferiority to an already approved product when the inclusion of an untreated control group is unfeasible.

There are still significant gaps in our understanding of many aspects of VBP transmission, such as transmission times of several VBPs (e.g., *A. platys*, *B. gibsoni*, *Ba. henselae*, *Rangelia vitalii* and *R. felis*) or even the vector identity of some agents (e.g., *A. platys* and *Onchocerca lupi*). We therefore advocate for future studies to increase such knowledge and to allow a continuous advancement as well as widening of these recommendations to include further relevant pathogens.

Declaration of Competing Interest

The authors declare no conflict of interest. JJF is employed by Clinvet USA, VL by Vetoquinol, France, MV by Ceva Santé Animale, France, JD by Elanco Animal Health, USA, BS and MP by Bayer Animal Health GmbH an Elanco Animal Health Company, Germany, TG by Zoetis, Belgium, RK by ParaC-consulting for Parasitology and Drug Discovery, Germany, ARH, by MSD Animal Health Innovation GmbH, Germany, FB by Boehringer-Ingelheim Animal Health, France and PH, PAH Consultancy Pty Ltd, Australia. No author received funding for her/his contribution to the article.

All authors have potential commercial interests (but none to specifically declare at the time of drafting or submitting this paper) but the paper represents the whole spectrum of scientific opinion and best practice. To create a guideline such as this the authors need to be experienced and well versed in the discipline, thus it is unexpected that they are within pharmaceutical companies or companies providing services to such companies.

Additionally, and very importantly, the draft paper was independently reviewed by 6 peer reviewers who offered independent and anonymous assessments. As such, considering all this, the WAAVP guideline sub-committee is satisfied on the objectivity and independence of the guidance offered up in this paper.

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Glossary

Vector: an organism (especially an arthropod) that carries and transmits a pathogen from one host to another
Anti-feeding effect: the action of a product that causes ectoparasites to fail to feed on the host
Control of ticks and fleas: the act of reducing the population of arthropods (both on animals and environment) or pathogen transmission by means of different approaches (e.g., the use of chemical insecticides and acaricides, vaccination or biological control)
Curative efficacy: the efficacy of an ectoparasiticide product to solve a pre-existing, already established infestation. Applicable to 'permanent' ectoparasites such as adult fleas, ticks, mites and lice
Efficacy: the extent to which a product achieves its intended effect under ideal circumstances (e.g., controlled clinical studies)
Incidence density rate: the number of new cases of disease during a defined period of time, divided by the sum of individual-time of the population at risk
Interrupted feeding: when an arthropod feeding process on a vertebrate host is interrupted after the initiation of the blood meal
Killing effect: the action of a product that causes the death of ectoparasites
Non-inferiority studies: studies designed to demonstrate that the test product is not inferior to the comparator (i.e. a product with proven efficacy). These studies require defining the non-inferiority limits
Persistent (residual) efficacy: extended activity of a product measured in days or weeks after initial treatment
Pre-activation: The time a pathogen requires in the vector to complete its development into a transmissible state
Prepatent period: the time between infection of the host and the earliest time at which parasitic immature forms (usually infective to the arthropod vectors) occur
Prevention: the act of preventing something, including ectoparasitic infestation and pathogen transmission
Repellent effect: the action of a product that causes ectoparasites to avoid or leave the host
Speed of kill: the time required for a product to kill a stated, non-negligible percentage (usually between 90 and 95 %) of ectoparasites
Transmission time: the time required from initiation of feeding by the arthropod vector to actual pathogen transmission to the host