10 October 2024
EMA/563432/2024
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

Withdrawal assessment report of an application for a grouped variation requiring assessment for Vectra 3D (EMEA/V/C/002555/VRA/0026/G)

International non-proprietary name: dinotefuran / pyriproxyfen / permethrin

Assessment report with all information of a commercially confidential nature deleted.

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1. Introduction

1.1. Procedural steps

In accordance with Article 64 of Regulation (EU) 2019/6, the marketing authorisation holder, Ceva Santé Animale (the applicant), submitted to the European Medicines Agency (the Agency) on 30 April 2024 an application for a group of variations requiring assessment for Vectra 3D.

On 27 November 2024, the applicant withdrew the application. In its letter notifying the Agency of the withdrawal of application, the applicant stated that "This withdrawal is based on the following reason: the CVMP considers that the data provided do not allow the committee to conclude on a positive opinion."

1.2. Scope of the variation

Variations requested	
G.I.7.a	Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one
G.I.4	Change(s) in the Summary of Product Characteristics, Labelling or Package Leaflet due to new quality, preclinical, clinical or pharmacovigilance data.

This grouped variation concerns change(s) to therapeutic indication(s) – addition of a new therapeutic indication or modification of an approved one: 'as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with *Dirofilaria immitis* via transmission by mosquitoes (*Aedes aegypti*)' and updates to the pharmacodynamics section of the SPC to include details on the activity of the product in relation to reducing the spread of vector-borne diseases leishmaniosis and dirofilariosis.

1.3. Changes to the dossier held by the European Medicines Agency

This application relates to the following sections of the current dossier held by the Agency:

Part 1 and Part 4.

1.4. Scientific advice

Not applicable.

1.5. Limited market status

Not applicable.

2. Scientific Overview

The product Vectra 3D spot-on solution for dogs contains the active substances dinotefuran, pyriproxyfen and permethrin, a combination of an insecticide, acaricide, repellent and insect growth regulator.

Vectra 3D is currently indicated for use in dogs for the treatment and prevention of flea infestation (*Ctenocephalides felis, Ctenocephalides canis*). The treatment prevents flea infestation for one month. It also prevents multiplication of fleas for two months after application by inhibiting egg hatching (ovicidal activity) and by inhibiting the emergence of adults from eggs laid by adult fleas (larvicidal activity). The product has persistent acaricidal and repellent efficacy against tick infestations (*Rhipicephalus sanguineus* and *Ixodes ricinus* for one month, and *Dermacentor reticulatus* for up to three weeks). Against flies, mosquitoes and stable flies, the treatment provides persistent repellent (anti-feeding) activity. It prevents biting from sand flies (*Phlebotomus perniciosus*), mosquitoes (*Culex pipiens, Aedes aegypti*) and from stable flies (*Stomoxys calcitrans*) for one month post-application. The treatment also provides persistent insecticidal activity for one month against mosquitoes (*Aedes aegypti*) and stable flies (*Stomoxys calcitrans*).

Vectra 3D is presented in five different strengths administered at a minimum dose of 6.4 mg dinotefuran/kg body weight (bw), 0.6 mg pyriproxifen/kg bw and 46.6 mg permethrin/kg bw.

The proposed grouped variation concerns change(s) to therapeutic indication(s) - addition of a new therapeutic indication or modification of an approved one for Vectra 3D spot-on solutions for dogs: "The veterinary medicinal product can be used as part of a prevention strategy against heartworm disease due to reduction of the risk of infection with *Dirofilaria immitis* via transmission by mosquitoes (*Aedes aegypti*) for one month. The effect is indirect due to the product´s activity against the vector."

Additionally, it is proposed to amend the pharmacodynamics section of the SPC to include details on the activity of the product in relation to vector-borne diseases leishmaniosis and dirofilariosis as follows: "In laboratory studies, the veterinary medicinal product was shown to contribute to the reduction of the spreading of vector-borne pathogens (*Leishmania* responsible for leishmaniosis, *Dirofilaria* responsible for dirofilariosis) from infected dogs by vectors (sand flies and mosquitoes, respectively). In the prevention of heartworm disease, the use of pyrethroids based products does not substitute the use of macrocyclic lactones, but it can be combined with the latest to lessen the risk of infection ("double defense")."

2.1. Safety (tolerance, user, environment)

No new pre-clinical or target animal safety studies were provided by the applicant for this variation application. Taking into account that no changes to the dose rate are proposed compared to that which has already been accepted by the CVMP, and the adverse events of diarrhoea, which occurred during one study, are already mentioned in the SPC, it can be accepted that no new concerns in terms of target animal tolerance/safety are raised.

Further, as the product will be administered to the same target species, using the same route of administration and at the same posology that have already been accepted by the CVMP, no new concerns in terms of user safety are considered to arise. That is, the user will not be exposed to a greater amount of the active substances or at a greater frequency than that which has previously been assessed and approved for the product. No change to the impact on the environment is envisaged.

Therefore, no further assessment is deemed necessary in respect of target animal tolerance, user safety or safety for the environment and it can be concluded that the introduction of the proposed change will not present an unacceptable risk for the animal, user or the environment.

2.2. Efficacy: change(s) to therapeutic indication(s) - addition of a new therapeutic indication or modification of an approved one: 'as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with Dirofilaria immitis via transmission by mosquitoes (Aedes aegypti)' (G.I.7.a)

To support the proposed change, the applicant presented scientific publications (reviews, studies and one case report) dealing with the epidemiological situation in different European countries, and the zoonotic potential of *D. immitis* and *D. repens*. In summary, these publications demonstrate that cases of dirofilariosis are increasingly occurring in more (northern) regions in Europe, which is probably due to several factors such as climate changes, presence of vectors in new areas, appearance of new competent vector species in the continent, increased movement of pets, urbanisation of rural areas or the creation of extensive areas of irrigated crops. Prudent use and further research regarding resistance mechanisms and the understanding of possible interactions between human and animal heartworm infections are necessary. It is however noted that *A. aegypti* is not mentioned as a documented vector of *D. immitis* in Europe in the presented publications and that data from the European Centre for Disease Prevention and Control (ECDC) show that *A. aegypti* is absent in most parts of mainland Europe.

In support of the proposed change to therapeutic indications and to justify the related amendment of the pharmacodynamics section of the SPC with regard to dirofilariosis, the results of one laboratory study conducted in the USA were provided.

This study was performed to support i) the proposed change to the SPC section on indications for use: "The veterinary medicinal product can be used as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with *Dirofilaria immitis* via transmission by mosquitoes (*Aedes aegypti*) for one month. The effect is indirect due the product´s activity against the vector." and ii) the addition of the following information in the pharmacodynamics section of the SPC: "In the prevention of heartworm disease, the use of pyrethroids based products does not substitute the use of macrocyclic lactones, but it can be combined with the latest to lessen the risk of infection ("double defense")."

In this pre-clinical, experimental study the efficacy of Vectra 3D and Vectra 3D in combination with a commercially available product containing milbemycin oxime (MBO) in blocking heartworm transmission and preventing heartworm disease by exposing heartworm-naïve dogs to infected mosquitoes was assessed when both products were administered according to their product information. The study design is considered adequate in terms of the selection of treatment groups to determine the efficacy of the veterinary medicinal product alone or in combination with milbemycin oxime. It is noted that, according to the relevant CVMP guideline (Guideline on data requirements for veterinary medicinal products intended to reduce the risk of transmission of vector-borne pathogens in dogs and cats; EMA/CVMP/EWP/278031/2015), pre-clinical studies should evaluate efficacy of the minimum authorised dose. The applicant stated that treating in line with the posology of the SPC would align with clinical field trial practices and reflect a "real-world usage" ensuring that study results are applicable to actual conditions of product use. Although the CVMP takes note of the applicant´s argumentation, the fact remains that the vector-borne pathogens guideline specifically mentions that for pre-clinical studies the established minimum recommended treatment dose should be used (section 5.3). Moreover, even in case of clinical trials, the guideline indicates that ´the chosen treatment dose should preferably be the established minimum recommended treatment dose (or a dose as close as possible to the minimum recommended dose)´ (section 6.2). Considering the above, the CVMP remains of the opinion that the minimum recommended therapeutic dose should have been used in the study.

Although the study was not performed under GCP-compliant conditions, it was conducted according to conditions considered sufficient to gain robust, high-quality results.

Thirty-two purpose-bred beagles, 5 to 7 months old (D0), weighing 3.4 to 6.2 kg, males and females (50:50) were randomly allocated to four treatment groups of 8 dogs each (untreated, Vectra3D, MBO, Vectra3D + MBO). The sample size of eight dogs per group is considered to be adequate. Animal selection, gender distribution and randomisation based on descending body weight within gender are considered acceptable.

Animals were housed individually in mosquito-proof indoor pens in a purpose-built building, with controlled temperature and ventilation systems, which is a deviation from Directive 2010/63/EU. Even though the pen dimensions, feeding, watering, and overall animal welfare practices met US requirements, the size of pens and the single housing in general did not comply with Directive 2010/63/EU, which should always be the case for studies submitted for a European authorisation procedure.

The JYD-34 strain of *D. immitis* was used; the *A. aegypti* (Liverpool Black-eyed strain) exposure was induced using laboratory reared, 4-to 5-days old adult female mosquitoes, which were infected on microfilaremic blood 16 days prior dog exposure. Infected mosquitoes were not older than 21 days, which is in line with the recommendation of the WAAVP guideline for studies evaluating the efficacy of parasiticides in reducing the risk of vector-borne pathogen transmission in dogs and cats (Otranto et al., 2021). Live mosquitoes were counted and categorized as live (engorged or non-engorged) or moribund (engorged or non-engorged). Dead mosquitoes were counted and categorized as crushed, engorged or non-engorged.

Dogs were exposed to 25±10 infected mosquitoes over a time frame of 60±10 minutes. It is noted that, according to the abovementioned WAAVP guideline, dogs should be exposed to not less than 50 mosquitoes for 1 h. Although only half of the recommended mosquito counts have been used for infection of dogs in this study, all control animals were adequately infected. Therefore, the reduced number of mosquitoes is acceptable.

Dogs were treated with Vectra 3D on D0 and/or with milbemycin oxime on D51. Mosquito infestations were performed on D21 and D28, which is three and four weeks after Vectra 3D treatment as well as three to four weeks before milbemycin oxime treatment, respectively. Taking into account that permethrin (a pyrethroid included in Vectra 3D) primarily acts as repellent and, according to the SPC of Vectra 3D, prevents infestation for one month after single administration, the treatment and infestation regimen is adequate. Macrocyclic lactones (e.g. milbemycin oxime) act as parasiticides against larval stages of *Dirofilaria immitis*. Usually, macrocyclic lactones should be administered after first possible exposure to mosquitoes, but not more than one month after exposure. Treatment should be continued at regular monthly intervals until 1 month after the last exposure to mosquitoes. Consequently, the chosen time point of treatment with milbemycin oxime 3 to 4 weeks after infestation with mosquitoes is adequate.

The following adverse events were observed: conjunctivitis, vomiting/regurgitation, diarrhoea/bloody diarrhoea, otitis and dermatitis (face). Although the applicant states that none was linked to the IVP, diarrhoea occurred more frequently in the treatment groups. However, as diarrhoea is already listed as an adverse effect in the SPC of Vectra 3D and the dosage and treatment regimens are identical to those already approved, no new safety concern is considered to arise.

At necropsy on D176/177, the untreated group presented with 8 (100%) animals infected (i.e. with at least 1 *D. immitis* worm in circulatory system at necropsy), while the Vectra 3D alone, MBO alone

and Vectra 3D+MBO groups presented with 3 (37.5%), 8 (100%) and 0 (0%) infected animals, respectively. The primary endpoint "percent efficacy of heartworm prevention" was 98% and 100% in groups treated with Vectra 3D and Vectra 3D+MBO respectively, using Abbott's formula based on geometric means of parasite counts on necropsy, compared to 0% efficacy in the untreated and the MBO group. The absence of efficacy in the MBO group was likely due to the use of *D. immitis* strain JYD-34, which is known to have varying degrees of resistance to all of the four macrocyclic lactones used for heartworm prevention. Since the study was performed with a *D. immitis* strain known to be resistant to macrocyclic lactones, it is questionable if an "add-on" effect of Vectra 3D when used together with macrocyclic lactones can be expected under European conditions. The revised wording proposed by the applicant to be included in the pharmacodynamics section of the SPC: *"The veterinary medicinal product, due to its mosquito repellent effect, can be used in association with a preventative heartworm medication to lessen the risk of transmission of Dirofilariosis."* could only be considered if the claim for dirofilariosis was accepted.

The secondary efficacy endpoint (percent blocking heartworm L3 transmission from mosquitoes to dogs) was 99.8%; 98.9% and 99.4% for D21, D28 and D21+28, respectively, using Abbott's formula based on geometric means of estimated numbers of L3 *D. immitis* heartworms transmitted from mosquitoes to dogs. This parameter was however considered uncertain since it is based on an estimate by comparing the average number of L3s in mosquitos before and after feeding on dogs. No data on the natural variation of L3s in infectious mosquitoes was presented. The results for the secondary efficacy parameter were therefore only considered supportive.

The results of the study indicated that a single administration of Vectra 3D used according to label reduced the transmission of heartworms to dogs for one month. The applicant provided scientific justification for the deviation from the guideline requirements for the calculation of efficacy with regard to the use of the total worm count instead of the number of infected animals. In the WAAVP GL it is stated that, in laboratory studies in which vector borne pathogens (VBPs) can be counted at necropsy (e.g. *D. caninum* and *D. immitis*), at the end of the observation period preventive efficacy is usually calculated using Abbott´s formula based on parasite counts. Furthermore, the WAAVP GL states that in cases when VBPs cannot be precisely counted or when necropsy is not an option (e.g. in field studies), the preventive efficacy can be calculated based on number of infected animals. Therefore, calculating efficacy using Abbott´s formula for the laboratory study is considered acceptable.

No clinical trials were conducted by the applicant in support of the proposed change to therapeutic indications. However, the WAAVP guidelines state for *D. immitis* that environmental stressors, like sunlight and bathing, are not normally applied under laboratory conditions. Environmental stressors 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 emphasizes the need for studies under field conditions. Taking into account the relevant CVMP guideline (EMA/CVMP/EWP/278031/2015) and the recommendations of the WAAVP, at least one clinical trial should have been conducted unless otherwise justified. No such justification regarding the impact of different breeds and environmental stressors in a laboratory setting was provided. As such, a major objection was raised by the CVMP on the lack of efficacy evaluation under conditions representative for European field conditions as addressed above.

In response to this major objection, the applicant referred to the results of a published clinical field study (Laidoudi et al., 2021), in which the combination of milbemycin oxime-praziquantel and dinotefuran-permethrin-pyriproxyfen (Vectra 3D) efficiently protected dogs from *Dirofilaria* spp. infection in a high-risk area. The study was conducted in Corsica (France) where the test group of 25 military working dogs was housed in wire cages and treated monthly with MBO-praziquantel and

Vectra 3D. The control group included 55 dogs that were also housed in wire cages or gardens but were treated only seasonally with various "real-life prophylactic treatments" to prevent vector biting or development of *Dirofilaria* larvae. All animals were followed up for one year, with blood collected at day 0, with follow-ups at 6 and 12 months and blood samples being tested for *Dirofilaria* spp. by species specific qPCR at every time point. At the end of the study no cases of *Dirofilaria* spp. were detected in the test group. In the control group the cumulative incidence of *Dirofilaria* spp. infection was 16.4%. The conclusion of the authors was that simultaneous administration of MBO-praziquantel and Vectra 3D protects dogs from *Dirofilaria* infections in high-risk area.

However, the CVMP identified the following shortcomings of the data presented: the dogs were not randomized to the treatment groups and furthermore the test group was very homogenous in terms of the following criteria: housing conditions, ownership and geographic localisation. Additionally, the test group was treated continuously, while the control group was only treated seasonally. These confounding variables cannot be discerned from the treatment effect. Furthermore, no information was provided with regard to the breeds, group baseline characteristics or various "real-life prophylactic treatments" of the control group and their duration. Due to the quality of the study, this publication could only be regarded as supportive and was not considered suitable to replace a pivotal clinical trial.

To summarize, the efficacy for the proposed change to therapeutic indications was not considered to have been demonstrated under field conditions. Since the raised major objection with regard to missing data representative for European field conditions was not satisfactorily addressed by the applicant, the proposed wording: *"The veterinary medicinal product can be used as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with* Dirofilaria immitis *via transmission by mosquitoes (*Aedes aegypti*) for one month."* was not considered to have been adequately justified and therefore could not be accepted.

Amendment of the pharmacodynamics section of the SPC

Related to the proposed change to therapeutic indications, following questions from the CVMP, the applicant proposed that the pharmacodynamics section of the SPC be updated with the following revised text: "*The veterinary medicinal product, due to its mosquito repellent effect, can be used in association with a preventative heartworm medication to lessen the risk of transmission of dirofilariosis*".

During assessment, a further revised wording was proposed by the applicant to be included in the pharmacodynamics section of the SPC: '*The veterinary medicinal product provides a repellent effect against Aedes aegypti, a mosquito vector responsible for transmitting Dirofilaria immitis, which causes cardiopulmonary dirofilariosis (heartworm disease). To effectively reduce the risk of dirofilariosis, the veterinary medicinal product must be associated with preventative drugs to eliminate the infecting larvae, providing both vector control and parasite elimination. Please seek veterinary advice to implement the most effective prevention strategy*.'.

However, since the activity of the product against *Dirofilaria immitis* transmission was not accepted, the above-mentioned information on its own would constitute a pseudo-claim, which was not considered acceptable in this section of the SPC, as indicated in the CVMP Q&A document on the information contained within section 5.1 of the SPC on pharmacodynamic properties for pharmaceutical products (EMA/CVMP/757903/2016). Therefore, inclusion of the respective information on *Dirofilari*a in the pharmacodynamics section of the SPC could not be accepted.

In conclusion, the data provided by the applicant in support of this variation (G.I.7.a) was not

considered acceptable by the CVMP.

On 27 November 2024, the applicant withdrew the variation application. In its letter notifying the Agency of the withdrawal of application, the applicant stated that the reason for the withdrawal was that the CVMP considered that the data provided would not allow the committee to conclude on a positive opinion.

2.3. Update of the pharmacodynamics section of the SPC to include details on the activity of the product in relation to reducing the spread of vector-borne diseases leishmaniosis and dirofilariosis (G.I.4)

The applicant proposed to include the following information in the pharmacodynamics section of the SPC: "*In laboratory studies, the veterinary medicinal product was shown to contribute to the reduction of the spreading of vector-borne pathogens (Leishmania responsible for leishmaniosis, Dirofilaria responsible for dirofilariosis) from infected dogs by vectors (sand flies and mosquitoes, respectively)*.".

2.3.1. Information regarding "*Leishmania* responsible for leishmaniosis"

To support the inclusion of the proposed information on the activity of the product in relation to leishmaniosis in the pharmacodynamics section of the SPC, the applicant provided scientific articles from the public domain and the results of one laboratory study.

Most of the submitted publications deal with the epidemiological situation in different European countries (based on literature reviews, questionnaires or case reports), but also with possible control and protective measures. To sum up, cases of leishmaniosis are increasingly occurring in more northern regions (northward spread) in Europe, which is probably due to changes in climate and environmental conditions. However, in some regions the occurrence of leishmaniosis is underestimated due to the absence of monitoring measures. Continuous surveillance is therefore essential in order to discover new or old unknown endemic areas.

The applicant also provided the results of one non-GCP compliant laboratory study assessing the effect of Vectra 3D on the potential *L. infantum* transmissibility from infected dogs via the vector *Phlebotomus perniciosus*. This was achieved by comparing the feeding, mortality and promastigote infection rate of sand flies that fed on the study dogs prior to and after IVP treatment.

This study was a monocentric non-blinded single-arm laboratory study using a single group of six owner dogs (5 males, 1 female), aged 1-7 years and weighing 7-19 kg. Only partial information on housing conditions was given. However, it was stated that the animals were housed and handled as per the study site standards procedures.

P. perniciosus sand flies originated from a colony in Italy. All used sand flies were confirmed as pathogen-free prior to inclusion in the study. Overall, the vector appeared suitable to be used in this study.

Approximately 98 unfed female of *P. perniciosus* aged 3-9 days plus about 10% males (to promote feeding behaviour) were allowed to feed for 90 min on caged dogs in pre-treatment tests performed 1-4 weeks before enrolment and on D1, D7 and D28 after treatment. Xenodiagnosis assays were performed in individual large exposure cages. Two hours before exposure to the vector, dogs received Adaptil Express Calming tablets (1 tablet/10 kg bw) and were allowed to acclimatize inside the exposure cage in the dark for 30 min before the release of sand flies. Live and dead sand flies

were counted.

The primary endpoint "Anti-transmissibility effect" was 100% at both D1 and D7 (no live engorged females were found, hence the vector-borne pathogen could not be transmitted) and 95.4% on average at D28 post treatment.

Neither study amendments and study deviations nor adverse events occurred during the study.

Several shortcomings were identified, which preclude the assessment of the impact of IVP on the potential transmissibility of *L. infantum* by infected dogs, and these are discussed below.

Without an untreated control group (untreated *Leishmania*-positive dogs infested with naïve sandflies), it is unclear whether the study model chosen is adequate and whether any observed effect is due to the treatment and not due to deficiencies of the study model. Single-arm trials lack crucial features to avoid bias stemming from e.g. sample selection, disease variability, regression to the mean, etc. and do not allow for precise quantification of the treatment effect and the associated sample variability, leading to less reliable effects. In this study, pre-treatment xenodiagnosis was not performed consistently for all animals (D-28 to D-7) so that changes in the post-treatment measures cannot be unequivocally attributed to the treatment instead to natural disease fluctuation. Moreover, the lack of randomisation of the animals into treatment and control groups precludes causal interpretation as an effect of the treatment, relying fully on knowledge from so-called historical controls, which might not necessarily be appropriate for the studied sample.

It is also noted that according to the relevant CVMP guideline on vector-borne pathogens in dogs and cats (EMA/CVMP/EWP/278031/2015), laboratory studies should evaluate efficacy of the minimum authorised dose. In the present study, treatment according to label instructions for Vectra 3D resulted in an average dose between 0.16-0.24 ml/kg bw, i.e. higher than the recommended minimum approved dosage of 0.12 ml/kg bw. Since the product does not have a corresponding leishmaniosis claim, the information cannot be included in SPC section 4.2 and this concern is not pursued further.

It is known that sand flies do not adapt well to laboratory conditions. The question therefore arises as to how viable *P. perniciosus* are after challenge and handling. Even if the standard rearing conditions for laboratory colonies are met and the sand flies are from the same laboratory colony, there may be differences in robustness/fitness between generations, which complicates the interpretation of parameters such as "insecticidal efficacy". And although the mortality rate of the sand flies of 20.1% on average before treatment gives some information regarding the general fitness, a direct comparison with an untreated group (*Leishmania* positive dogs infested with naïve sandflies) at the same time of the study and under the same conditions was not possible due to the uncontrolled study design.

It is furthermore unclear whether the observation time point of 96 h (4 days) post blood meal on infected dogs is sufficient to determine the promastigote stages in *P. perniciosus*. From scientific literature, 96 hours appear to be a very short time period for the development from amastigote to promastigote stages (the infective, transmissible forms). This is also in line with the ESCCAP Guideline 05, where it is stated that the parasite development in the vector is temperature dependent and lasts around 7-14 days at temperatures above 18°C. However, this information relates to natural conditions, whereas in laboratory studies constant environmental conditions (24-28°C and 70-80% relative humidity (RH) for rearing conditions (Lawyer et al., 2017)) may shorten the time required for promastigote development.

Since the primary endpoint "Anti-transmissibility effect" was calculated on the basis of the proportion of promastigote infection rate in the total number of females used in the challenge, it is

unclear if the chosen time point of 96 hours post sand fly challenge was sufficient for the development from amastigote to promastigote stages in sand flies under the proposed study conditions.

It needs to be noted that there is a critical distinction between the minimum number of data points required to perform a certain statistical computation and the minimum number of data points necessary to make a statistical claim regarding the efficacy of a treatment. The latter hinges on an adequate sample size that should be based on the expected effect size, statistical power and Type I error rate (WAAVP guideline, Otranto et al., 2021). The very small sample size of this study suggests a severe lack of statistical power, with the known attached increased risks of bias, chance observations, decreased positive predictive value etc., and thus the reliability and validity of the reported results is highly questionable.

It is further noted that, in line with the CVMP Question and answer document on the information contained within section 5.1 of the SPC on pharmacodynamic properties for pharmaceutical products (EMA/CVMP/757903/2016), "Any data mentioned in SPC section 5.1 (*4.2 for QRD 9.0*)) is to be considered as additional information aiming to provide further details on the scientific basis of the indication(s), as presented in section 4.1 (*3.2 for QRD 9.0*), for the target species as presented in section 4.1 (*3.1 for QRD 9.0*). Information in section 5.1 (*4.2 for QRD 9.0*) should not constitute a new indication or a widening or restriction of an approved indication." Moreover, the appropriateness of including this information on efficacy against transmission of vector-borne pathogens from dogs to vectors in SPC section 5.1 (*4.2 for QRD 9.0*) is considered questionable since the effect is not relevant for the treated dog and there is a risk that this could be misinterpreted, especially taking into account the non-prescription status of the product.

Since the applicant was not able to substantiate the new information regarding the spread of *Leishmania* in SPC section 4.2, the addition of this information in the product information was not considered acceptable by the CVMP.

2.3.2. Information regarding "*Dirofilaria* responsible for dirofilariosis"

To support the inclusion of the proposed information on the activity of the product in relation to dirofilariosis in the pharmacodynamics section of the SPC, the applicant provided the results of one non-GCP laboratory study.

According to the study protocol, the aim of the study was to evaluate the model of heartworm microfilaremic dog exposure to mosquitoes by assessing the performance of the IVP in the TRS dog/mosquito/laboratory exposure model. The results of this study were intended to provide supportive information for the proposed wording in the pharmacodynamics section of the SPC: *"In laboratory studies, the veterinary medicinal product was shown to contribute to the reduction of the spreading of the vector-borne pathogens (…Dirofilaria responsible for dirofilariosis) from infected dogs by vectors (…mosquitoes…)."*

This study was a negative controlled, single-site, clinical efficacy study using a randomized block design with blocks based on pre-treatment, average gut microfilaria counts on engorged mosquitos. However, due to the flaws in the study design with respect to the limited data set resulting from a small number of study animals and the missing statistics (see details below), this study could only be considered as pilot study.

Six beagle dogs (≥ 12 weeks, bodyweight 6.6-11.0 kg on D-7), healthy and microfilaria positive (infected with *D. immitis* JYD-34 isolate) were included in the study, randomized into either a

treated or untreated group of three dogs each. Due to the small number of study animals no statistical analysis could be performed, instead only individual data were assessed.

As per the relevant WAAVP guideline (Otranto et al., 2021), the applicant should have performed and reported a sample size estimation based on the expected effect size, statistical power and Type I error rate. Given the extremely small group size, it could only be concluded that the study lacked sufficient statistical power to make any (efficacy) claim and thus the reliability and validity of the reported results was highly questionable, due to well-known issues of underpowered studies (increased chance of bias, chance results, effect overestimation, etc.).

Although the study protocol was approved by an ethics committee (TRS Labs' Institutional Animal Care and Use Committee), deficiencies with regard to animal housing were noted (see below). It should be noted that, according to Annex II of Regulation (EU) 2019/6, section I 'General principles and requirements', for studies presented in support of applications for marketing authorisation "All experiments on animals shall be conducted taking into account the principles laid down in Directive 2010/63/EU, notwithstanding the place of conduct of the experiments.".

In the provided study, animals were housed individually in cages or runs that prevented contact between dogs from time of treatment until study end. This is not in line with the requirements of Directive 2010/63/EU. Even though the pen dimensions, feeding, watering, and overall animal welfare practices met US requirements, the size of pens and the single housing in general did not comply with Directive 2010/63/EU, which should always be the case for studies submitted for an European authorisation procedure.

Dogs in the treated group were administered Vectra 3D; the treatment was performed according to the label instructions. According to CVMP guideline EMA/CVMP/EWP/278031/2015 on vector-borne pathogens in dogs and cats, the minimum approved dose should be used in laboratory studies. In the present study, the administered dose ranged between 0.19 – 0.34 ml/kg bw, i.e. up to more than double the approved minimum dose of 0.12 ml/kg bw.

Mosquitoes were 4- to 5-day-old female *A. aegypti* (Liverpool black-eyed strain) fed on water with sugar until exposure time. Each microfilaria-positive dog was placed in a dedicated mosquito-proof chamber, exposed to 80±20 unfed mosquitoes for 60 minutes at weekly intervals on D-7, D7, D14, D21 and D28. Mosquitoes were assessed visually and counted as live, moribund or dead, furthermore fed and unfed. Live and moribund mosquitoes were collected for viability assessment. According to WAAVP guidelines for studies evaluating the efficacy of parasiticides in reducing the risk of vector-borne pathogen transmission in dogs and cats (Otranto et al., 2021), "dogs or cats should be exposed to not less than 50 mosquitoes for 1h, under sedation, within individual mosquitoes-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". Thus, the number, age of mosquitoes and infestation design is in line with the recommendation of the relevant W.A.A.V.P. guideline and can therefore be accepted.

No adverse events occurred during the study.

Prior to treatment, 95% of the engorged mosquitoes in both groups had microfilariae. After treatment, engorgement rates for the treated group were 0%, 2.3%, 2.7% and 2.2% for D 7, D 14, D 21 and D 28, respectively, with anti-feeding efficacy (repellency) of 100%, 98.0%, 95.8% and 97.0%, respectively. A total of 22 mosquitoes fed on treated dogs; most of them were dead within 24 h, and all were dead within 72 h. Only 2 unfed mosquitoes exposed to treated dogs survived the incubation period and no L3 were found in them. A total of 121 of the 132 (91.6%) surviving mosquitoes that had engorged on untreated dogs had an average of 12.3 *D. immitis* L3 per

mosquito. However, due to the small number of study animals per group (n=3) the results should be interpreted with caution. In addition, in contrast to the relevant CVMP guideline, the dosing followed the dosing instructions given in the SPC. The applicant argued that treating in line with the posology of the SPC would align with clinical field trial practices and reflect a "real-world usage" ensuring that study results are applicable to actual conditions of product use. Although the CVMP takes note of the applicant´s argumentation, the fact remains that the vector-borne pathogens guideline specifically mentions that for pre-clinical studies the established minimum recommended treatment dose should be used (section 5.3). Moreover, even in case of clinical trials, the guideline indicates that 'the chosen treatment dose should preferably be the established minimum recommended treatment dose (or a dose as close as possible to the minimum recommended dose)' (section 6.2). Considering the above, the CVMP remains of the opinion that the minimum recommended therapeutic dose should have been used in the study.

The CVMP considered that the proposed new information in the pharmacodynamics section of the SPC with respect to reducing the spread of *D. immitis* from infected dogs via mosquito vectors was not sufficiently supported by the results from the provided study due to shortcomings in the study design, including the limited number of animals. In addition, the appropriateness of including this information on efficacy against transmission of vector-borne pathogens from dogs to vectors in SPC section 4.2 is considered questionable since the effect is not relevant for the treated dog but there is a risk that this could be misinterpreted, especially taking into account the non-prescription status of the product. Usually, large-scale field studies or modelling would be required for such information. Since the applicant was not able to provide an adequate justification for the inclusion of the new information in SPC section 4.2, the addition of this wording regarding the spread of *Dirofilaria immitis* was not considered acceptable by the CVMP.

In conclusion, the data provided by the applicant in support of this variation (G.I.4) was not considered acceptable by the CVMP.

On 27 November 2024, the applicant withdrew the variation application. In its letter notifying the Agency of the withdrawal of application, the applicant stated that the reason for the withdrawal was that the CVMP considered that the data provided would not allow the committee to conclude on a positive opinion.

3. Benefit-risk assessment of the proposed change

Vectra 3D is authorised in dogs for the treatment and prevention of flea infestation (*Ctenocephalides felis* and *C. canis*). The product has persistent acaricidal and repellent efficacy against tick infestations (*Rhipicephalus sanguineus, Ixodes ricinus* and *Dermacentor reticulatus*) and also provides persistent repellent (anti-feeding) activity and prevents biting from sand flies (*Phlebotomus perniciosus*), mosquitoes (*Culex pipiens*, *Aedes aegypti*) and stable flies (*Stomoxys calcitrans*), and persistent insecticidal activity against mosquitoes (*A. aegypti*) and stable flies (*S. calcitrans*). The active substances are dinotefuran (an insecticide), pyriproxyfen (an insect growth regulator) and permethrin (a synthetic pyrethroid). Vectra 3D is available as spot-on solution in applicators of five different strengths for use on dogs of different weights.

The proposed variation concerned change(s) to therapeutic indication(s) – addition of a new therapeutic indication or modification of an approved one: 'as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with *Dirofilaria immitis* via transmission by mosquitoes (*Aedes aegypti*)' and updates to the pharmacodynamics section of the SPC to include details on the activity of the product in relation to reducing the spread of vector-

borne diseases leishmaniosis and dirofilariosis.

3.1. Benefit assessment

Direct therapeutic benefit

As this was a variation to introduce changes to therapeutic indications for existing presentations of the product Vectra 3D, the direct benefits would arise from the inclusion of this change, which was investigated in one laboratory study.

The following wording was proposed for inclusion in SPC section 3.2: 'The veterinary medicinal product can be used as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with *Dirofilaria immitis* via transmission by mosquitoes (*Aedes aegypti*) for one month. The effect is indirect due to the product's activity against the vector'.

Following assessment of the dossier, the CVMP concluded that the proposed change was not sufficiently substantiated, as data representative for European field conditions were not provided by the applicant. As such, the proposed change was not considered acceptable and therefore no additional direct therapeutic benefit was foreseen. Similarly, the data provided to support the proposed update to the pharmacodynamics section was not considered to be adequate.

Additional benefits

No additional benefits were foreseen.

3.2. Risk assessment

As this was a variation to introduce changes to therapeutic indications for existing presentations of the product Vectra 3D, the risk assessment would normally focus on potential risks arising from the introduction of these changes. As the product would have been administered to the same target species, using the same route of administration and at the same dose rate as already approved, no new risk was considered to arise in terms of user safety, target animal tolerance, potential for resistance development or for the environment. However, the proposed change to therapeutic indications was not considered acceptable.

3.3. Risk management or mitigation measures

The proposed change to therapeutic indications: "*as part of a prevention strategy against heartworm disease due to the reduction of the risk of infection with Dirofilaria immitis via transmission by mosquitoes (Aedes aegypti)*" was not considered to be compatible with the non-prescription status of the product, as there is a risk that the owner misunderstands the indication and the need for a combination treatment with preventative heartworm medication. The proposed wording was not considered to provide sufficient information to the owner on how the product should be used the reduce the risk of dirofilariosis. Whether this issue could be sufficiently mitigated by including additional text in the product information was not further discussed, as the proposed change to therapeutic indications was not considered acceptable.

3.4. Evaluation of the benefit-risk balance

In the presence of major objections and other concerns, no conclusion could be reached on the benefit-risk balance of the product and the applicant chose to withdraw the application.

4. Conclusion

Based on the original and complementary data presented on efficacy, the Committee for Veterinary Medicinal Products (CVMP) was not able to recommend approval of the application for variation to the terms of the marketing authorisation for Vectra 3D since "major objections" had been identified. Since the application was subsequently withdrawn, a final conclusion on the benefit-risk balance of the application was not reached.