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Guidelines on specific efficacy requirements for ectoparasiticides in cattle

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This guideline replaces the guidelines on specific efficacy requirements for ectoparasiticides in cattle (EMEA/CVMP/625/03-FINAL).

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*The current revision consists of administrative changes made in order to align the guideline to the new definitions and terminology provided by Article 4 of Regulation (EU) 2019/6. The references to the legislation applicable and other scientific guidelines have also been updated. As no changes were made to the scientific content, no concept paper and no public consultation were deemed necessary.

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Guideline on specific efficacy requirements for ectoparasiticides in cattle

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1. General requirements

1.1. Introduction

This guideline gives guidance on how to study the efficacy of products in cattle against all arthropod species that need animal involvement for completing their life cycle, i.e. where at least one parasitic stage occurs in/on the animal or feeds on the animal. Guidance is aimed at the principal ectoparasites found in cattle (mites, lice and ticks) but could be adapted to study the efficacy of products against less common (regional) ectoparasites and arthropod-related disorders, providing that any adjustments to the methods are justified. The scope of this guideline includes nuisance and biting flies because similar principles exist for the demonstration of efficacy. This guideline should be interpreted in conjunction with the existing general guideline on the demonstration of efficacy of ectoparasiticides 7AE17a, 1994.

The guideline is written principally for the treatment methods currently available, but the same principles could be employed for testing novel formulations and active ingredients. This guideline gives some species-specific information to help in the practical implementation of the general principles outlined. However alternative approaches may be used where scientifically justified by the applicant.

1.2. General principles

The aim of treatment is to eliminate or to reduce arthropod parasites or to protect animals from them, in order to maintain animal health and to prevent losses in production. For those parasite species that are permanent parasites this requires an overall efficacy of more than 90% up to 100%, depending on the parasite species. For parasites that only complete part of their life cycle on the animal, treatment should be directed towards elimination of parasitic stages. In the case of the latter, treatment should at least result in resolution of clinical signs or the significant reduction of nuisance. This can be achieved by reducing parasite burdens on the animal to clinically irrelevant levels, by preventing active stages from settling on the host by repelling or by environmental control.

Regarding the life cycle and the impact on animal health, the overall efficacy of treatment, expressed as relative reductions, should be approximately 100% for all lice species and *Sarcoptes scabiei*. For *Psoroptes (b)ovis, Chorioptes bovis* and ticks an efficacy of more than 90% should be achieved. For *Demodex* treatment should result in elimination of clinical signs. For *Haematobia*, *Stomoxys* and larval arthropods (*Hypoderma* spp.) reductions should preferably be about 100%, but always more than 90%. For *Musca and Hydrotea* spp reductions should be 80%-100%, but preferably more than 90%.

1.2.1. Protocols

When designing study protocols the mode of action exhibited by the active substance (e.g. killing, repellent, anti-feeding) as well as the life cycle of the parasite (e.g. length, seasonality, parasitic stages) should be taken into account.

Statistically adequate numbers of treated and control animals should be included in each study. The applicant is required to justify the group sizes and is recommended to seek the advice of a statistician.

Two types of studies should be performed: efficacy studies under controlled conditions to establish immediate and persistent efficacy of a product, depending on the claim, and clinical trials to confirm the results of the studies under controlled conditions. It is acknowledged that for some species e.g. hornfly, Culicoides and warble fly, it is not possible, as yet, to conduct pre-clinical studies. In these cases, controlled clinical trials could be conducted on a farm in a region with a history of infestation.

Animals should be infested with a suitable number of parasites. The adequacy of infestation should be addressed in terms of the statistical, parasitological and clinical relevance of the level of infestation. For a study to be considered valid, parasites should be recovered from at least 80% of the negative control animals at each post-treatment observation.

All studies should be conducted using the final formulation intended for marketing. The use of alternative formulations is only be acceptable if adequate justification for doing so, is provided.

Methods used for the assessment of efficacy should be relevant for the parasite species involved and for the level of efficacy to be demonstrated. Although methods assume equal distribution and availability of active substance over the entire body, concentrations of active substance on different body sites can vary considerably, e.g. due to the formulation used or the way treatment was performed. This should be taken into account.

It should also be noted that most arthropods show marked seasonal activity. Depending on the mode of action of the active substance and the target parasite stage, clinical trials should be carried out in the season in which activity is maximal, unless out of season treatment is more effective, e.g. due to specific parasite stage-active substance interaction.

Untreated control groups should be used provided there are no serious welfare implications of the disease. (Where there is no control, Formula 1 (see appendix) would have to be used for calculating efficacy).

For products intended for external use, the final formulation intended for marketing should be tested for its photostability (e.g. by UV radiation under laboratory conditions) and water stability (e.g. the impact of exposure to rainwater should be evaluated).

1.2.2. Assessment of efficacy

Where a control group is employed, the percentage efficacy should be calculated using the Abbott Formula (see appendix) but in the absence of a control group, Formula 1 should be used for calculating efficacy. It should be noted that all the formulae mentioned in the appendix, refer to the relative reduction that can be obtained for a given parameter (e.g. parasite count, animals still infested) in relation to the method applied (usually one treatment) and the observation period used.

Any statistically significant difference between the treated and the control group should always be interpreted in terms of biological and clinical significance.

The interpretation of reductions of less than 100% should be made with consideration to the parasite species involved. If reduction after one treatment is less than 100%, and occurs in a study of a permanent parasite where complete elimination is required, recommendations for treatment should be such that elimination is obtained, e.g. repeating the treatment.

It should be noted that because differences exist between parasite species (e.g. in feeding habits) accurate extrapolation between species (especially in mites) cannot be assured and so efficacy should be demonstrated for each parasite species claimed.

For parasitic infestations that create skin lesions, it is also useful to record the site and size of the lesions at the beginning and end of the study.

1.3. Dose determination / dose confirmation studies

Dose determination and dose confirmation studies, involving acaricides or insecticides administered by any route should be conducted under controlled conditions against natural or artificial (where feasible) infestations of the target parasite. The most usual method is to mix naturally infested with uninfested

animals; infestation is obtained by transmission through contact. It is accepted that artificial infestation is more difficult to achieve, except for ticks and flies and, to a lesser extent, Psoroptes (b)ovis.

Dose determination studies should be carried out using at least four groups of infested animals, unless otherwise justified, treated with the proposed dose, half the recommended dose and twice the recommended dose administered by the recommended route and an untreated/vehicle-treated control group respectively. Unless welfare issues are a significant factor, then an untreated control group should be included. Delayed treatment of the untreated control group should be considered after an appropriate time period.

It is preferable that the animals should have no history of treatment with acaricide/insecticides or injectable, topical or oral endecto-parasiticides. Prior treatment with acaricide/insecticides or endectocides may be acceptable provided there is a sufficient washout period to guarantee the absence of residual efficacy from any previous treatment. It is acknowledged that naturally infested animals may carry more than one species of ectoparasite. This is acceptable provided that the parasites can be distinguished.

Each treatment group should be housed in separate pens throughout the course of the studies to prevent cross infestation or cross contamination. Animals should be treated with the test product once the infestation has become established and, if appropriate (e.g. for mites), they exhibit clinical signs of infestation.

To establish a claim, two dose confirmation studies should be conducted with adequately infested animals. A dose determination study can be used in place of one of the dose confirmation studies, if the final formulation was used and administered under label conditions.

1.4. Persistent Efficacy Testing

Persistent efficacy is a measurement of the product's continued efficacy in the face of continuing challenge by the target parasite. Persistent efficacy implies the presence of an ectoparasiticide at levels that are relevant in terms of efficacy in time. If the product is claimed to be effective for a seasonal period of pest activity, the study must be conducted over the entire season.

As the dosage needed for relevant protection can be different to the dosage needed for initial treatment, specific dose determination studies are necessary in relation to persistence of efficacy, unless the initial treatment dosage is used. It should be noted that products achieving complete elimination by a single treatment are likely to produce protection for a period of time as well. However, if both claims (treatment and prevention) are made, this should be demonstrated accordingly.

Volatile active substances that are applied topically to the animal may produce a repellent effect, preventing parasites from making contact with the animal. Non-volatile substances lack a repellent effect and are only effective after the parasite has come into contact with the substance or ingested the substance. These differences should be taken into account when designing studies for a persistent efficacy claim.

A persistent efficacy is useful for two reasons:

- To achieve complete parasite control in infested animals with a single treatment only. This depends on the characteristics of the active substance, the formulation and dosage used and the animal species involved.
- To protect parasite-free animals from becoming (re)infested.

As described for dose confirmation, a persistence claim should include 2 studies, each with a non-treated and treated group of adequate size and adequately infested animals. Such studies can be a continuation of a controlled efficacy study.

Following treatment, cattle should preferably be artificially challenged by placing a suitable number of live parasites directly on to the skin at predilection sites (and the areas recorded on a body map in the study protocol), or into isolation cells fixed on to the skin or coat, depending on the mode of action of the substance.

Where artificial challenge is not possible, challenge by contact with cattle carrying natural infestations is acceptable. The reasons for use of this method of challenge should be adequately justified.

Approximately 25-50% of the herd should be left untreated as a reservoir of infestation. Untreated animals should be examined for the presence of live parasites prior to being re-introduced to the main infested group.

Before these examinations and depending on the mode of action of the active substance, it is permissible to separate the treated animals from the untreated for a period of 1-2 days as appropriate for the proposed claim. The numbers of live parasites should be counted as described above.

The duration of the post-treatment interval before the first challenge will depend upon the proposed claim, and should be justified by the applicant. Subsequent challenges should be made, within the same area at weekly intervals (or more frequently if justified) post-treatment, depending on the claim.

Animals should be examined for the presence of live ectoparasites and the development of any lesions after each challenge. A breakdown in residual activity is recorded when live parasites are detected and, in the case of ticks, are attached and feeding. The actual length of protection is recorded as the last date of challenge that failed to initiate an infestation. For example, in conducting a study where a challenge is made once a week, if live parasites are observed 49 days post treatment, then the period of persistent efficacy is 35 days since the breakdown could have occurred subsequent to the challenge on day 35.

1.5. Clinical Trials

Clinical trials are normally carried out on identified infested herds. Preferably, herds should not have been treated with any acaricidal or insecticidal spray, pour-on, injection or drench unless a sufficient washout period has elapsed to guarantee the absence of residual efficacy from any previous treatment prior to the trial.

Preferably cattle should only be infested with ectoparasites belonging to the same order, i.e. infestation with lice only and not with lice and mites. However, it is acceptable for more than one species to be present. In this case, all species should be documented and the dose and treatment schemes should be known for each ectoparasite species. The product should cover all parasite species present or relevant at the moment of treatment and be carried out in accordance with the label recommendations of the final product.

Where an untreated control group is not justified because of animal welfare reasons, a positive control using an established product may be included. It should be noted that the non-inclusion of control animals is justified in exceptional cases only, e.g. sarcoptic mange and psoroptic mange.

The number of trials to be conducted and animals involved in each trial will depend on the ectoparasite species, the geographical location and local/regional situations. However, usually they should be conducted in at least 2 different geographical and climatic regions.

The choice of sampling times should be justified e.g. in respect to the seasonal or daily time of maximum infestation with ectoparasites, taking into account sites of predilection. Efficacy should be demonstrated in at least two different common breeds to represent the target population.

For topical products (e.g. spray, pour-on), the effects of coat length and density should be considered. Climatic conditions (rainfall, relative humidity, sunshine etc.), and faecal contamination, dirtiness of the coat should be documented to assess any effect of these parameters, if relevant.

2. Parasite species-specific information

2.1 Mites (Chorioptes bovis / Psoroptes bovis / Psoroptes ovis / Sarcoptes sp. / Demodex bovis / Neotrombicula sp.)

Dose determination / dose confirmation studies

Cattle should be naturally challenged (by transmission through contact with free moving, infested animals) or artificially challenged, where feasible, by placing a suitable number of live adult female mites directly on to areas of skin known to be predilection sites. The areas should be recorded on a body map in the study protocol. An acclimatisation period of the included animals is necessary, to be sure that the infestation can also persist in the study environment. Sites of challenge should be examined for presence of live mites and the development of any lesions following challenge. Infestation should be allowed to progress until a suitable lesion(s) has developed before treatment. Should it be necessary to use animals that have natural infestations then these should, if possible, have a single specific infestation.

Each treated animal should be inspected at suitable intervals for the duration of the study (e.g. weekly) for at least two complete parasite life cycles post-treatment. The animals should be inspected for the presence of live mites by parting the coat at intervals and performing skin scrapings. It should be noted that whilst a clinical cure may be obtained earlier, a considerably longer period of observation might be required to demonstrate eradication of mites. Therefore, the applicant should justify the period of observation with reference to the proposed claims. Samples should preferably be examined within 24 hours. A suitable area should be scraped from the periphery of at least 3 lesions; the same area should not be scraped repeatedly. It is noted that mite counts are not always a highly sensitive test and that mites surviving after treatment might be missed. Additionally, lesions might be caused by hypersensitivity reactions triggered by small numbers of mites and/or their metabolic products. Taking these factors into account the applicant should appreciate that the correlation between mite count and evaluation of efficacy might not be sufficient. Methods to supplement the evaluation of efficacy could include e.g. mapping affected areas of skin over a grid diagram for each animal and then calculating the percentage body area affected (clinical index). Changes can then be monitored.

It should be noted that purposive treatment of affected areas or over sampling of predilection sites could affect the reliability of sampling results.

Lesion areas should be assessed both prior to treatment and at the end of the study period by measuring the length and width of each lesion. Finally, all cattle should be thoroughly examined for secondary lesions and the presence of live parasites on both primary and secondary lesions by skin scrapings. The presence of any lesion within the challenged areas should be recorded and the lesion area calculated for each animal. Lesion areas can be calculated by measuring their length and width using tuberculosis calipers and the rate of lesion growth calculated using formula 2 (see appendix).

Infestation with *Chorioptes bovis* is more prevalent and mite populations are greater during colder weather and this should be taken into account when study protocols are designed.

Persistent Efficacy Testing

See section 1.4.

Clinical Trials

Efficacy should be assessed using herds naturally infested with mites, and should utilise a Critical Study Group (CSG), within the herd, comprising at least 15 cattle each carrying an infestation of at least 25 live mites and showing clinical signs, at the time of treatment. For those parasite species where these numbers might not be feasible e.g. *Sarcoptes* and *Demodex*, the number of cattle used must be justified. The CSG cattle should be individually identified by tags.

Each animal in the CSG should be visually inspected prior to treatment and at intervals post-treatment until the end of the assessment period (usually at least two complete parasite life cycles post-treatment) when any lesions should be evaluated by measuring length and width of each lesion. Additionally, each animal should be inspected for the presence of live mites and where appropriate, all stages of development should be present to indicate an active infestation. An estimate should be made of mite numbers made along the periphery of each lesion by parting the coat at regular intervals over the body. The predilection sites of all cattle in the CSG should also be examined for the presence of live mites at the start and termination of the trial. If appropriate, skin scrapings should be taken from all unresolved lesion areas and examined for live mites.

2.2 Lice (e.g. Linognathus vituli / Solenopotes capillatus / Haematopinus eurysternus / Bovicola (Damalinia) bovis)

Dose determination / dose confirmation studies

Cattle should be naturally challenged (by transmission through contact with free moving, infested animals) or artificially challenged, where feasible, by placing a suitable number of live adult lice of mixed sex directly on to the withers or other areas of skin known to be predilection sites. Lice burdens should be monitored on both treated animals, and control animals, for at least two complete parasite life cycles post-treatment. The lice burden should be recorded by parting the coat at designated points over the neck, poll, brisket, back and flanks or other predilection sites, and counting the number of live parasites.

A variety of factors cause fluctuations in cattle lice populations; nutrition, sunlight, temperature, humidity, crowding, host skin reaction, hair condition, hair length and shedding, animal grooming and animal and breed resistance. These should be taken into account when designing studies and should be documented in the protocol; the disease is more prevalent and lice populations are greater during the winter.

Persistent Efficacy Testing

See paragraph 1.4.

Clinical Trials

See paragraph 1.5.

2.3 Tick (e.g. Ixodes spp. / Dermacentor spp. / Rhipicephalus spp. / Haemaphysalis spp. / Boophilus annulatus)

Dose determination / dose confirmation studies

In the assessment of efficacy, the applicant should distinguish between claims of repellent and/or acaricidal effect. A repellent effect means that no tick will attach to the animal and ticks already on the animal will leave it soon after treatment. In evaluating the acaricidal efficacy of the product, the feeding or engorgement of the ticks should be taken into consideration in addition to the rate at which ticks are killed. Indications such as 'to prevent' or 'for prophylactic use' should be omitted if the effect is purely acaricidal because as a rule, the acaricidal substance does not prevent an attachment of the tick.

Infestation is considered to be established when attachment of the ticks occurs.

Single host ticks (e.g. *Boophilus annulatus*) should be counted for a suitable and justified period after treatment, e.g. for one parasite life cycle. Single host ticks should be counted repeatedly at regular intervals during this period and note should be taken of their engorgement.

Multihost ticks should be counted at 24-48 hours after treatment and note should be taken of their engorgement.

Persistent Efficacy Testing

Where it is inappropriate to perform artificial challenges, clinical trials may be carried out in herds, which are carrying natural infestations of ticks. However, an untreated control group should be included to monitor the natural challenge. Ticks should be counted periodically, e.g. 3-5 times a week, for the proposed time of persistent efficacy and note should be taken of their engorgement.

Clinical Trials

Clinical trials are carried out in herds, which are carrying natural infestations of ticks.

See also paragraph 1.5.

2.4 Flies (non-biting flies – **Musca domestica / Hydrotea irritans** *[head fly] and biting flies* – **Haematobia irritans** *[horn fly],* **Stomoxys calcitrans** *[stable fly] and* **Culicoides** *sp. [biting midges] and warbles [***Hypoderma bovis / H.lineatum***])*

Dose determination / dose confirmation studies

Studies can be performed as described for ectoparasites.

Persistent Efficacy Testing

Studies can be performed as described for ectoparasites.

Clinical Trials

Flies

Where pasture trials involve different regions, pastures should be comparable with respect to topography and insect activity. Meteorological variables such as temperature, wind-force, relative humidity, presence and absence of sunshine or rainfall should be monitored throughout the trial. The

studies should include an untreated control group to monitor insect activity. Appropriate distance between groups should be maintained to avoid cross contamination.

Flies should be counted prior to treatment and then at weekly intervals after treatment. Pre-defined areas should be chosen depending on the behaviour of the fly-species and areas should be marked on a body map in the protocol. The study period depends on the treatment period claimed by the applicant. The species and number of flies on the animal can be determined by visual counting (with the aid of binoculars) or by making photographs of pre-defined areas (including predilection sites for the different fly species) of the animal's body.

The differences between treated and control animals should be statistically analysed; the duration of the fly season should be taken into account when noting any reduction in fly numbers in response to treatment.

Warbles

Cattle can be selected from herds with a history of warble infestation at a time when the larvae are expected to be in the first instar stage. During the subsequent period of emergence, the numbers of warbles parasitising animals in treated and non-treated groups can be compared.

APPENDIX – Calculation formulae

Abbott Formula

$$\text{Efficacy (\%)} = 100 \times (m_c - m_t)/m_c$$

Control group (m_c): Mean number of live parasites on the host animals

Treatment group (m_t): Mean number of live parasites on the host animals

Arithmetic means are usually acceptable for this calculation. If geometric means are used, the transformation should be justified and the arithmetic means also recorded.

Formula 1

$$\text{\% Efficacy} = \frac{\text{(Total number of animals treated)} - \text{(Number of animals still infested after treatment)}}{\text{(Total number of animals treated)}} \times 100$$

Losson, B.J. and Lonneux, J.F. (1992) Vet. Rec. 131, 73-75.

Formula 2

$$\text{Rate of lesion growth } (\text{cm}^2\ \text{day}^{-1}) = \frac{\text{(Lesion area at time of final assessment)} - \text{(Lesion area at Day 0)}}{\text{(Number of days of assessment)}}$$