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Guidelines on specific efficacy requirements for ectoparasiticides in sheep

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This guideline replaces the guidelines on specific efficacy requirements for ectoparasiticides in sheep (EMEA/CVMP/411/01-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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Guidelines on specific efficacy requirements for ectoparasiticides in sheep

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1. General requirements

1.1. Introduction

These guidelines cover the principal parasites found in sheep but could be adapted to study the efficacy of products against less common (regional) ectoparasites (e.g. sarcoptic and chorioptic mange), providing that any adjustments to the methods are justified. This guideline should be interpreted in conjunction with the existing general ectoparasiticide guideline[1].

With most ectoparasites at least 90% efficacy is required. However, for *Psoroptes ovis* infections 100% efficacy is considered necessary for the treatment of this commercially important disease of sheep.

These guidelines are written principally for the treatment methods currently available but novel formulations and actives could be tested using the principles contained within this guideline.

1.2. General principles

Statistically adequate numbers of animals representative for the intended use (e.g. with an appropriate range of bodyweights), should be used in studies (which may use artificially or naturally infested animals). The animals should have no history of treatment with acaricide/insecticides or injectable, topical or oral endecto-parasiticides. In studies that use animals that have natural infestations of ectoparasites only a single infestation should be present (e.g. sheep scab alone and not sheep scab and biting lice). In studies using animals that have natural infestations prior treatment with acaricide/insecticides or endectocides may be acceptable, provided a sufficient washout period is employed to guarantee the absence of residual efficacy from previous treatment.

Dose determination studies should be carried out using at least four groups of infested animals, unless otherwise justified, treated with the proposed dose (or dip concentration), 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 (e.g. sheep scab), then an untreated control group should be included. Delayed treatment of the untreated control group should be considered after appropriate time period. For topical products the effect of the fleece should be considered. Climatic conditions (rainfall, sunshine, etc.), and faecal contamination and dirtiness of the fleece should be documented to assess any effect of these parameters, if relevant. For dipping studies, groups of unshorn sheep should be used. Sheep should be dipped for a suitable period of time, with the head immersed at least twice.

Dose determination and dose confirmation studies, involving either acaricides or insecticides administered as plunge dips (where animals are immersed in a treatment bath), injectable endecto-parasiticides or any other formulation that can be justified, should be conducted under laboratory conditions against artificial infestations or if necessary, natural infestations of the target parasite.

In each study carried out the percentage efficacy can be calculated using the Abbott formula (see appendix). Other formulae (Formula 1) can be helpful for assessing the cure rate.

[1] Guideline on the demonstration of efficacy of ectoparasiticides, 7AE17a, 1994

1.3 Louse (e.g. Linognathus spp., Damalinia (Bovicola ovis)), ked (Melophagus ovinus) and tick (Ixodes spp., Dermacentor marginatus, Rhipicephalus bursa).

1.3.1 Dose determination and Dose confirmation studies

Animals should be naturally or artificially infested with 25-50 parasites of the target species and should be housed in groups, in separate pens throughout the course of the studies to prevent cross infestation or cross contamination.

Animals should be treated when the infestation has become established. In the case of ticks this will be when attachment is established.

Louse or ked burdens should be monitored on both treated animals, and control animals, for up to 70 days post-treatment. Counts should be recorded by parting the hair or fleece at designated points over the neck, poll, brisket, back and flanks or other predilection sites, and counting the number of live parasites present.

Ticks should be counted daily for a suitably justified period and note should be taken of their engorgement.

1.3.2 Persistent Efficacy Testing

Persistent efficacy is a measurement of the products continued efficacy in the face of continuing challenge by the target parasite. Therefore, studies designed to demonstrate persistent efficacy need to include a method of challenge at regular intervals throughout the defined period post-treatment.

In the experimental challenge studies, animals should be artificially challenged by placing 25-50 parasites of the target species directly onto the animals.

The first challenge, depending on the proposed claim, should be initiated at approximately 7 days post-treatment. Subsequent challenges should be made at least weekly or more frequently depending on the claim. Animals should be examined for the presence of live parasites prior to each challenge. A breakdown in residual activity is recorded as when live parasites are detected. The actual length of protection is recorded as the last date of challenge that failed to initiate an infection. For example, if live parasites are observed 49 days post-treatment, then the period of persistent efficacy is 35 days as the breakdown could have occurred subsequent to the challenge on day 35.

However, where it is inappropriate to perform artificial challenges, clinical trials may be carried out in flocks, which are carrying natural infestations of lice, keds (flocks only) or ticks. The animals to be treated should not have been treated with any insecticide or acaricide or any endecto-parasiticide for a sufficient wash-out period to guarantee the absence of residual efficacy from any previous treatment prior to the start of the trial. At least half of the herd or flock should be left untreated as a reservoir of infection. Following treatment, the treated animals should be separated from the untreated animals for a period of time (1-2 days) and examined for the presence of live parasites prior to being re-introduced to the main infected group. Examinations should be carried out at weekly intervals at least for the claimed period of persistence. Before these examinations 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.

2. Recommendations for studies of specific ectoparasites

2.1 Sheep Scab (Psoroptes ovis)

2.1.1 Dose determination / Dose confirmation studies

Sheep should be artificially challenged by placing at least 25 live adult female mites directly onto a small area over the withers and the areas marked. Sites of challenge should be examined for presence of live mites and the development of scab lesions following challenge. Infestation should be allowed to progress until a suitable lesion(s) has developed, which typically may take 21-35 days, before treatment. Should it be necessary to use animals that have natural infestations of sheep scab then these must only have this specific infestation (e.g. sheep scab alone and not sheep scab and biting lice).

Each treated animal should be inspected weekly until 56 days post-treatment, for the presence of live mites, by parting the wool at intervals, and the numbers of live mites estimated and recorded.

At the end of the study period, the cryptic sites (i.e. the pinnae, the auditory canal, infra-orbital, and inguinal fossae) should also be examined. All sheep should also be examined for secondary lesions and skin scrapings taken from any unresolved lesions. Where no lesions are present and no parasites can be found in the cryptic sites whole body examinations should be performed.

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. Lesion growth with time (days) can be calculated by Formula 2.

The overall efficacy against sheep scab must be 100%.

Due to the serious welfare implications of this disease untreated control groups should not be used.

2.1.2 Persistent Efficacy Testing

For studies involving the use of systemic endecto-parasiticides, sheep can be prepared by gluing isolation cells onto the clipped backs of sheep using a suitable adhesive. Usually, 10 cells are normally applied, 5 evenly spaced each side of the spine.

Following treatment, sheep should be artificially challenged by placing at least 25 live adult female mites directly onto a small area over the withers and the areas marked, or into the cells for systemic studies.

The first challenge, depending on the proposed claim, should be initiated at 7 days post-treatment. Subsequent challenges should be made, within the same area at weekly intervals (or more frequently if justified) post-treatment, depending on the claim. Sites of challenge should be examined for the presence of live mites and the development of scab lesions after each challenge. A breakdown in residual acaricidal activity (scab protection) is recorded as when active sheep scab (lesion plus live mites) is initiated at a site of challenge. The actual length of protection is recorded as the last date of challenge that failed to initiate disease. For example, if live mites are observed 49 days post-treatment, then the period of persistent efficacy is 35 days as the breakdown could have occurred subsequent to the challenge on day 35.

At the end of the study period, all sheep should be thoroughly examined for secondary lesions and the presence of live mites on both primary and secondary lesions. Skin scrapings should be taken from lesions where mites are not observed in situ. Lesion area should also be recorded for each sheep.

For systemic treatments, the presence of any scab lesion within the challenged isolation cells should be recorded and the lesion area calculated. Lesion areas can be calculated by measuring their length and width using tuberculosis callipers and the rate of lesion growth calculated using formula 2 (see appendix).

Due to the serious welfare implications of this disease untreated control groups should not be used.

2.1.3 Clinical trials

Clinical trials are normally carried out on identified infested flocks which should not have been treated with any acaricidal or insecticidal wash, spray, dip, pour-on, injection or drench for a sufficient wash-out period to guarantee the absence of residual efficacy from any previous treatment prior to the trial.

Efficacy should be assessed using flocks naturally infested with sheep scab mites utilising a Critical Study Group (CSG), within the flock, comprising thirty to fifty sheep each carrying an infestation of at least 25 live mites at the time of treatment. The CSG will be treated with the remainder of the flock at specific time points however, during the course of the trial the CSG should be kept separated from the remainder of the flock.

For plunge dip formulations, sheep should be dipped for a suitable period of time, with the head immersed twice. Pairs of CSG sheep should be dipped prior to replenishment of acaricide after approximately 25%, 50% and 75% of the remainder of the flock have been dipped. Remaining CSG sheep should be dipped last and according to the dipbath volume (i.e. it is recommended that only two sheep be dipped per 5 litres of dipwash, on account of the build-up of organic matter). If the test flock exceeds the recommended number of sheep dipped per volume of dipwash, the dipbath should be emptied, cleaned and the remainder of the flock dipped in fresh dipwash. Dipwash samples should be taken after the initial make up and before and after every replenishment for laboratory analysis.

Injectable endecto-parasiticides can be assessed in a similar manner with the CSG sheep treated randomly with other sheep in the flock.

CSG sheep should be individually identified by tagging, and examined for live mites and the resolution of disease at appropriate intervals post-treatment.

Each sheep in the CSG should be visually inspected prior to treatment and at intervals post-treatment until the end of the assessment period (usually 10 weeks but as appropriate for the proposed claim) when scab lesion areas for each sheep should be calculated by measuring length and width of each lesion. Additionally, each animal should be inspected for the presence of live mites and where present all stages of development should be present (instars and adults) to indicate an active infestation. An estimate should be made of mite numbers made along the periphery of each lesion by parting the wool at regular intervals over the body. The cryptic sites (i.e. the pinnae, auditory canal, infra-orbital and inguinal fossae) of all sheep 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 only.

Due to the serious welfare implications of this disease untreated control groups should not be used.

2.2 Sheep Blowfly (Lucilia spp.)

2.2.1 Dose determination / Dose confirmation

Sheep should be artificially challenged to induce blowfly strike; for example with approximately 150-200 newly emerged larvae of the blowfly, *Lucilia sericata* (or other species if appropriate), placed on a

marked, abraded area of skin on each sheep and held in place by a small moist cotton wool plug and an elastic band.

Each challenge site should be examined at daily intervals for 1 week and the number of live *Lucilia* larvae counted. First instar and early second instar larvae are usually found within the area of challenge but late second and third instar larvae may have migrated away from the challenge site. Thus, areas distant from the challenge sites should be examined carefully.

2.2.2 Persistent efficacy testing

Sheep should be artificially challenged with approximately 150-200 newly emerged larvae of the blowfly, *Lucilia sericata* (or other species if appropriate). These should be placed on a marked, abraded area of skin on each sheep and held in place by a small moist cotton wool plug and an elastic band.

Each challenge site should be examined at daily intervals for 1 week following infestation and the presence of live and dead *Lucilia* larvae recorded. First instar and early second instar larvae are usually found within the area of infestation but late second and third instar larvae may have migrated away from the infection sites. Thus, areas distant from the infection sites should be examined carefully.

Subsequent challenges should be made at weekly intervals on separate subgroups within the treatment groups such that individual sheep are only challenged every 3 weeks. Where individual sheep show larval survival for two consecutive weeks no further challenges should be attempted, as protection has been lost. A breakdown in residual insecticidal activity is recorded as when live larvae are found at a site of challenge. The actual length of protection is recorded as the last date of challenge that failed to initiate disease. For example, if live larvae are found 49 days post-treatment, then the period of protection is 35 days, as the breakdown could have occurred subsequent to the challenge on day 35.

2.3 Specific Requirements For Testing Dip Formulations (depletion "Stripping" trials)

The efficacy of plunge dip formulations needs to be assessed regarding their depletion ("stripping") following initial charging and replenishment.

"Stripping" trials should be conducted on full-fleeced longwool and closewool breeds in at least two separate geographical locations and involving a minimum of 200 animals, but based on a minimum of 2 sheep per 5 litres of dip bath.

Dip samples should be taken for laboratory analysis before dipping, before and after each replenishment, midway between replenishments and at the end of dipping, to determine the concentration of active substance in the dipwash. Samples must always be taken after the contents of the dip bath have been thoroughly stirred.

Representative fleece samples should be taken from sheep dipped just before every replenishment and the percent moisture prior to dipping calculated. Climatic data should be recorded, especially if rain precipitation occurs during dipping trials.

Information on the number of sheep dipped prior to replenishment, the volume of dipwash before replenishment and the amount of concentrate and volume of water at each replenishment should be recorded.

Dipwash should generally be replenished after 40 sheep or when the dip volume falls by 10%. As a general guide dips containing lipophilic active substances, which strip out of the dipwash should be

replenished at 1.5 times the initial charge rate; non-stripping dips should be replenished at the same rate as the charge rate.

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 I

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

Formula II

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