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Guideline for the demonstration of efficacy for veterinary medicinal products containing anticoccidial substances

Draft

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This guideline will replace the current Guideline on anticoccidials used for the therapy of coccidiosis in chickens, turkeys and geese (7AE15a).

Comments should be provided using this template. The completed comments form should be sent to vet-guidelines@ema.europa.eu

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Guideline for the demonstration of efficacy for veterinary medicinal products containing anticoccidial substances

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Executive summary

This guideline provides guidance in respect to the documentation required to demonstrate the efficacy of veterinary medicinal products (VMPs) containing anticoccidial substances. The previous guideline on anticoccidial products (7AE15a) was limited to poultry. The current guideline covers a wider range of mammalian and avian target species, now containing general data requirements for all target species, as well as specific requirements for poultry, ruminants, pigs, rabbits, dogs and cats.

1. Introduction (background)

The objective of this guideline is to specify the data required to demonstrate efficacy of VMPs containing anticoccidial substances. The following sections provide guidance on the essential elements which the applicants should cover in order to demonstrate efficacy, i.e. pharmacokinetics (PK), pharmacodynamics (PD) including resistance mechanisms, dose determination, dose confirmation, and clinical trials.

Coccidia are parasitic microorganisms and some features of anticoccidials may bear more similarity to antiparasitics than to antimicrobials. However, Regulation (EU) 2019/6 specifically includes anti-protozoals (and thus anticoccidials) in the category of antimicrobials (according to the definition in Article 4(12)).

The term coccidia will be used in the following for apicomplexan parasites belonging to the genera *Eimeria* and *Cystoisospora*. The life cycle of all coccidia – after oral uptake of infective oocysts – is constituted by a limited number of asexual reproductive cycles mainly in the intestinal or the bile duct (rabbits) epithelium, called merogony, followed by a single sexual cycle (gamogony), and shedding of a new generation of oocysts. Sporogony, typically occurring in the environment, is the process in which oocysts formed during gamogony develop into sporozoites, which are the infectious stage and can be ingested by a new host to continue the life cycle. Coccidia have immunogenic properties and consequently, following a sufficient level of exposure to coccidia, animals develop an immune response and hence a certain degree of immunity to cope with subsequent infections.

As coccidiosis is a significant disease in many target animal species, the scope of the guideline, which historically only concerned chickens, turkeys and geese, has been extended to include other avian and mammalian target animal species. The new guideline contains general data requirements as well as specific requirements for poultry, ruminants, pigs, rabbits, dogs and cats (please refer to sections 10 to 14 and also to the annex for a list of some relevant coccidian species).

2. Scope

The guideline aims to provide guidance in respect to the data required to demonstrate the efficacy of veterinary medicinal products containing anticoccidial substances intended to be used in mammalian and avian target animal species. It applies to veterinary medicinal products covering all routes of administration and all pharmaceutical forms, where data have to be presented to support clinical efficacy.

The guideline focusses on coccidiosis caused by *Eimeria* spp. and *Cystoisospora* spp. (syn. *Isospora* spp.) of the family Eimeriidae, although the general principles may also be applicable to other apicomplexan parasites affecting the gastrointestinal tract (such as *Cryptosporidium and Tyzzeria*).

Data requirements for 'coccidiostats' used as feed additives, which are addressed by Regulation (EC) No 1831/2003, are outside the scope of this guideline.

3. Legal basis

This guideline replaces the Guideline for anticoccidials used for the therapy of coccidiosis in chickens, turkeys and geese (7AE15a; 1993), and should be read in conjunction with Regulation (EU) 2019/6.

In accordance with Annex II of Regulation (EU) 2019/6, all experiments on animals should be conducted taking into account the 3Rs principles (replacement, reduction and refinement) as laid down in Directive 2010/63/EU on protection of animals used for scientific purposes.

Applicants should also refer to other relevant European and VICH guidelines, including those included in the reference list of this document.

4. General considerations

In the planning of efficacy studies, the following should be taken into account:

- Adequate pharmacokinetic and pharmacodynamic data should be provided demonstrating at which stage in the life cycle of the parasite the active substance under investigation is effective, and if the mode of action is predominantly coccidiostatic or coccidiocidal;
- Safety data obtained during pre-clinical efficacy studies and clinical trials should be used to complete the data generated from target animal safety studies (refer to VICH GL43);
- Studies should be performed without concomitant use of other products with an action against coccidia (i.e. feed additives) or vaccination against coccidia;
- 3Rs principles should be followed through standardised methodology for infection and efficacy calculation, by avoiding mortality rate as primary endpoint and by using other appropriate endpoints (e.g. lesions scores assessed in chickens euthanised prior to spontaneous death), where possible.

The required efficacy data for a veterinary medicinal product claiming efficacy against coccidia involve three types of studies in the target animal species:

1. At least one dose determination study testing at least three different dose levels;
2. At least two dose confirmation studies;
3. At least one multicentre clinical trial (actual use conditions) in at least two different geographical areas representative for European conditions.

The omission of a type of study (laboratory or field conditions) may be accepted, if appropriately justified and where the provided data are sufficiently robust to demonstrate efficacy.

Efficacy data have to be provided for each coccidian species claimed.

5. Preclinical studies

5.1. General principles

Pharmacological and pre-clinical safety studies shall be carried out in conformity with the provisions related to Good Laboratory Practice (GLP).

For pre-clinical efficacy studies, it is recommended to follow the requirements for Good Clinical Practice (GCP) and/or Good Laboratory Practice (GLP), as appropriate (depending on the nature of the studies). In case GCP and/or GLP is not applied for pre-clinical efficacy studies (e.g. absence of certified GLP

status), traceability, accuracy, integrity and correctness of data should be ensured, and the use of such data in pivotal studies should be justified.

5.2. Pharmacodynamics

The pharmacodynamic properties of the active substance should be adequately documented. The mode of action against the targeted stage(s) of coccidia should be stated, and the anticoccidial class should be defined. It should be demonstrated at which stage in the life cycle of the parasite the active substance under investigation is effective (target of the active substance), e.g. using PCR or histopathological examinations with identification of endogenous coccidian stages performed during and after the proposed dosing interval. This will demonstrate the activity of the anticoccidial substance for each of the stages and coccidian species which are claimed.

It should be highlighted if the active substance shares a mode of action with other anticoccidial substances and if cross-resistance is likely to occur (see also 5.4).

Potential interactions and incompatibility with feed additives should be addressed primarily with regard to feed additives with anticoccidial properties.

5.3. Pharmacokinetics

The pharmacokinetic properties of the active substance should be adequately documented. Pharmacokinetic data on the absorption, distribution, metabolism and excretion of the active substance should be provided.

Specifically, the applicant should specify if the active substance acts primarily locally in the intestine or systemically after absorption, and how long therapeutic concentrations are maintained.

For the conduct of pharmacokinetic studies please also see the CVMP guideline on conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/99).

5.4. Resistance

Current information on potential resistance to the active substance or active substance class should be provided. This should also include potential cross-resistance to relevant active substances commonly used in the same target animal species as anticoccidial feed additives according to Regulation (EC) 1831/2003. Where possible, information on the resistance mechanism(s) should be provided and discussed. This information may come from literature (peer-reviewed journals) or proprietary studies. Information on risk of development of resistance towards microorganisms other than coccidia should be considered for those products having a potential action against microorganisms other than coccidia.

If resistance of the coccidian isolate used in laboratory studies against other active substances is known, this should also be reported.

5.5. Dose determination and dose confirmation studies

5.5.1. Specific considerations on dose determination studies

Unless otherwise justified, dose determination studies should be conducted under laboratory conditions. Studies performed with different doses of the investigational veterinary product (IVP) are required to determine if the proposed dose regimen is appropriate for the selected clinical endpoints. Test doses must be calculated as actual intake of the active substance per kilogram of body weight.

Dose determination studies should be carried out with at least three dose levels (usually 0.5, 1, 2 times the proposed dose) of the active substance, and one infected placebo-treated group. Unless otherwise justified, the final formulation of the product should be used.

Infective dose, number of animals and endpoints should be considered carefully to take account of the 3Rs principles, whilst adhering to statistical principles and allowing for robust data.

Unless otherwise justified, dose determination studies are required for each claimed species of coccidia.

Single cell isolations are not required, i.e. 100% purity is not mandatory, but the degree of impurity should be identified and justified. When testing field isolates, it is acknowledged that the presence of non-pathogenic coccidian species in the isolate cannot be avoided. If clearly distinguishable, these non-pathogenic species oocysts should be identified and mentioned when expressing the number of infective oocysts for the species of interest.

The optimal time of administration(s), duration of treatment and dosing intervals in relation to the time of infection should be adequately justified. The selection of the duration and interval of administration can also be based on a combination of pharmacokinetics for the IVP and experimental parasitological data on life cycle and pathology. The timing of administration(s) should be evaluated in relation to the claim, for example if the product is intended to prevent clinical signs, it has to be administered during the prepatent period.

The applicant should also address if the administration, and in particular exposure to the active substance early in parasite life cycle, interferes with the development of acquired immunity to the coccidian species.

5.5.2. Specific considerations on dose confirmation studies

At least two dose confirmation studies should be carried out with the final product formulation per each coccidian species. Preferably, these are laboratory studies, in which the infection model mimics the field conditions. Dose confirmation studies can also be conducted in the field with naturally-infected animals, provided study conditions are appropriately controlled. One of these dose confirmation studies might be substituted by a dose determination study, if the final formulation was used, the product was administered according to the intended posology, and if the infection level and the number of animals tested were adequate. Separate studies are required for the different stages of infection for which the product claims efficacy.

Dose rate and duration of administration should reflect the proposed final use of the product, including the stages of the coccidian life cycle which are targeted. The study may be carried out in artificially or naturally infected animals. The experimental design should include an infected group treated with the IVP and an infected placebo-treated control group. In case of administration via feed or water, the study should take reduced intake of feed and water into account.

The infection should result in clinical signs of coccidiosis and oocyst shedding in the control animals.

If the study is intended to support a claim to prevent clinical signs by administering the veterinary medicinal product during the prepatent period, clinical signs and oocyst shedding should be present in the untreated control group.

If the study is intended to support a treatment claim, treatment should not be initiated until clinical signs occur.

The duration of the study should be sufficient to determine if relapse occurs.

For group-housed animals, each administration should be replicated over several pens. Pen or individual animal weights should be recorded at appropriate time points. Feed conversion rate should be calculated for the species where this is relevant.

Environmental and husbandry conditions should be similar for both treated and untreated groups.

All animals that die during the experiment should be necropsied as soon as possible, and it should be determined whether the cause of death is related to the disease or the treatment. Macroscopical and histopathological changes should be listed, and the species of coccidia specified.

5.5.3. Coccidia isolates

Efficacy for each coccidian species claimed on the label should be confirmed by appropriate data. The most relevant coccidian species for different target animal species are listed in the annex.

Resistance in field isolates may change over time, and isolates susceptible to the active substance should be used. The pathogenicity of the isolate should be evaluated to determine the adequate infective dose that depends upon the inoculated species and can vary within the species (see host-species information in sections 9-13).

For dose determination studies, it is acceptable to use laboratory strains of coccidian species. However, for dose confirmation studies, recent (less than 5 years unless otherwise justified) field isolates exposed to commonly administered anticoccidials are preferred as the inoculum. The isolates should be representative of the EU area. The history of the isolates should be included, i.e. where and when it was isolated, the name of the anticoccidial applied at the time of the outbreak, if any, and the predominant coccidian species involved. Isolates should not originate from vaccinated farms as in these cases the susceptibility of the isolated strains might not be representative for wild strains.

The isolates should be passed through susceptible animals, cultures built up, sporulated oocysts collected at appropriate time points, and titration for appropriate morbidity or other relevant endpoints performed before initiating the pivotal study/studies.

Freshly passaged isolates of sporulated oocysts should be used.

The number of oocysts to be administered per animal should be determined based on their virulence, and titration studies should be done in advance of pivotal study/studies in the target animals to determine the pathogenicity of the inoculum. The infective dose of the laboratory strain used for oral inoculation should be adequate to artificially induce clinical signs of coccidiosis, taking into account the pathogenicity of the chosen strains.

Different infective doses of the same coccidian species may be necessary when examining acute disease, oocyst production and mode of action (e.g. which stages of the life cycle are targeted).

5.5.4. Adequacy of infection

It is generally up to the applicant to demonstrate that clinical signs of coccidiosis can be artificially induced with the strain used for oral infection. Individual factors, health conditions and genetic background of the animals to be infected may also be considered at study commencement.

The adequacy of infection criteria and number of adequately infected control animals should be defined *a priori,* taking into account the statistical, parasitological and clinical relevance of the infection level in individual control animals. For some coccidian species and for some target animal species, the criteria may include clinical signs such as diarrhoea in ruminants.

To confirm the adequacy of infection in naturally infected animals, the diagnosis of the infection and/or potential major co-infections should be made in order to exclude any other causes of the clinical signs.

5.5.5. Study animals

The age, sex and production type of the study animals should be representative for the target population.

In laboratory studies, animals that will be experimentally infected should not have been exposed to coccidia prior to the study and should be free of other infections. The absence of coccidian oocyst prior to the experiment should be confirmed, taking into account that infected animals can be immune and have reduced oocyst output. The animals should usually be weighed individually at the beginning and during the experiment, if possible. Measures may be taken to ensure a comparable distribution of baseline characteristics such as body weight, between the treatment and control group.

5.5.6. Endpoints and timing of efficacy assessment

The primary and secondary endpoints should be clearly defined in the study protocol. In general, a parasitological parameter (oocyst shedding) should be used as a primary endpoint; in case a clinical indication is claimed, the primary endpoints should also include a relevant clinical parameter as co-primary endpoint. The endpoints will depend on host species and coccidia species.

The endpoints may include clinical signs, mortality, macroscopical and histopathological changes, or body weight gain, depending on the target animal species (see below in the target species specific section). Animal welfare should be considered when establishing endpoints.

The timing of the assessment of an endpoint in relation to time of administration(s), time of infection, and/or time of appearance of clinical signs should be explained.

The selection of primary endpoints should reflect the proposed claim, e.g. prevention of clinical signs.

The most relevant (co-)primary endpoints should be used, e.g. reduction of oocyst shedding (OPG=Oocyst per gram of faeces), body weight gain and reduction of morbidity (diarrhoea incidence, faecal scores or days with diarrhoea).

Post-treatment follow-up should be performed to assess the risk for relapse after the effects of treatment are expected to have ceased. The timing and duration of the follow-up measurements should be considered carefully.

5.5.6.1. Pathological findings and lesion scores

In most target animal species lesion scoring is not well established. However, the examination of lesion scores may be essential in the efficacy evaluation of anticoccidials in chickens. In this species, reliable lesion scoring systems are available and standardised for some species of *Eimeria,* that allow direct estimation of the severity of the pathological changes. Lesion scores should be examined in freshly dead or necropsied animals.

Similar scoring systems are currently not available for other target animal species. However, appropriate pathological findings could also be used in other species as an aid for the diagnosis.

Lesion score data should be analysed with appropriate statistical methods for ordinal data, which will often be non-parametric tests.

5.5.6.2. Oocyst counts

Faecal samples for oocyst counts should be taken daily.

For dose determination and dose confirmation studies, individual faecal samples (e.g. by rectal sampling) are generally necessary for oocysts quantification. In certain circumstances such as in group-housed animals, pooled samples or litter samples can be used for the detection of oocysts, but not for quantification of oocysts, unless the method is validated.

If relevant, the diurnal rhythm of shedding should be considered, when oocyst shedding (or dropping score) is examined.

The McMaster method is the preferred method for oocyst quantification, but other quantification methods can be used, if validated.

Oocyst reduction should be assessed by the area under the OPG-time curve (AUC) of the daily mean per group during the defined post-treatment follow-up period. The duration of follow-up should be given in the study protocol and should preferably cover the assumed period of oocyst shedding. For rabbits, the total oocyst shedding per animal is a much more reliable measure (see section 13.4).

Alternatively, oocyst counts can be expressed on a day-to-day basis, but it should be stated which days of the shedding period are pivotal and clinically relevant. Otherwise, it may inflate the number of statistical comparisons. In case of mixed infections, oocyst counts should be calculated separately for each coccidian species claimed.

For studies with a negative control group, reduction in oocyst shedding (% efficacy) should be calculated using Abbott's formula either based on AUC or counts on individual days:

$$\%\, efficacy = \frac{Mean\ (control)\ -\ Mean\ (treatment)}{Mean\ (control)},$$

where the mean is the arithmetic mean of the negative control or the treated groups.

The efficacy in reducing oocyst shedding should be at least 90%.

Additionally, the percent reduction of the number of shedding days per group can be presented as a secondary efficacy parameter.

5.5.6.3. Morbidity and mortality

Clinical signs should be monitored throughout the study, including faecal consistency scores (e.g. dropping scores) and thriftiness. Diarrhoea incidence but also its severity and duration should be considered (e.g. number of days with diarrhoea in piglets). Faecal consistency should be scored using an appropriate scoring system. In some species such as rabbits and lambs it may be difficult to note the faecal score individually, and in these species 'perianal faecal soiling scores' could also be used.

Mortality and morbidity data should be analysed with statistical methods for categorical data analysis such as logistic analysis.

5.5.6.4. Animal performance

Generally, animal performance should not be a primary endpoint; however, it can be used as a secondary endpoint.

Animal performance indicators such as body weight gain or feed/water intake and feed conversion rate may be more relevant for species with a high growth rate, where a substantial growth can be expected within the experimental period.

6. Clinical trials

6.1. General principles

Multicentre clinical trials should be conducted in line with the principles of VICH GL9 (Good clinical practices) in at least two geographical areas representative for European conditions for the purpose of determining the efficacy and safety of the IVP under field conditions. Sites with a confirmed presence of oocysts of the relevant species should be selected. Concomitant use of other anticoccidial substances is not accepted during the trial, and details of any potential prior use (routine or not) of anticoccidial feed additives on the site should be reported. The housing and rearing practices should reflect the recommended use of the veterinary medicinal product.

Clinical trials should be conducted in the animal species, age group, and under husbandry conditions representative of the intended use of the veterinary medicinal product (see section 6.3). The proposed timing of administration(s) for the claimed indication should be justified.

As often under field conditions the infection pressure can be variable (e.g. in poultry farms a disease outbreak cannot be predicted from one batch to another), the adequacy of infection should be demonstrated. Inclusion of a negative control group or sentinel animals might be considered. For animal welfare reasons, this group should be as small as possible, but large enough to maintain statistical power.

For anticoccidials with a prophylaxis claim, the IVP should be compared to a placebo treated control. For animal welfare reasons, this group should be as small as possible, but large enough to maintain statistical power. If available, a positive control product can be used in a three-arm study for non-inferiority or superiority comparisons.

Animals should be carefully examined for any suspected adverse effect of the veterinary medicinal product. Dead or euthanised animals should be necropsied and the cause of death should be determined.

6.2. Adequacy of infection

The adequacy of infection criteria and number of adequately infected control animals should be defined *a priori,* taking into account the statistical, parasitological and clinical relevance of the infection level in individual control animals. For some coccidian species and for some target animal species, the criteria may include clinical signs such as diarrhoea in ruminants.

To confirm the adequacy of infection in naturally infected animals, the presence of the infection and absence of any potential major co-infection that could cause the clinical signs should be demonstrated.

6.3. Study animals

The age, sex and production type of the study animals should be representative for the target population.

Measures may be taken to ensure a comparable distribution of baseline characteristics such as body weight between the treatment and control group.

6.4. Endpoints and timing of efficacy assessment

The principles relevant for pre-clinical studies (please refer to section 5.5.6) also apply to clinical trials. Relapses should be carefully evaluated to determine the cause, as relapses can be caused by lack of efficacy of the candidate veterinary medicinal product (e.g. caused by resistance of the coccidia strain) or by reinfection.

7. Safety parameters

Safety of an anticoccidial should also be evaluated during pre-clinical efficacy studies and clinical trials, in particular when the margin of safety is narrow. Clinical parameters likely to be related to the properties of the active substance need to be monitored during all efficacy studies.

Clinical assessment with the aim to detect adverse events should be conducted before and after treatment and documented in the study report.

8. General statistical principles

Reference should be made to the CVMP guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (CVMP/EWP/81976/2010).

The number of animals included in a study should be calculated and justified by the applicant *a priori* to enable evaluation of statistical significance. For dose determination/confirmation studies and in studies where the individual animal is the experimental unit, at least six animals per group are required. However, more animals may be needed if e.g. there is a large inter-individual variability with regard to the primary endpoint or if the statistical analysis has to be based on non-parametric tests. Where animals are housed in groups, the design should take the between-group variability into account and the statistical model should take the pen effect into account, or – if appropriate – the pen should be defined as statistical unit.

Wherever possible, parametric tests should be used; however, for certain (i.e. categorical and ordinal) evaluation criteria (e.g. lesion scores, oocyst counts) non-parametric tests are a suitable alternative.

9. Summary of product characteristics (SPC)

The SPC for veterinary medicinal products containing anticoccidial substances should contain the information laid down in Article 35 of Regulation (EU) 2019/6. The SPC should contain specific information in accordance with the Guideline on the summary of product characteristics (SPC) for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/383441/2005). However, as the resistance profile of coccidia may bear more similarity to antiparasitics than to antimicrobials, the Guideline on the summary of product characteristics for antiparasitic veterinary medicinal products (EMA/CVMP/EWP/170208/2005) should also be taken into consideration. In addition, other relevant guidelines should be considered when compiling the SPC.

SPC Section 3.2 Indications for use for each target species

Information on the efficacy of the product against the different stages of coccidia and the optimal timing of administration(s) in the life cycle of the parasite should be included. The target coccidian species should be clearly stated. The efficacy claims should be in accordance with the findings of the efficacy studies. In this context, each claim should be accompanied by details on the 'conditions of use', such as administering the product *"in farms with a confirmed presence of <target coccidia*

species> oocysts". In case of a prophylaxis claim, wording requiring the administration of the product "*during the prepatent period of infection for the prevention of clinical signs*" could for instance be used.

SPC Section 3.4 Special warnings

If the product targets early stages in the life cycle (prepatent period), and no effect is demonstrated at later stages, a sentence should be added such as "*The administration of the VMP will reduce the spread of infection but the product has not been demonstrated to be effective against the clinical signs of infection in animals already diseased.*". If necessary, the SPC should state that "*maximum benefit will be seen if the veterinary medicinal product is administered to animals in the group before onset of clinical signs*".

If the immune response of the animal against coccidian infection is suppressed when an anticoccidial VMP is administered, this should be mentioned in the SPC.

The SPC should also address the need for hygienic measures, herd management and/or pasture management.

Other warnings related to the effective use of the VMP should be included in SPC section 3.4:

- "*Repeated use for an extended period, particularly when using the same class of substances, increases the risk of resistance development. The decision to use the product should be based on confirmation of the coccidian species and burden, or of the risk of infection based on its epidemiological features, for each <individual animal/herd/flock>* [depending on the target species]."
- "*Unnecessary use of antiprotozoals or use deviating from the instructions given in the SPC may increase the resistance selection pressure and lead to reduced efficacy.*"
- "*If resistance to {anticoccidial substance / class} is present, it should be considered to use an antiprotozoal from another class/with a different mechanism of action.*"
- "*This veterinary medicinal product should not be used together with feed additives or other veterinary medicinal products containing coccidiostats or histomonostats.*"

The following warnings concerning responsible use of anticoccidials should also be included in SPC section 3.4[1]:

- "*Use of the product should be based on identification and susceptibility testing of the target pathogen(s). If this is not possible, therapy should be based on epidemiological information and knowledge of susceptibility of the target pathogens at farm level, or at local/regional level.*"
- "*Use of the product should be in accordance with official, national and regional antimicrobial policies.*"
- "*The veterinary medicinal product should not be used as part of herd health programmes.*"

SPC Section 3.5 Special precautions for use

Special precautions for safe use in the target species

Special precautions for safe use in the target species should be included in SPC section 3.5.

[1] It is acknowledged that the *CVMP Guideline on the summary of product characteristics (SPC) for veterinary medicinal products containing antimicrobial substances* (EMA/CVMP/383441/2005) indicates that the warnings concerning responsible use of antimicrobials should be included under SPC section 3.5. However, for VMPs containing anticoccidial substances it is considered appropriate that these warnings are included under SPC section 3.4, as they are considered more related to the effective use.

For instance, product-related information restricting prophylactic and metaphylactic use linked to Articles 107(3) and 107(4) of Regulation (EU) 2019/6 should appear in this section. Repetition of content across several SPC sections should be avoided.

10. Specific requirements for poultry

10.1. General information

Coccidiosis in poultry is caused by species belonging to the genus *Eimeria*. Please see the annex for a list of the most important pathogenic species. These species vary with respect to their localisation in the intestinal tract and the age of the target animal at which an outbreak of coccidiosis is most likely to occur.

Data should be provided on the excretion of the anticoccidial active substance or its metabolites in droppings, which may contaminate the litter or the floor, as such contamination may result in a risk of increased (re)uptake by treated and/or untreated animals.

10.2. Study animals

The category of target animals (e.g. broilers or replacement chickens) for which the veterinary medicinal product is intended to be marketed should be used. Between these categories, extrapolation is possible if justified (e.g. from broiler chicken to broiler breeders).

In mixed groups/flocks of male and female animals, individual weights by sex and sexed-weighted averages should be established. Individual weights are preferred, but if birds are of equal weight or of the same sex per pen, pen weights are satisfactory.

10.3. Laboratory studies

10.3.1. Inoculum

The pathogenicity of the coccidian isolate used for the challenge has to be tested prior to each experimental infection.

The previous guideline specified a threshold for mortality for the most pathogenic coccidian species in chickens as a measure of adequacy of infection. However, well-characterised disease models are now available, and it is possible to determine the adequacy of infection by requiring a minimum lesion score (e.g. a group mean of 2 on a scale of 1 to 4). To minimise variability between studies, the quality of the inoculum is of critical importance. Adequacy of infection should be determined by the minimum lesion score, oocyst shedding and possibly weight reduction in target animals. Furthermore, the timing of necropsy should be accurately defined in the study protocol, as timing of lesion scoring post infection can influence the scores considerably.

10.3.2. Experimental design

In addition to the IVP group and the infected placebo-treated control group, inclusion of the following two additional test groups should be considered mainly for chickens and other fast-growing species, where "growth" is a secondary endpoint. The inclusion of these groups will allow to determine any direct active substance-related effects on growth:

- Non-infected, non-medicated controls (animals should be kept isolated from the infected groups to avoid infection from the experimental strain or by natural infection);

- Unless otherwise justified, non-infected medicated controls (a small number or satellite group should be included in order to clarify active substance-related effects in the target species).

All animals having died or being euthanised during the experiment(s) should be necropsied as soon as possible. Differential diagnosis should be established and the cause of death recorded.

10.4. Endpoints

As for chickens a standardised lesion score system has been developed for most coccidian species, it is recommended that the primary endpoint for chickens should be lesion scores for those coccidian species, whereas oocyst shedding, diarrhoea, and body weight gain can be included as secondary endpoints. Mortality is not required as primary endpoint. For *E. mitis*, *E. praecox* and *E. maxima* a lesion score system might not be relevant, and oocyst shedding, clinical signs, and mortality could be used as primary endpoints.

For other poultry species, clinical scoring may be used as primary endpoint.

OPG-time curve (AUC) of the daily mean per group is not an adequate primary endpoint for poultry as the concentration of oocysts is different depending upon the consistency of the droppings.

11. Specific requirements for ruminants

11.1. General information

Coccidiosis in ruminants is caused by a number of *Eimeria* species (see the annex for the most relevant species). Different coccidian species predominantly affect different target species, age groups and/or husbandry systems; thus, depending on the indication applied for, the appropriate study design/prevailing coccidia species should be considered.

11.2. Study animals

Coccidia-naïve animals aged from 3 weeks to 6 months can be enrolled, since at this age, the immune system is often still developing. Preferably animals should be in the post-weaning period, the most critical time point for infection, as lambs, calves and goat kids are highly sensitive to coccidan infections during this period.

11.3. Laboratory studies

11.3.1. Inoculum

The nature of clinical signs in infected lambs, calves or goat kids are comparable (e.g. diarrhoea, reduced body weight, reduced feed conversion rate) irrespective of the inoculated infective dose, but the onset and severity of clinical signs are dose dependent.

Trickle infections may mimic natural exposure to oocysts. Even trickle infections with low doses of parasite may induce clinical coccidiosis, but severe disease is generally related to high infection pressure.

11.3.2. Experimental design

Prophylaxis claims:

For prophylaxis claims, administration of the veterinary medicinal product to exposed animals should take place during the prepatent period. The timing of treatment should be driven by the life cycle(s) of the coccidia targeted by the IVP, usually the prepatent period of the *Eimeria* species concerned, which is in cattle about 18 to 21 days for *E. bovis,* 15 to 17 days for *E. zuernii* and approximately 1 week in *E. alabamensis*. Thus, treatment(s) of the animals up to D+14 after inoculation are considered appropriate to assess a claim for the prevention of clinical signs caused by the relevant coccidia species *E. bovis* and *E. zuernii*. To claim a reduction of oocyst shedding, oocyst shedding should be measured depending on the life cycle, e.g. in regard to *E. bovis* and *E. zuernii* for at least 5 weeks post artificial infection, since haemorrhagic diarrhoea may last for up to 36 days in infected non-treated calves.

Metaphylaxis claims

For metaphylaxis claims, administration of the veterinary medicinal product should take place in a group of animals in which some animals show clinical signs, whereas most animals are in the prepatent period.

Treatment claims:

If the claim is to reduce clinical signs in clinically sick animals (e.g. in case of cryptosporidiosis), the veterinary medicinal product should be administered after the prepatent period.

11.4. Endpoints

It is recommended that a primary efficacy parameter for prophylaxis or metaphylaxis (administration of the veterinary medicinal product during the prepatent period) should be faecal scoring (1 = Normal to pasty, 2 = Liquid, 3 = Liquid with blood, 4 = Liquid with blood and tissue) as in ruminants diarrhoea is considered the key symptom related to clinical coccidiosis. In addition, the reduction of oocyst shedding after treatment should be consistently calculated as co-primary endpoint.

Inappetence linked with weight depression and dehydration is a main and consistent effect of clinical coccidiosis in calves, irrespective of the *Eimeria* spp. involved. Weight loss is apparent at times of peak oocyst shedding, also death caused by coccidiosis might occasionally occur. Thus, all clinical signs observed during the study other than diarrhoea can be considered as secondary endpoints.

12. Specific requirements for pigs

12.1. General information

The predominant pathogen in pigs is *Cystoisospora suis*. Naïve piglets are infected around birth and usually recover within 2 weeks post-infection (p.i.). Neonatal suckling piglets between 7 and 11 days of age are the most affected age group while older pigs are less susceptible and excrete few or no oocysts without clinical signs. Oocyst shedding starts 5-6 days p.i. and frequently occurs in two peaks at 5-9 and at 11-14 days p.i. Clinical signs can be seen as early as 3 days p.i.

12.2. Study animals

Newborn pigs of the same age (from birth to 4 days old depending upon study design and intended claim) should be used. Healthy, coccidia-free animals of both genders should be used. Animals should be randomised to the treatment group based on birth weight within litter using a complete randomised block design. As contaminated farrowing pens are an important source of infections for the piglets, the pen/litter effect should be taken into account in the experimental design.

12.3. Laboratory studies

12.3.1. Inoculum

In artificial challenge models, piglets should be orally infected once with sporulated oocysts of *C. suis*. The infective dose should be justified depending on the intended claim and the virulence of the strain used. High infection doses may lead to an unacceptable high mortality rate in the piglets, which is usually not observed in the field. Models using lower doses which induce oocyst shedding and diarrhoea are preferred to mimic natural infection. The origin of the strain and the number of passages through piglets without anticoccidial treatment should be documented.

12.3.2. Experimental design

In laboratory studies, experimentally infected animals rather than naturally infected animals are preferred. For infections with *C. suis*, an experimental model mimicking the field situation of cystoisosporosis is available. Dose confirmation studies can also be conducted under field conditions.

The timing of infection and treatment should be justified depending on the proposed claim and the dose regimen recommendations.

12.4. Endpoints

Endpoints should be defined depending upon the intended claim and the aim of the study. The oocyst count reduction (see section 5.5.6.2) should be selected as the primary endpoint in a dose determination or a dose confirmation study. A relevant clinical parameter (e.g. diarrhoea) as co-primary endpoint is needed to show the prevention of that clinical sign if claimed.

In clinical trials, it is recommended to use the percentage of piglets not affected by diarrhoea associated with coccidiosis as primary endpoint to demonstrate the efficacy in preventing clinical signs. A co-primary endpoint should then be the reduction in oocyst shedding.

Secondary endpoints could include faecal scores, reduction of the number of days with oocyst shedding, percentage of piglets with oocyst shedding, mortality rate caused by coccidiosis and bodyweight gain.

13. Specific requirements for rabbits

13.1. General information

There are two forms of coccidiosis in rabbits:

- Hepatic coccidiosis caused by *Eimeria stiedai,* which may lead to severe pathological changes both in bile ductus and liver parenchyma especially in young animals in case of high infective doses of oocysts;
- Intestinal coccidiosis caused by various species in different parts of the intestine (see annex).

Clinical signs of the disease include diarrhoea, loss of weight, poor feed conversion rate, ascite, icterus, distended abdomen, and possibly death. The faeces are generally dry, but a short period of diarrhoea can be observed, e.g. more hydrated (*E. intestinalis*, *E. magna*) or liquid (*E. flavescens*). In rabbits the peak of oocyst shedding is of short duration, about 48 h (intestinal coccidiosis). Part of the faeces (caecotrophes) is re-ingested by the animal, and oocysts in the faeces can, therefore, only be detected during a certain period (afternoon until next morning).

Data should be provided on the excretion of the anticoccidial active substance or its metabolites via faeces or urine, which may contaminate the litter or the floor, as such contamination may result in increased (re)uptake by treated and/or untreated animals.

13.2. Study animals

Rabbits become immunised even with small doses of oocysts, and thus the study animals should be coccidia-free prior to study initiation. Under field conditions, this is usually achieved by the use of feed with coccidiostats. The administration of in-feed coccidiostats should be ceased in advance of infection to avoid any carry-over effect.

Animals are most sensitive after weaning and hence rabbits aged 4-6 weeks old should be enrolled. The rabbits must be weaned at least four days before the experiment, but not before 28 days of age.

13.3. Laboratory studies

13.3.1. Inoculum

To mimic natural infection, the oocysts are preferably inoculated under the tongue in a small volume. Only if it is impossible to concentrate the desired quantity of oocysts in this volume, the animals should be infected via gavage.

13.3.2. Experimental design

The litter of origin may have a considerable role (also in SPF animals). Rabbits should be randomised to each treatment group based on birth weight within litter using a total randomised block design.

The experimental design should include the following three test groups:

- Infected and treated,
- Infected and untreated control,
- Unless otherwise justified, a non-infected and treated control group (a small number or satellite group) should be included to clarify active substance-related effects in the target species.

Having only one animal per cage is to be avoided in view of the gregarious nature of rabbits and to avoid stress.

13.4. Endpoints

It is recommended that the primary endpoint is the reduction in oocyst shedding. In addition, weight gain or a clinical sign of the disease could be selected as co-primary endpoints.

Although there is no correlation between oocyst shedding and the severity of the disease, treatment must effectively suppress development of the parasite in the host. Therefore, total oocyst shedding during the first three days after beginning of the patent period, or OPG values must be reduced at least by 90%. Total oocyst shedding per animal is much more reliable, namely due to caecotrophy and diurnal periodicity of oocysts shedding connected with this phenomenon. Total oocyst shedding should preferably be assessed by a method that is validated or described in peer-reviewed literature.

Secondary endpoints could include feed conversion rate and macroscopic and histopathological changes that will depend on the target pathogen, e.g. gastro-intestinal gross lesions (with intensity being parasite species-dependent) for intestinal coccidiosis or liver lesions for hepatic coccidiosis.

Rabbits after weaning grow rapidly and hence their weight gains are in practice one of the most reliable criterion of their health status. Feed conversion rate should also be calculated. The performance of animals must be checked for at least three weeks after challenge.

14. Specific requirements for dogs and cats

14.1. General information

The predominant enteric coccidia in dogs and cats are *Cystoisospora* spp. (*C. canis, C. ohioensis, C. neorivolta, C. burrowsi* in dogs, and *C. felis, C. rivolta* in cats). In both dogs and cats, coccidiosis has a higher prevalence in young animals and among breeding colonies or shelters where hygiene is deficient or difficult to maintain.

In cats, kittens less than six months of age have shown higher rates of oocyst shedding. Most infections are mild or subclinical, especially in adult cats. In some cases, the disease can be severe and complicated by other factors (e.g. immunocompromised animals). In these cases, haemorrhagic enteritis, dehydration, anaemia, anorexia, weight loss and emesis can be observed. Stress factors, e.g. moving the animals into another environment, might trigger clinical disease.

In dogs, puppies under four months of age are more susceptible to develop the disease, especially in large kennel situations and dog breeder facilities. The common clinical signs include diarrhoea, which may be bloody, with varying degrees of abdominal pain, anorexia, anaemia, and weight loss. In rare cases, fatal infections have been reported. Under experimental conditions, where diarrhoea was induced in neonatal puppies with *C. ohioensis* oocysts, clinical disease was not observed in similarly exposed weaned puppies and young dogs.

14.2. Study animals

The laboratory studies should be performed with healthy weaned kittens/puppies up to 4 months of age.

14.3. Laboratory studies

14.3.1. Inoculum

The faecal oocyst counts considered for establishment of the disease is 500-1000 OPG.

14.3.2. Experimental design

See general part.

14.4. Endpoints

It is recommended that the primary endpoint for dogs and cats is the reduction of faecal oocyst counts. As co-primary endpoint, a clinical sign of the disease (e.g. incidence of diarrhoea) could be used. Secondary endpoints such as body weight gain and frequency of diarrhoea could be selected.

Definitions

Anticoccidial product: In the context of this guideline, an anticoccidial product is an antimicrobial veterinary medicinal product developed for the prophylaxis, metaphylaxis, and/or treatment of coccidiosis.

Coccidiocidal: For the purpose of this guideline, coccidiocidal is an active substance with coccidiocidal action, which kills or irreversibly damages most of certain coccidian stages, without evidence of clinical relapse after drug withdrawal.

Coccidiostatic: For the purpose of this guideline, coccidiostatic is an active substance with coccidiostatic action, which inhibits the development of certain coccidian stages in a reversible way; thus, withdrawal of the active substance may lead to completion of the life cycle and possibly both the appearance of clinical signs and shedding of oocysts several days after medication is discontinued.

Dose-limiting parasite: In the context of this guideline, a dose-limiting parasite is the least susceptible parasite species in a claimed indication for a determined dose of a VMP.

Metaphylaxis: The administration of a medicinal product to a group of animals after a diagnosis of clinical disease in part of the group has been established, with the aim of treating the clinically sick animals and controlling the spread of the disease to animals in close contact and at risk and which may already be subclinically infected (definition provided for in Article 4(15) of Regulation (EU) 2019/6).

Prepatent period: For the purpose of this guideline, a prepatent period is a period between the initial infection with oocysts and the shedding of viable oocysts in the faeces. It represents the period in time it takes for the parasites to complete their life cycle within the host, including multiplication and development, before they become detectable in the host's faeces.

Prophylaxis: The administration of a medicinal product to an animal or group of animals before clinical signs of a disease, in order to prevent the occurrence of disease or infection (definition provided for in Article 4(16) of Regulation (EU) 2019/6).

Treatment: For the purpose of this guideline, treatment means the administration of a veterinary medicinal product after the onset of a disease for curative purposes.

References

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CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (CVMP/EWP/81976/2010).

CVMP Guideline on the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/1999).

CVMP Guideline on the summary of product characteristics (SPC) for veterinary medicinal products containing antimicrobial substances (EMA/CVMP/383441/2005).

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Annex

Examples of the most common coccidia species considered of clinical relevance within the scope of this guideline:

Host species	Species of coccidia considered of clinical relevance
Chickens	*Eimeria tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti, E. mitis*
Turkeys	*E. adenoeides, E. meleagrimitis, E. gallopavonis*
Geese	*E. anseris, E. truncata* (coccidiosis of the kidney)
Ducks	*E. kotlani, E. danailova, Tyzzeria perniciosa*
Cattle	*E. bovis, E. zuernii*, *E. alabamensis*
Sheep	*E. crandallis, E. ovinoidalis (highly pathogenic), E. ovina, E. parva, E. intricata, E. bakuensis, E. ahsata*
Goats	*E. alijevi, E. ninakohlyakimovae, E. arloingi, E. caprina, E. christenseni*
Pigs	*Cystoisospora suis* (*Isospora suis*)
Rabbits	Hepatic coccidiosis: *E. stiedai* Intestinal coccidiosis: *E. exigua, E. perforans, E.vejdovskyi* (slightly pathogenic) *E. irresidua, E. magna, E. media, E. piriformis* (mildly pathogenic) *E. intestinalis*, *E. flavescens* (highly pathogenic)
Dogs	*Cystoisospora (C. canis, C. ohioensis, C. neorivolta, C. burrowsi)*
Cats	*Cystoisospora (C. felis, C. rivolta)*