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# 4 Guideline for the demonstration of efficacy for veterinary 5 medicinal products containing anticoccidial substances

6 Draft

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8 This guideline will replace the current NtA Guideline on Anticoccidials used for the Therapy of
9 Coccidiosis in Chickens, Turkeys and Geese (7AE15a), which was adopted prior to 1995.

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

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30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
**Telephone** +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
**Send a question via our website** www.ema.europa.eu/contact

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

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# Executive summary

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

# 1. Introduction (background)

The objective of this guideline is to specify the data required to demonstrate efficacy for the prevention or reduction of clinical signs of coccidiosis, and the reduction of oocyst shedding. Thus, the following sections provide guidance on the essential topics which the applicant should cover in order to demonstrate efficacy i.e. pharmacokinetics (PK), pharmacodynamics (PD) including resistance mechanisms, dose determination, dose confirmation, and clinical field trials.

The life cycle of all coccidian species – after oral uptake of infective oocysts – is constituted by a limited number of asexual reproductive cycles in the intestinal or the bile duct (rabbit) epithelium, called schizogony, followed by a single sexual cycle (gamogony), and shedding of a new generation of oocysts. Coccidia have immunogenic properties and consequently, following a sufficient level of exposure to a coccidian species, animals develop an immune response and hence a certain degree of immunity to cope with subsequent infections. Coccidiosis is generally managed by different methods, including hygiene and herd management measures to limit the uptake of coccidia oocysts, and the preventive use of feed-additives or veterinary medicinal products. Veterinary medicinal products are used to prevent or reduce the clinical signs of coccidiosis, and to reduce the shedding of oocysts. Indications will depend on the target species, and the data provided. Vaccines are available for chickens only and at present mostly used for replacement birds.

As outbreaks of clinical coccidiosis occur in most common livestock requiring treatment with veterinary medicinal products, the scope of the guideline has been extended to include other avian and mammalian target species. The new guideline contains general data requirements as well as specific requirements for poultry, ruminants, dogs and cats, pigs and rabbits (please refer to section 8 to 12 and the annex for a list of some relevant coccidian species).

# 2. Scope

The guideline aims to provide specific guidance in respect to the documentation required to demonstrate the efficacy of veterinary medicinal products intended to be used to prevent or reduce clinical signs in mammalian and avian target species, and to reduce oocyst shedding. It applies to veterinary medicines 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 species of the family Eimeridae and Cystoisospora spp. (syn. Isospora spp.); although the general principles may also be applicable to other coccidia affecting the gastrointestinal tract (such as Cryptosporidia).

Data requirements for ‘coccidiostats’ used as feed additives and which are addressed by regulation (EU) No 1831/2003, are outside the scope of this guideline.

# 3. Legal basis

This guideline replaces the current NtA guideline for Anticoccidials used for the therapy of coccidiosis in chickens, turkeys and geese (7AE15a; 1993), and should be read in conjunction with Directive 2001/82/EC, as amended.

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes, the 3R principles (replacement, reduction and refinement) should be applied (see also section 6.1.2).

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 substance under investigation is effective, and if the mode of action is predominantly coccidiostatic and/or coccidiocidal;

– the safety for the target animal should be established via tolerance data (e.g. VICH GL 43) completed with data derived from laboratory and field efficacy studies;

– studies should be performed without concomitant use of other anticoccidials (i.e. feed additives) or vaccination against coccidian;

– 3R principles should be followed through standardised methodology for infection and efficacy calculation and by substituting mortality with more humane endpoints (e.g. lesions scores in the case of chickens).

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

1. At least one dose determination study (DDS) testing at least three different dose levels;

2. At least two dose confirmation studies (DCS);

3. At least one multicentre field trial (actual use conditions) in at least two different European geographical areas.

Clinical efficacy data should be provided for each coccidian species claimed.

# 5. Pharmacology

The pharmacokinetic and pharmacodynamic properties of the active substance should be adequately documented. 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).

## *5.1. Pharmacodynamics (PD): Mode and mechanism of action*

The mode of action against the targeted stage(s) of coccidia should be stated, and the anticoccidial class should be defined. In order to demonstrate at which stage in the life cycle of the parasite the substance under investigation is effective (target of the substance), histopathological examinations

with identification of endogenous coccidian stages should be 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 proposed in the application.

It should be highlighted if the candidate substance shares a mode of action with other anticoccidial substances and if cross-resistance is likely to occur (see also 5.3).

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

### *5.2. Pharmacokinetics*

Pharmacokinetic (PK) data on the absorption, distribution, metabolism, and excretion of the active substance should be provided.

Specifically, the applicant should specify if the substance acts primarily locally in the intestine or systemically after absorption, and how this will influence the duration of therapeutic concentrations.

### *5.3. Resistance*

Current information on potential resistance to the substance or substance class should be provided. This should also include potential cross-resistance to relevant substances commonly used in the same target 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.

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

## 6. Efficacy studies

### *6.1. General principles*

Efficacy studies should be conducted according to the principles of GLP and/or GCP. In case of older studies where GLP and/or GCP principles are not applied, traceability and integrity of data should be adequately guaranteed by other means. For clinical field trials, GCP compliance is required.

### *6.2. Coccidia isolates*

Efficacy for each parasite 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 substance should be used. The pathogenicity of the infective strains should be representative of the respective coccidian species (see host-species information in sections 8-10).

For dose determination studies it is acceptable to use laboratory strains of coccidian species. However, for dose confirmation trials recent (less than 5 years unless otherwise justified) field isolates exposed to commonly administered anticoccidials should be preferred as the inoculum. The strains should be representative of the EU area. The history of the isolates (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) should be included.

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 the principal study/studies is initiated.

Freshly passaged isolates of sporulated oocysts, stored for a maximum period of 3 months, should be used.

The number of oocysts to be administered per animal should be determined by their virulence, and titration studies should be done in advance of pivotal study (ies) 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 may be necessary when examining acute disease, oocyst production and mode of action (stages of the life cycle) with the same parasite species.

## *6.3. Adequacy of infection*

It is generally up to the applicant to demonstrate that clinical signs of coccidiosis can be artificially induced with the laboratory 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 coccidia 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.

## *6.4. Study animals*

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

Naturally infected or experimentally infected animals can be used. In laboratory studies, animals that will be experimentally infected should not have been exposed to coccidian prior to the study and should be free of other infections. The absence of coccidian oocyst prior to the experiment should be confirmed. These animals should usually be weighed individually at the beginning and during the experiment, if possible. To reduce within group variability, the heaviest and lightest animals may be excluded at the beginning of the trial.

## *6.5. Dose determination studies*

Studies with different doses of the investigational veterinary product (IVP) are required to determine if the proposed dose regimen is adequate for the selected clinical endpoints. Test doses must be calculated as intake of the active substance per kilogram body weight. For medication in feed or water, the proposed dose level should also take into consideration a potentially reduced feed or water intake when animals become sick.

Dose determination studies should be carried out with at least three dose levels (usually 0.5, 1, 2 x of the proposed dose) of the active substance using the final formulation of the VMP, if possible, and one infected placebo-treated group.

Infective dose, number of animals and endpoints should be considered carefully to take account of 3R principles.

For each claimed species of coccidia, separate dose determination studies are required; however, single cell isolations are not required, i.e. 100% purity is not obligatory but the degree of impurity should be identified and justified. When testing field strains, non-pathogenic species in the isolate cannot be avoided. If clearly distinguishable, these non-pathogenic oocyst species can be excluded when expressing the number of infective oocysts for the species of interest.

Scientific data should be provided to determine the optimal time, duration and frequency of treatment in relation to the time of infection. This can be obtained through a combination of pharmacokinetics for the IVP and experimental parasitological data on life cycle and pathology. The timing of treatment should be evaluated in relation to the claim, i.e. whether the product is for administration during the prepatent period to avoid clinical signs or whether the claim is for therapeutic treatment.

The applicant should also address if the treatment, and in particular treatment early in parasite life cycle, interferes with the development of acquired immunity to coccidia species.

## *6.6. Dose confirmation studies*

At least two dose confirmation studies should be carried out per coccidian species with the final product formulation. One of these dose confirmation studies might be substituted by a dose determination study, if the final formulation was used, the product was applied according to the labelling and infection level, and the number of animals tested is considered adequate. Separate studies will be needed for the different stages of infection for which the product claims efficacy.

Dose rate and duration of administration via feed or water should reflect the proposed final use of the product, including the stages of the coccidian life cycle which are targeted, and also take reduced intake of feed and water into account. The study may be carried out in artificially or naturally infected animals. The experimental design should include:

- An infected group treated with IVP;
- An infected placebo-treated control group.

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

If the study is for a claim to prevent clinical signs by treating during the prepatent period, clinical signs and oocyst shedding may only be present in the untreated control group.

If the study is for a therapeutic claim (e.g. reduction of clinical signs), treatment should not be initiated until clinical signs occur. The duration of the study should be sufficient to determine if there is a relapse.

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

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

All animals dying during the experiment should be necropsied as soon as possible, macroscopical and histopathological changes should be determined, and coccidia specified. The cause of death should be stated.

## 6.7. Field efficacy studies

Multicentre field trials should be conducted in line with the principles of VICH GL9 (Good clinical practices) in at least two EU representative areas for the purpose of determining the efficacy and safety of the IVP under field conditions. Sites (e.g. farm, kennel) with a history of clinical coccidiosis caused by the relevant species should be selected. Concomitant use of other anticoccidial substances is not accepted during the study, and details of any potential prior use (routine or not) of anticoccidial feed additives on the site should be reported.

Field trials should be conducted in the animal species, age group, and husbandry conditions for which the veterinary medicinal product is intended. The proposed timing of treatment administration for the claimed indication should be justified.

For anticoccidials used preventatively, 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 reference product can be used in a three-arm study for non-inferiority or superiority comparisons.

Flocks/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 identified.

## 6.8. 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 differ between host species and coccidia species.

The endpoints may include clinical signs, macroscopical and histopathological changes, or body weight gain, depending on the target animal species (see below in the target species specific section).

The timing of the assessment of an endpoint in relation to both time of infection and time of treatment should be explained.

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

In chicken, lesion scoring is available as a reliable endpoint, while in other species lesion scoring is not well-established. The most relevant (co-)primary endpoints should be used, e.g. reduction of oocyst shedding (OPG=Oocyst per gram faeces) and morbidity (diarrhoea incidence, faecal scores or days with diarrhoea).

### 6.8.1. Pathological findings and lesion scores

The examination of lesion scores may be essential in the efficacy evaluation of anticoccidials in chicken as reliable lesion scoring systems are available and standardized and 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 species. However, appropriate pathological findings could also be used in other species to support the efficacy of a new product. For example, in severely affected piglets with *Cystoisospora suis*, typical histological lesions are confined to the jejunum and ileum, and characterized by villous atrophy, blunting of villi, focal ulceration, and fibrinonecrotic enteritis with parasite stages in epithelial cells. In the same way, young rabbits infected

with *Eimeria stiedai* have typical gross lesions affecting the liver, gall bladder and bile ducts; these lesions allow differential diagnosis of infection with *Eimeria stiedai* and infection with other intestinal coccidian species of rabbits.

Lesion score data should be analysed with appropriate statistical methods for categorical, ordinal data or by non-parametric tests.

### 6.8.2. Oocyst counts

Faecal samples for oocyst counts should be taken daily. For DDS and DCS, individual faecal samples (e.g. by rectal sampling) are preferred. In field studies or in group-housed animals, pooled samples or litter samples are allowed.

If relevant, the diurnal rhythm of shedding should be considered when oocyst shedding (or dropping scores) 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 the most reliable measure (see section 11.4).

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 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 to be indicated.

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 secondary efficacy parameter.

### 6.8.3. Morbidity and mortality

Clinical signs should be monitored throughout the study including faecal consistency scores (e.g. dropping scores) and unthriftiness. Diarrhoea incidence but also its severity and duration should be considered (e.g. number of days with diarrhoea in piglets). Faecal consistency is normally expressed on a scale from 1 to 4. In some species, such as rabbits and lambs, 'perianal faecal soiling scores' could also be used although it may be difficult to note the faecal score individually.

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

Due to the 3R principles, mortality should not be the primary endpoint in the study.

### 6.8.4. Animal performance

Animal performance indicators such as body weight gain or food/water intake may be more relevant for species with a high growth rate (e.g. poultry and rabbits), where a substantial growth can be expected within the experimental period, but will be less relevant for other species, e.g. calves. Generally, animal performance should only be a secondary endpoint.

## *6.9. General statistical principles*

Reference is made to the principles of the CVMP guideline on statistical principles for veterinary clinical trials (CVMP/EWP/81976/2010).

The number of animals included in a study should be calculated and justified by the applicant to enable evaluation of statistical significance. For dose finding/confirmation studies and where the individual animal is the experimental unit, at least six animals should be used for each treatment level. However, higher numbers may be needed if there is a large between-animal variation 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 variation into account and the statistical model should take the pen effect into account.

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

# 7. Summary of product characteristics (SPC)

The SPC should include information on the efficacy of the product against the different stages of coccidia and the optimal timing of treatment in the life cycle of the parasite.

The SPC efficacy claims should be in accordance with the primary endpoints in the clinical studies and the claims should be restricted to the findings of the efficacy studies. The target coccidian species should be clearly stated.

In general, claims would be for the prevention or reduction of clinical signs of coccidiosis, and the reduction of oocyst shedding.

A claim for the reduction in oocyst shedding and the prevention of clinical signs of coccidiosis should be accompanied by the criteria for initiation of treatment, with a sentence such as ‘use the product in farms with a confirmed history of coccidiosis due to <*target coccidia species*>’.

If the treatment targets early stages in the life cycle (prepatent period), and no effect is demonstrated at later stages, a sentence should be added such as “although treatment will reduce the spread of infection, it may not 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 all animals in the group are treated before onset of clinical signs.

If the therapy supresses immune response of the animal against coccidia infection, this should be mentioned in the SPC.

The SPC should include sentences mentioning the consequences of misuse of the VMP Responsible/prudent use in regard to resistance development should be addressed.

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

Warnings related to the safe use of the VMP should be included in section 4.5:

– "As with any antiparasiticide, frequent and repeated use of antiprotozoals from the same class may lead to the development of resistance." And "If resistance is present it should be considered to use other antiprotozoal from another class/mechanism of action.";

– "This VMP should not be used together with feed additives/or other veterinary medicinal products that might interfere with the efficacy of the product, like 'coccidiostats' and 'histomonostats'".

# 8. Specific requirements for poultry

## *8.1. 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 stains. These species vary with respect to their localisation in the intestinal tract and the age 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 via faeces or urine, 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.

## *8.2. 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. 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 the same sex per pen, pen weights are satisfactory.

## *8.3. Laboratory studies*

### 8.3.1. Inoculum

The previous guideline specified a threshold for mortality for the most pathogenic coccidian species in chicken 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). Adequacy of infection should be determined by the minimum lesion score, oocyst shedding and possibly weight reduction in target animals.

### 8.3.2. 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 chosen secondary endpoint. The inclusion of these groups will allow to determine any direct 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 substance-related effects in the target species).

All animals dying or euthanised during the experiment(s) should be necropsied as soon as possible. The coccidian species should be determined and attempts should be made to establish and record the cause of death.

### *8.4. Endpoints*

As a standardised lesion score system has been developed for chickens, it is recommended that the primary endpoint for chickens should be lesion scores, whereas oocyst shedding, diarrhoea, and body weight gain can be included as secondary endpoints. Mortality should no longer be required as primary endpoint.

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

## 9. Specific requirements for ruminants

### *9.1. General information*

Coccidiosis in ruminants is caused by a number of Eimeria species (see annex, listing the most relevant species). Different pathogen 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.

### *9.2. Study Animals*

Coccidia-naïve animals ageing from 3 weeks to 6 months can be enrolled, since their immune system is often still developing. The period after weaning, during which lambs, calves and goat kids are highly sensitive to coccidian infections, is the most critical time point for infection.

### *9.3. Laboratory studies*

#### 9.3.1. Inoculum

The nature of clinical signs in infected lambs or kids are comparable (e.g. diarrhoea, reduced body weight) 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.

#### 9.3.2. Experimental design:

*Prevention or reduction of clinical signs:*

To prevent or reduce clinical signs due to coccidiosis, exposed animals should preferably be treated during the prepatent period rather than after onset of clinical signs. The timing of treatment should be driven by the live 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- 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 adequate to assess a claim for the prevention of clinical signs caused by the relevant coccidian species *E. bovis E. bovis* and *E. zuernii*.

*Reduction of oocyst shedding:*

Oocyst shedding should be measured depending of 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.

### *9.4. Endpoints*

It is recommended that a primary efficacy parameter for treatment 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 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 of coccidian, also death caused by coccidiosis might occasionally occur. Thus, all other clinical signs during the study can be considered as secondary endpoints.

## 10. Specific requirements for pigs

### *10.1. General information*

The predominant pathogen in pigs is *Cystoisospora suis*. Naïve piglets are infected around birth and usually recover within 2 weeks post-infection. (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 post-infection and frequently occurs in two peaks at 5-9 and at 11-14 days post-infection. Clinical signs can be seen as early as 3 days p.i.

### *10.2. 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 total 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.

### *10.3. Laboratory studies*

#### 10.3.1. Inoculum

In artificial challenge models, piglets are 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.

The timing of infection and treatment should be justified depending on the proposed claim. For treatment with the aim to prevent or reduce clinical signs, animals should be treated during the prepatent period i.e. 4-6 days post-infection.

### 10.3.2. Experimental design

In laboratory studies, experimentally infected animals rather than naturally infected animals are preferred. For infections with *Isospora suis*, an experimental model mimicking the field situation of cystoisosporosis has been described (Mundt *et al.*, 2006). Dose confirmation studies can also be conducted in field conditions.

## *10.4. Endpoints*

Endpoints should be defined depending upon the intended claim and the aim of the study. The oocyst count reduction (see section 6.8.2) should be selected as the primary endpoint in a dose determination or a dose confirmation study. However, for a field trial 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: histopathology, faecal scores, reduction of days with diarrhoea, reduction of the number of days with oocyst shedding, percentage of piglets with oocyst shedding, mortality rate caused by coccidiosis and bodyweight gain.

# 11. Specific requirements for rabbits

## *11.1. General information*

There are two anatomical forms of coccidiosis in rabbits:

- hepatic coccidiosis caused by *Eimeria stiedai* may lead to severe pathological changes both in bile ductus and liver parenchyma especially in young animals if high infective doses of oocysts are used;
- 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, ascites, 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.

## *11.2. Study animals*

Rabbits become immunised even with small doses of oocysts, and the study animals should be coccidia-free prior to study begin. Under field conditions, this is usually achieved by the constant 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.

## *11.3. Laboratory studies*

### 11.3.1. Inoculum

The oocysts are preferably inoculated under the tongue in a volume of 0.2 ml. Only if it is impossible to concentrate the desired quantity of oocysts in this volume, the animals should be infected via gavage.

### 11.3.2. 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, non-infected and treated control (a small number or satellite group should be included to clarify 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.

## *11.4. Endpoints*

It is recommended that the primary endpoint should be the reduction in oocyst shedding and as co-primary endpoint a clinical sign of the disease.

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 the method described by Coudert *et al.* (1995). Only if the quantity of faeces is too high (in field studies), or if animals are placed on litter, faecal samples may be randomly collected to assess the OPG value.

Secondary endpoints:

Secondary parameters would include body weight, growth rate and macroscopic and histopathological changes that will depend on the target pathogen, e.g. GIT 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. Food conversion should also be calculated. The performance of animals must be checked for at least three weeks after challenge.

# 12. Specific requirements for dogs and cats

## *12.1. General information*

In both dogs and cats the disease has a higher prevalence in young animals and among breeding colonies or shelters when hygiene is deficient or difficult to maintain.

In cats, kittens less than six months 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 4 months are more susceptible to develop the disease, especially in large kennel situations and dog breeder facilities. The common clinical signs consist of 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.

## *12.2. Study animals*

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

## *12.3. Laboratory studies*

### 12.3.1. Inoculum

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

### 12.3.2. Experimental design

See general part.

## *12.4. Endpoints*

It is recommended that the primary endpoint for dogs and cats is the reduction of faecal oocyst counts, and as co-primary endpoint a clinical sign of the disease (e.g. incidence of diarrhoea); secondary endpoints being the body weight gain and frequency of diarrhoea.

# Definitions

Anticoccidial medicinal product: In the context of this guideline, an anticoccidial medicinal product is a veterinary medicinal product developed for the prevention or reduction of clinical signs of coccidiosis, and/or the reduction of oocyst shedding.

Coccidiocidal: Active substance with coccidiocidal action, which kills or irreversibly damages most of certain parasite stages, without evidence of clinical relapse after drug withdrawal.

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

Dose-limiting parasite: The least sensitive parasite species in a claimed indication for a determined dose of a VMP.

# References


Anticoccidials used for the therapy of coccidiosis in chickens, turkeys and geese (7AE15a; 1993 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004441.pdf )

Coudert P, D Licois, F Drouet-Viard (1995): *Eimeria* species and strains of the rabbits. In: J. Eckert, R. Braun, M.W. Shirley and P. Coudert (Eds.), Guidelines on techniques in coccidiosis research. European Commission, Directorate-General XII, Science, Research and Development Environment Research Programme, pp. 52–73.

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

CVMP Guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/99)

Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products, as amended.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Mundt, H.C., Joachim, A., Becka, M., Daugschies, A., 2006. *Isospora suis*: an experimental model for mammalian intestinal coccidiosis. Parasitol Res 98, 167-175.

VICH GL 9 Good clinical practices (CVMP/VICH/595/1998)

VICH GL 43: Target animal safety: pharmaceuticals (CVMP/VICH/393388/2006)

VICH GL 52 Bioequivalence: blood level bioequivalence study (EMA/CVMP/VICH/751935/2013 – Corr.1).

# Annex

Examples of the most common coccidian 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* |
| **Cattle** | *E. bovis, E. zuernii* (causing coccidiosis in stabled calves), *E. alabamensis* (typically causing coccidiosis on pasture) |
| **Sheep** | *E. crandalis and E. ovinoidalis (highly pathogenic), E. ovin, E. parva, E. intricata, E. bakuensis, E. ahsata* |
| **Goats** | *E. alijevi*<br>*E. ninakohlyakimovae, E. arloingi, E. caprina, E. christenseni* |
| **Pigs** | *Cystoisospora suis* (*Isospora suis*) |
| **Rabbits** | Hepatic coccidiosis: *E. stiedai*<br>Intestinal coccidiosis:<br>*E. exigua, E. perforans, E.vejdovskyi* (slightly pathogenic )<br>*E. irresidua, E. magna, E. media, E. piriformis* (mildly pathogenic )<br>*E. intestinalis*, *E. flavescens* (highly pathogenic ) |
| **Dogs** | *Cystoisospora (C. canis, C. ohioensis, C. neorivolta* and *C. burrowsi)* |
| **Cats** | *Cystoisospora (C. felis* and *C. rivolta)* |