ANTICOCCIDIALS USED FOR THE THERAPY OF COCCIDIOSIS IN CHICKENS, TURKEYS AND GEESE

Guideline Title	Anticoccidials used for the Therapy of Coccidiosis in Chickens, Turkey and Geese
Legislative Basis	Directive 81/852/EEC as amended
Date of First Adoption	November 1992
Date of Entry into Force	May 1993
Status	Last revised November 1992
Previous Titles	Anticoccidials used for the Therapy of Coccidiosis in Chickens, Turkey and Geese and Pigeons
Other References	III/9289/90
Additional Notes	The objective of this document is to provide specific guidance in respect of the documentation of the efficacy of new veterinary medicinal products developed for therapy of coccidiosis. It should be read in conjunction with Directive 81/852/EEC as amended, and the note for guidance on *Good Clinical Practice for the Conduct of Clinical Trials on Veterinary Medicinal Products in the European Union.*

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ANTICOCCIDIALS USED FOR THE THERAPY OF COCCIDIOSIS IN CHICKENS, TURKEYS AND GEESE

For poultry raised for food production most effort is directed toward prevention of coccidiosis by administration of coccidiostats in the form of additives mixed with complete feed. Outbreaks of clinical coccidiosis occur and require treatment with veterinary medicinal products approved in accordance with Directives 81/851/EEC and 81/852/EEC.

Although all claims for efficacy of the product against particular species of Eimeria must be validated, among the Eimeria species the following ones are of importance in the European Union:

Chickens:

– E. tenella
– E. necatrix
– E. acervulina
– E. maxima
– E. brunetti
– E. mitis

Turkeys:

– E. adenoeides
– E. meleagrimitis
– E. galiopavonis

Geese:

– E. anseris
– E. truncata (coccidiosis of the kidney)

Although this note for guidance is not directly applicable to products for pigeons, its general principles may be applicable.

New veterinary medicinal products developed for therapy of coccidiosis will have to satisfy all the usual requirements of approval. This guideline is not intended to replace the note for guidance: *Good Clinical Practice for the Conduct of Clinical Trials on Veterinary Medicinal Products in the European Union*, but is intended to provide special guidance in respect of the documentation of the efficacy of such veterinary medicinal products, and should be applied together with Directive 81/852/EEC and the note for guidance: *Good Clinical Practice for the Conduct of Clinical Trials on Veterinary Medicinal Products in the European Union.*

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

– data which demonstrate at which stage in the life cycle of the parasite the substance under investigation is effective (target of the substance). That means that sufficient histopathology in recently dead or culled birds, time titration, and anticoccidial withdrawal studies must be available which confirm where in the life cycle of the

parasite the anticoccidial is effective and if it is predominantly coccidiostatic or coccidiocidal.

– the safety for the target animal;

– interactions with feed additives;

– data on residues which may contaminate the litter or the floor. Such contamination may result in a recycling of active substance(s)/metabolite(s) any may have consequences for utilisation of the excreta.

To ensure that the anticoccidial will control resistant Eimeria spp., efficacy studies must be conducted using carefully characterised isolates of recent origin.

When generating data for a medicinal product claiming therapy of coccidiosis, the required efficacy data involve 3 stages of target animal experimentation:

1. controlled battery experiments (single and mixed infections);
2. controlled floor pen studies (simulated use conditions);
3. field trials (actual use conditions)

1. CONTROLLED BATTERY EXPERIMENTS

1.1 Animals

Susceptible female and male target animals should be used whose individual weights by sex and sexed-weighted averages should be established. In the case of replacement chickens, trials are to be conducted solely in female birds. The class of target animals (e.g. broilers or replacement chickens) for which the veterinary medicinal product is to be marketed should be used. Extrapolation is possible, if justified (e.g. from broiler chicken data in the case of broiler breeders). It may be not practical to keep adult geese or turkeys in batteries. Thus, trials may be conducted while keeping the birds in floor pens.

Birds should be individually weighed and identified at the beginning of the experiment. At this time the heaviest and the lightest birds should be discarded (approximately 10% of each). Individual weights are preferred but if birds are of equal or of one sex per pen, pen weights are satisfactory. It is important to obtain healthy birds so that concomitant infections (i.e. bacterial or vial) do not confuse coccidiostat evaluations.

1.2 Environment and facilities

There should be uniform environments for all experimental groups. Non-infected non-medicated controls should be maintained strictly isolated in an effort to preclude their accidental infection and possible contamination with medicated feed or water, unless batteries are specifically designed to prevent this from happening.

Uniform lighting per group should be used. the number of hours of light per day should be noted.

Papers under the birds should be changed at appropriate times prior to scoring droppings.

Feeders should not be allowed to become empty, but if feed efficiency is being measured they should not be over 1/2 full. It is important to prevent medicated feed from being "billed out" so it will not fall from one experimental feeder to the other. The feed should not contain substances which may interfere with the purpose of the trial. This is to be considered in the light of the fact that feed may be contaminated with additives and that a few grams of some anticoccidial agents or growth promoters per ton of feed have anticoccidial activity. One

must use judgement regarding random selection of pens per group versus preselection. The final method must be justified.

1.3 Inoculum

Freshly passaged isolates should be used. They should have been multiplied using the target animals. Titration studies should be done in advance of principal experiment(s) in a few target animals to determine the pathogenicity of the inoculum. The number of oocysts which will be administered per bird is determined by their pathogenicity. Different doses will be necessary when examining acute disease, oocyst production and mode of action (stages of life cycle) with the same species.

1.4 Single infections

These are to be single species infections; however, single cell isolations are not required, i.e. a 100% purity is not obligatory. The degree of impurity should be identified and justified. For E. tenella and E. necatrix, there should be at least 30% coccidiosis mortality in the infected non-medicated controls. With E. brunetti and E. maxima, coccidiosis mortality should be 5 to 10%. Where a well characterised disease model is available, it may be possible to have much lower mortality rates than those stated and still be able to demonstrate efficacy. With the other species which do not usually cause death, there should be at least 10 – 20% weight reduction. Evaluation against less severe coccidiosis mortality and/or morbidity is also suggested. This may be particularly important in the case of a potential anticoccidial for layer replacement chickens and breeder chickens.

In the early stages of anticoccidial evaluation it is perfectly permissible to use laboratory strains of Eimeria species; however, it is imperative that as the battery studies continue, that stocks of recent field isolates exposed to commonly administered anticoccidials be used as the inoculum. They should originate from different geographic areas. The history of the isolates (i.e. where and when it was isolated, the name of the anticoccidial reportedly in the feed at the time of the outbreak, and the predominant species involved) should be included. The isolates should be passed through susceptible birds, cultures built up, oocysts collected at appropriate times, and titration for appropriate morbidity or mortality performed before the principal experiment(s) is initiated. Each species claimed on the label should be confirmed by appropriate data with several stocks of recent field isolates, in which that particular species is the predominant one. If the anticoccidial under investigation is intended to control normally resistant Eimeria species, such species must be used for infection.

1.5 Mixed infections

Since under field conditions the existence of single species infections is unusual, it is essential that the applicant uses various Eimeria spp. in the infection experiments. In the case of mixed infections of chickens, E. tenella should be predominant (at least 25% - 30% of the sporulised oocysts) to achieve adequate mortality in the infected non medicated controls. In turkeys the same applies to E. adenoeides. Experiments with inoculum prepared by deliberate mixing of different species (combinations) should also be conducted.

1.6 Experimental design

Groups

1. Non-infected non-medicated controls (birds should be kept in isolation)
2. Infected non-medicated controls

3. Infected medicated group(s)

4. Non-infected medicated controls (a small number or satellite group should be included in order to differentiate between direct, substance-related effects and indirect, treatment-related effects)

Where appropriate, birds should be infected by resistant strains of Eimeria spp..
The bird numbers, replicate numbers per experimental group and the number of times experiments should be repeated depend upon the expected differences.

1.7 Medication

Time titration studies, in birds infected with laboratory strains or recent isolates exposed to commonly administered anticoccidials with each species on the label, should be designed to determine how long after coccidial inoculations birds may be effectively treated with the proposed veterinary medicinal product. A "therapeutic" anticoccidial should usually be administered 48 hours and 72 hours following challenge. However, when choosing the time for medication the stage of action of the substance under investigation can be taken into consideration.

Anticoccidial withdrawal studies are necessary to determine if there is recrudescence of infection.

All experimental feed or water should be assayed for anticoccidial content each time it is mixed.

Confirmatory tests utilising either E. tenella or E. necatrix with at least 30% mortality in the infected non-medicated controls must confirm its activities against coccidiosis in chickens. However, where a well characterised disease model is available, a lower mortality rate may be sufficient.

1.8 Parameters

The following parameters should be detailed:

Clinical signs, coccidiosis mortality, feed and water intake, feed conversion (this includes weight gains), lesion scores, dropping scores, oocyst counts and total mortality.

The examination of lesion scores is obligatory because other parameters (e.g. excretion of oocysts) may be negative if the anticoccidial is effective. Lesion scores should be examined in freshly dead or culled birds. The diurnal rhythm of excretion must be considered when dropping scores and oocyst excretion are examined.

In order to assess the safety for target animals simultaneously within trials on efficacy, the following additional data should be evaluated:

– Fattening birds: interference with growth and feed conversion

– Replacement layers: interference with growth and feed conversion

– Laying hens: feed conversion, effects on egg production and egg quality

– Breeding birds: interference with growth and feed conversion effects on egg production, egg quality, fertility and hatchability

1.9 Diagnostic work

All birds dying during the experiment(s) should be necropsied as soon as possible. Attempts should be made to establish and record etiologic diagnoses. In some experiments, an extra set of replicates should be maintained so that they can be sacrificed in the acute infection, lesions scored, and wet mount microscopic examinations conducted. In mixed infections, attempts should be made to document that the Eimeria species inoculated were causing the mortality and/or morbidity.

1.10 Dose titration studies

Titration studies are required to determine whether or not the proposed dose level is the optimal one. Test doses must be calculated as intake of the active substance per kilogram body weight per time. Therefore, data on light regimen and dosage regimen are necessary. Furthermore, data on pharmacokinetics and bioavailability can provide useful information. The investigation should include oocyst counts. The proposed dose level should take into consideration that reduced feed or water intake, mixing errors etc., can cause a theoretically optimal dose level to be suboptimal.

One of the criteria for establishing lower limits of acceptance (specifications) is that the product is, in fact, efficacious at this limit. Data generated while titrating the proposed use level is acceptable for this requirement.

2. FLOOR PEN TRIALS

These should be designed to confirm efficacy under carefully controlled simulated use conditions.

Before stocking the floor with birds, the premises should be cleaned and disinfected to avoid any interference with the purpose of the trial.

There are several methods for exposing birds to infection in floor pen studies. Coccidiosis mixed infections utilising all species claimed on the label should result from appropriate methods, i.e. synchronous infection via oocysts in the feed, individual administration of the infectious inoculum, exposure to coccidia-infected seeder birds, oocysts in the litter, or other appropriate methods. Attempts should be made to titrate oocyst dosage so that each species claimed on the label is causing pathology. This should be demonstrated on wet mount examination and attempted speciation of the coccidia. It is known that the degree of mortality and morbidity in floor pen experimentation is unpredictable so that time should be spent in development of appropriate methodology.

The infection should result in clinical signs of coccidiosis in some birds. Medication should be delayed until then. The duration of the experiment should be sufficient to determine if there is a resurgence of infection.

Pens should be replicated, pen or individual weights should be taken at appropriate times, and feed conversion calculated. The data must be statistically analysed (significant differences at 5% level of probability) with the method of statistical analysis shown. Environmental conditions should be similar.

All birds dying during the experiment should be necropsied as soon as possible, wet mount examinations made, and coccidia speciated. Etiologic diagnoses should be attempted with appropriate diagnostic tests.

Small pens of 60 to 120 birds of equal sex each (with the exception of replacement birds) are recommended which are replicated. Also randomisation utilising sex, breed etc., as factors is encouraged. Commercial birds of the class in which the veterinary medicinal product is to be used should be utilised in this type of experimentation. However, because they are not practical, efficacy floor pen studies in broiler breeders are not required provided their efficacy has been demonstrated in broilers.

2.1 Groups

a) Non-infected, non-medicated controls (birds should be kept isolated)

b) Infected non-medicated controls

c) Infected medicated group(s), recommended dosage regimen

d) Non-infected medicated controls (a small number or satellite group should be included in order to differentiate between direct, substance-related effects and indirect treatment-related effects)

Experiments should be conducted with stocks of recent isolates exposed to commonly used anticoccidials. Stocks must be described sufficiently (e.g. origin, passages, specificity, resistance, etc.). Infections induced with laboratory strains or Eimeria species are unsatisfactory except if it is with strains resistant to anticoccidials.

Facilities should be cleaned and disinfected and clean litter used in each experiment.

Birds should not be used for more than one trial.

Feed or water should be assayed each time it is mixed including non-medicated feed for anticoccidial content.

2.2 Parameters

Coccidiosis mortality, weight gains, lesion scores, feed conversions and results of wet mount examinations of sample birds removed at random from each pen and coccidiosis infected birds dying during the experiment, other mortality, and subjective observations of the investigator.

Data on target animal safety should be reported as required for controlled battery experimentation.

3. FIELD TRIALS

Field trials should be conducted in at least 2 geographic areas of intensive production for the purpose of determining the utility of the anticoccidial agent under actual commercial conditions and to determine adverse effects of the veterinary medicinal product, if any, under these conditions.

Field trials should be conducted in the class of birds the veterinary medicinal product is intended for.

Where possible, a control group should be included even though the number of birds in the control group need not be large. There are many variables and inconsistencies in obtaining clinical coccidiosis in field trials. The feed or water should be assayed each time it is mixed. The proposed formulation should be used. Flocks should be carefully examined for any suspected adverse effect of the veterinary medicinal product. Birds dying should be necropsied and etiologic diagnoses attempted.

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