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GUIDELINE FOR THE CONDUCT OF EFFICACY STUDIES FOR INTRAMAMMARY PRODUCTS FOR USE IN CATTLE

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GUIDELINES FOR THE CONDUCT OF EFFICACY STUDIES FOR INTRAMAMMARY PRODUCTS FOR USE IN CATTLE

This note for guidance replaces the three previous guidance notes (III/9041/90, III/3576/91, III/3173/92) in Volume 7A (1998).

1. OBJECTIVE

The objective of the trial is to assess the efficacy of a medicinal product administered via the teat duct for the treatment of clinical and/or subclinical mastitis during lactation, or for the elimination of udder infections at drying-off and the prevention of new infections during the dry period. Clinical mastitis is defined as mastitis with clinical signs in the quarter (swelling, heat, pain) and/or changes in the appearance of milk (clots or flakes, watery appearance, discoloration), with or without general signs (fever, loss of appetite). Subclinical mastitis is defined as mastitis without clinical signs but a high milk somatic cell count in the quarter and a positive bacteriological isolation from the milk.

2. METHODS AND TREATMENTS

2.1 Comparative trials

Comparative trials should be carried out in two groups of cows:

- cows/quartars treated with the test product,
- control cows/quartars treated with an authorized product, placebo or no treatment. The choice of the type of control group should be justified by the applicant.

The trial must be carried out on a sufficient number of herds and cows. The described statistical methods should include definition of study population, sample size calculation, randomization and blocking procedures, confounding factors, statistical models, and level of significance. Cows selected in each herd should not exceed 20% of the total number of cases treated in the complete study, unless justified.

Treatment should be allocated within each herd in a random manner using blinding as far as is practically possible. The balancing of the groups to include control and treated cows must be carried out at the individual herd level and on the total population.

The milk samplings and microbiological investigation must be carried out in accordance with the methods recommended by the International Dairy Federation (IDF) (see Bulletin No 132, 1981), or by other adequate reference.

2.2 Reference product

Where possible, it is advisable to use a reference product with the same claims, approved in accordance with Directive 2001/82/EC.

2.3 Test product

The conditions of administration, dosage and frequency of administration should be justified, described and should be those proposed.

2.4 Milk withdrawal period

In a blinded study, the milk withdrawal period should be the same for each product (test and reference products) and correspond to the product with the longest withdrawal period. If blinding is not used, then the withdrawal period of the individual products should be observed.

In dry cow treatment, the products must be given to cows with dry periods of a sufficient length to ensure that antibiotic residues will not persist in the milk intended for human consumption following calving.

2.5 Treatment unit

In clinical mastitis the treatment and statistical unit is the individual udder quarter, and only one quarter is to be treated in any one cow.

In the dry cow treatment and subclinical mastitis, the treatment unit is the cow but the statistical unit is the individual quarter. In the dry cow all four quarters receive the same treatment.

3. SELECTION OF ELIGIBLE HERDS AND COWS

3.1 Identification

Study cows should be selected from herds with proper cow identification and recording of health data.

3.2 Defining inclusion/exclusion criteria

The population of eligible cows must be described and the inclusion/exclusion criteria defined for:

- cows which are to be included in the trial;
- cows which are to be excluded from the trial;
- cows which are to be excluded post inclusion from the trial.

In subclinical mastitis trials, all lactating cows with presence of pathogens in conjunction with quarter somatic cell count (SCC) > 300 000 cells/ml in both pre-treatment milk samples, may be eligible for the trial.

In clinical mastitis trials, all lactating cows with clinical mastitis which can be treated with intramammary treatment only are eligible for the trial. Cows with severe systemic signs or toxic mastitis should not be included.

In dry cow treatment, all lactating cows approaching the end of lactation are eligible for the trial.

3.3 Exclusion criteria

The following cows are to be excluded from the trial:

- cows with intercurrent diseases;
- cows which were given systemic or intramammary anti-infectious or anti-inflammatory treatments within a 30-day period before the trial;
- cows with visible teat damage;
- in clinical mastitis: cows with mastitis in two or more udder quarters;
- in clinical and subclinical mastitis: cows with a daily milk yield less than 5 litres of milk prior to onset of clinical signs.

3.4 Herd and cows information

The history of the herd and cows must be recorded after the inclusion of a cow in the trial and before the commencement of the treatment.

Farm:

- name and address of herd owner;
- number of dairy cows;
- methods of herd management, milking, and dry cow management;
- teat disinfection procedures if practised;
- bulk milk SCC in the herd over preceding months (if available).

Cows:

- name or identification number;
- breed;
- number of lactations;
- date of calving;
- estimated or measured milk yield at time of treatment;
- cow milk SCC if available during preceding months;
- history of previous mastitis treatments if available;
- in clinical mastitis: carefully recorded clinical signs at the time of treatment;
- in the dry cow treatment: the milk yields of cows at drying-off and the method of drying-off (e.g. once-a-day milking, abrupt cessation of milking, etc.).

4. REPORTING

4.1 Data required

Data required from each treated cow:

Clinical mastitis:

The cow is examined clinically (general condition, milk quality, udder consistency) just before treatment, and in connection with the first bacteriological post-treatment sampling. In addition to these examinations, clinical examination should be made in cases where clinical developments make it necessary. If no clinical cure has occurred at the first post-treatment sampling, the case is excluded from further sampling.

Milk samples for bacteriological analysis are taken before treatment and two times after treatment. The post-treatment samples should be taken at least 7 days apart between day 14 and day 28 from the cessation of the treatment. Quarter milk SCC is determined from the second post-treatment sample.

Subclinical mastitis:

Milk samples for bacteriological analysis are taken twice before treatment and twice after treatment. Two positive isolations of the same pathogen before treatment are required (two consecutive samples taken with one day apart; if only one sample out of these two is positive, diagnosis must be confirmed with a third sample). The post-treatment samples should be taken at least 7 days apart between day 14 and day 28 from the cessation of the treatment. Quarter milk SCC is determined from the pre-treatment samples, and from the second post-treatment sample.

Dry cow treatment:

Milk samples for bacteriological analysis must be taken before drying-off and after calving from all the quarters. Two samplings before treatment are required (two consecutive samples taken with one day apart within one week before drying-off; if one sample out of these two is positive, diagnosis must be confirmed with a third sample). The first post-treatment sample is taken before the first regular milking after calving, and the second post-treatment sample 4 to 7 days later. Cow milk SCC should

be determined from the second post-treatment sample. The cow must be clinically examined after calving for any pathological changes of the udder or of the appearance of the milk. Cases of clinical mastitis occurring during the dry period and during the post-calving investigational period shall be recorded.

The *in vitro* antimicrobial susceptibility of the bacteria isolated from the pre-treatment sample to the antimicrobials used in treatment should be determined using accepted procedures.

4.2 Admission criteria for cows or quarters in the final analysis of data

- a) Cases which are uninterpretable, due to a lack or loss of information, shall be listed in the final report and their distribution in each group shall be analysed.
- b) Data collected from any udder/quarter of a cow, which had to be treated with additional antibiotic or other supportive treatment associated with the mastitis within the individual cow experimental period, should be included in the final data analysis. These cases should be classified as treatment failures. Cows treated with antibiotics or anti-inflammatories due to intercurrent diseases during the experimental period should be excluded from the trial. Data from cows in which additional quarters had to be treated within the individual cow study period shall be excluded from the final analysis and listed separately for each treatment group.

5. INTERPRETATION OF RESULTS

5.1 Bacteriological status

Bacteriological status is the key parameter in evaluating success of treatment. Bacteriological cure must be evaluated for each treated infected udder quarter and must be based on the total elimination (eliminated from both post-treatment samples) of the pathogens which were present at the time of treatment. In clinical and subclinical mastitis claims, and in treatment claim of dry cow treatment, quarters with new infections in the originally infected, treated quarters (growth of bacterial species different from the original ones) in one or both post-treatment samples can be classified as a bacteriological cure. The number and type of these occurrences in each treatment group should be included in the final study report. A high frequency of these occurrences is not acceptable and needs further clarification.

5.2. Clinical mastitis

In clinical mastitis studies, the clinical cure must be evaluated for each infected quarter and must be based on the return to normal of the parameters concerning the cow's general condition, the quality of the milk and the consistency of the udder. A case is regarded as a clinical cure if the milk has a normal appearance and the condition of the udder and the cow's general condition are normal in the clinical examination at time of the first post-treatment sampling. If there is no clinical cure, the case is to be excluded from the second sampling and classified as treatment failure. Only cases of mastitis in which bacteria are isolated in the pre-treatment sample will be used in calculating cure rates. Results from quarters with no growth in the pre-treatment sample can be reported separately.

5.3. Clinical and subclinical mastitis

In clinical and subclinical mastitis trials, individual quarter milk SCC is determined from the second post-treatment samples. Mean SCCs are calculated from the results for each treatment group and separately for bacteriologically cured and not cured quarters. The mean SCC results for each treatment group will be used to give numerical trends, but these data are generally not included in the final judgement criteria.

The data should be expressed as numbers of quarters and number of cows cured clinically, bacteriologically, and based on individual quarter milk SCC (subclinical mastitis only), see table 1. For clinical mastitis studies, cows with no clinical cure in the clinical examination at the first post-

treatment sampling are included in the final calculations of the bacteriological cure rates as failures. For subclinical mastitis studies, it is preferable to present combined cure rates based on individual quarter data (bacteriological cure + quarter milk SCC < 300 000 cells/ml).

Table 1. An example for data presentation for each treatment group (further details are given in the text).

		Post-treatment Cure					
		Clinical cure		Bacteriological cure		Bacteriological + SCC cure ¹	
	No of quarters/cows	n	%	n	%	n	%
Treatment A							
Treatment B							

¹ For subclinical mastitis only

5.4 Dry cow treatment

An application for a dry cow treatment may support either a claim for cure or a prevention claim or both cure and prevention claims together. However, efficacy of treatment (claim for cure) and the prevention of new infections (prevention claim) must be evaluated separately.

Treatment effect is evaluated for each pathogen over all the treated udder quarters. A new infection corresponds to the appearance of a bacterial species different from that isolated at drying-off or the appearance of bacterial growth in a quarter not infected at drying-off. Mean cow milk SCCs for each treatment group determined from the second milk samples after calving are calculated for each treatment group. The mean SCC results will be used to give numerical trends, but these data are generally not included in the final judgement criteria. In the treatment claim, if the bacterial species growing in one or both post-calving sample(s) is the same as the original one at drying-off, it is a “treatment failure”; if the post-calving sample(s) has growth of other bacterial species, it is considered as a new infection and “treatment success”.

For the prevention claim, only healthy quarters with no bacterial growth at drying-off are eligible. The same quarter cannot be taken into account both for prevention and treatment claim. No growth in both post-calving samples is “prevention success” for prevention claim and “cured i.e. treatment success” for treatment claim. For prevention claim, bacterial growth in one or both post-calving samples is always “prevention failure”.

6. PRESENTATION OF DATA

The data to be presented are described in the annex to Directive 81/852/EEC as modified by directive 92/18/EEC.

A record from each individual case should be presented in the final report.

In particular, the data on the bacteriological response for each organism for each treated quarter must be summarized and tabulated separately for each bacterial species and treatment group. Results from the *in vitro* susceptibility are enclosed in the final report.