Antibiotic Residues in Milk: Dye Marking Versus Direct Control

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ABSTRACT

Advantages and disadvantages of control for antibiotic residues in milk by compulsory dye marking of anti-mastitis preparations or by direct tests utilizing microbiological methods are reviewed. It appears feasible to construct a very strict direct control system utilizing highly sensitive microorganisms in laboratory methods or incorporated in industrially-made test kits. Useful methods whereby a dramatic decrease in both the incidence and amount of antibiotic residues can be attained are described. Compared to these systems, the compulsory addition of dye-markers to anti-mastitis preparations is unnecessary and even undesirable.

Antibiotic residues in milk are undesirable on the grounds of public health and good dairy practice. Therefore, in most countries an increasingly intensive control is being imposed on these residues. Control can be achieved in both a direct and an indirect manner. In direct control, use is made of microbiological test methods whereby the presence and, if necessary, concentration and nature of any antibiotics can be demonstrated. For indirect control, a marker dye is added to the antibiotic preparation such that the excretion rate of the dye correlates, as far as is possible, with that of the preparation administered. Most countries with intensive dairy farming use a direct control procedure, but indirect methods have been applied in the State of Victoria, Australia, since 1962 and later also in New Zealand, France and South Africa.

While in a growing number of countries the problem of control has been, or is being, reduced to acceptable proportions by detection based exclusively on microbiological methods, there are other countries in which an intention to introduce non-microbiological methods is still under discussion. It is therefore opportune to detail the various arguments in support of, or against, either system in the light of practical experience.

This paper will not deal with the already extensively described details of the various test methods, nor with equally well documented excretion patterns of many different antibiotics in milk; reference to two reviews will suffice (6, 18).

INDIRECT CONTROL

[DYE-MARKING OF ANTIBIOTIC PREPARATIONS]

Locally administered antibiotics infused into the udder are responsible for the vast majority of incidences of residues in milk at concentrations which, even after high dilution on bulking, may remain detectable. In areas where use of a marker dye is compulsory, the immediately visible color warns against use of that milk and prevents its addition to bulk milk. This is an advantage over direct control methods as these are generally not applied until herd yields have been bulked. Added dyes, usually Brilliant Blue FCF ("Food Blue 3") and Green S ("Food Green 4"), can still be observed visually at a concentration of about 0.1 ppm. Concentrations as low as 0.005 ppm can be detected using ion exchange resin methods.

With such dye marking, even if there is no "unit-by-unit" excretion of dye and antibiotic (2), more than 95% of all antibiotics administered in combination with a dye can be excluded from milk intended for consumption. Compulsory dye marking has, however, some attendant serious disadvantages.

(a) Although most investigators (3, 10, 14) have noted a reasonable correlation between the excretion rates of dyes and antibiotics, marked deviations have also been reported. This is especially true for certain antibiotic substances (9) and in cases where vehicles having a slow excretion rate are included in the formulation (4).

(b) As residues in the milk of animals treated with antibiotics given other than by the intramammary route, e.g. by subcutaneous, intramuscular, or intra-uterine administration, cannot be detected by an indirect control, direct techniques cannot be dispensed with.

(c) Addition of the dye in itself constitutes a residue problem and can lead to discoloration of milk products should the dye enter fat globules and remain undetected (7). In a number of countries where milk is considered to be a basic food which should be kept as pure as possible, residues of this kind are in principle not allowed (24).
(d) Use of dye markers will have the effect of increasing the cost of antibiotic preparations, not only on account of the cost of providing and incorporating the marker, but also due to mandatory requirement to evaluate the excretion rate of each individual substance. Also, as there is no international harmonization of regulations governing detection of residues, costs are further increased by the resultant requirement to develop preparations to satisfy various standards. This inhibits large-scale batch production of each preparation.

(e) With certain antibiotics addition of a dye will decrease the stability of the product and thus shorten the shelf-life (10,13).

(f) As a result of contamination of clothing and hands, dairy farmers find it unpleasant to work with dye-marked preparations especially as high concentrations (up to 250 mg per instillation) are frequently needed.

(g) One disadvantage, however, may be even more important than those previously discussed. Where dye marking and indirect controls are made obligatory, direct control will be less intensive or even ignored. This would open the way for abuse of control procedures by use of non-dyed preparations obtained by official and unofficial channels of distribution. Also the imposition of dye marking requirements constitutes ethical pressure on veterinarians (23) which however is misplaced in countries where veterinarians have little or no influence on the distribution of antibiotics. It is true that in areas where compulsory dye-marking applies, about 95% of antibiotics administered locally as dye-marked preparations can be excluded from milk for consumption. However, milk from one quarter treated with a non-colored preparation is sufficient to vitiate the control procedure of a tanklot of milk.

DIRECT CONTROL PROCEDURES

Preparations containing penicillin still account for 80-90% of the locally applied anti-mastitis treatments. Therefore, it is not only the intensity of testing but also the sensitivity of the method as regards penicillin that determines the impact of control on the occurrence of antibiotic residues in milk. This explains why, in recent years, public health authorities of many countries have changed to test methods which are much more sensitive for penicillin. Levels of detection have decreased from 0.05 IU/ml (Bacillus subtilis) through 0.02 - 0.01 IU/ml (Sarcina lutea and Streptococcus thermophilus) to 0.003 IU/ml (Bacillus stearothermophilus var. calidolactis). This last test organism is utilized in the assay procedure described by Galesloot and Hassing (7) and a modification of this method is accepted as the official test by the International Dairy Federation.

In addition to these methods, which can only be pursued at well equipped laboratories, industries have developed ready-for-use test kits based on S. thermophilus, such as the “Interest” (15), or on B. stearothermophilus var. calidolactis, as in the “Delvo-test-P” (20), “Enterotox” (16) and “Thermocult” (22). Several of these test meet many or all requirements; they are highly sensitive for penicillin, display satisfactory sensitivity for other antibiotics, are reliable and can be used practically in a rapid and economic fashion. B. stearothermophilus var. calidolactis has been accepted as the test microorganism of choice in most Western European countries and has recently been considered for acceptance in the USA (17).

The direct applicability of industrially-produced test kits makes it possible to check all incoming bulk milk. In addition individual supplier samples can be required and can be examined should the incoming bulk milk prove positive; this would enable suppliers of the positive portion of the bulk milk to be identified. Similarly, checks of farm milk are facilitated in that they can be carried out at more irregular intervals. Such test systems would permit appropriate measures to be taken more easily to prevent further incidents.

A major argument against direct control systems has always been that a risk exists that residues in bulk milk will be detected only after dilution with residue-free milk. However, experience has shown that initiation of direct control, based on a “sample-hold, bulktest, check-sample” procedure leads to a dramatic decrease in both the incidence and levels of antibiotic residues such that the total residue level of the bulked milk falls to levels generally far below acceptable or detectable levels. As a final check the finished dairy product can be tested.

DISCUSSION AND PRACTICAL EXPERIENCE

Compulsory dye-marking seemed a useful aid to control as long as no efficient direct test method existed. As indicated above, dye marking has a number of serious disadvantages. Thus with development of more sophisticated direct control systems, dye marking of antibiotic preparations seems to become obsolete.

Against direct control it has been argued, in particular by the dairy industry, that the necessary education of the pharmaceutical industry (which has to establish excretion rates) for this method, the veterinarians (who must instruct the farmer) and the farmer (who must carry out the instructions) would be too time-consuming, if not impossible. However, the major manufacturers of ethical veterinary preparations have done sufficient research on their products so that specific advice can be given to both veterinarians and farmers. Furthermore experience with some large cooperatives in various countries has shown that, using intensive checking with appropriate sanctions and combined with good instruction and technical support, the number of positive samples can be decreased markedly, even in a short period (19). Despite intensive control, the incidence of positive samples in, for example, The Netherlands and Denmark has stayed at around 0.2% for a number of years and it would seem that since such a level persists any further reduction is difficult to achieve. It is interesting to note that use of milking-cow preparations accounted for 63% of these
positive samples, dry-cow preparations for 9%, systemic therapy for 8% and penicillin test-channel treatments for 5% of this incidence according to a regional study in the Netherlands (18). According to another report, 45% of positive samples appeared to result from a lack of communication between the farmer and the milker (12). Thus it seems that to reduce the incidence of positive samples the need to clearly identify treated cows should be stressed in the information provided.

In addition to provision of appropriate information, good service facilities provided by the dairy industry itself can stimulate appreciation of a control system. Examination, at the request of the farmer, of suspect milk samples can prevent economic sanctions and unnecessary waste of milk. In a collaborative study of this aspect, a dairy industry was found to obtain highly reliable results using an industrial test carried out by dairy technicians (21). In this way results of locally performed tests can be made available to the farmer before delivery is made. Legal sanctions against the supplier of contaminated milk are generally based on identification of penicillin residues. Where such regulations are closely restricted to residues of penicillin only, a shift in favor of the use of other antibiotics may occur. This accounts for the need for more simple methods by which the residues of other antibiotics can be identified.

As well as being highly sensitive for antibiotics some modern methods are sufficiently selective to be unaffected by other, naturally occurring, substances present in milk and thus avoid “false-positive” reactions. In one of the previously mentioned test methods (Delvotest-P) interference by such substances is rare, whether in bulk or farm milk and irrespective of preheating of samples (20). Even lysozyme concentrations which have an inhibitory effect in the disc assay utilizing B. stearothermophilus var. calidolactis do not interfere with this test method (8).

Finally, although further harmonization of test methods used in various countries is undoubtedly necessary, B. stearothermophilus var. calidolactis seems to be the test microorganism of choice.

REFERENCES