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Depletion time of antibiotic residues in milk from Manchega dairy ewes

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SUMMARY – The use of pharmacological products, which may contaminate milk and affect its quality, is a standard practice to treat various sheep diseases. So far, little research has been done on depletion times of antibiotic residues as well as on the techniques to detect them in sheep milk. The aim of the study was to analyse the depletion time of an antibiotic solution injected in ewes to treat mastitis. Twelve Manchega ewes at the end of the lactation period (5th month) were injected with a colistine, spiramycin and dexametasone solution (Mames parenteral). Milk samples from a single daily milking (morning) were collected after 24 hours from the antibiotic treatment and for the following 14 days and analysed by the microbiological methods BRT and Delvotest . Our preliminary results indicated that depletion time of colistine and spiramycin in milk collected from treated ewes, determined by BRT and Delvotest methods, was 10 days, and it depended on the daily milk production of the ewes.

Key words: Dairy ewes, antibiotic residues, screening methods.

RESUME – *"Temps d'élimination de résidus antibiotiques dans le lait de brebis laitières de race Manchega". Dans le cas des brebis l'utilisation de produits pharmaceutiques, dont les résidus peuvent apparaître dans le lait et altérer sa qualité, est une pratique habituelle dans le traitement de différentes pathologies, mais les études réalisées sur l'élimination des produits utilisés et les méthodes de détection chez les brebis sont très peu abondantes. À ce propos, l'objectif de cette étude a été d'analyser le temps d'élimination d'une solution d'antibiotiques injectable utilisée dans le traitement des mammites chez les brebis en fin de lactation. Douze brebis de race Manchega en fin d'allaitement ont été utilisées. Celles-ci furent injectées avec une solution de colistine, spiramycine et dexametasone (Mames parenteral). Les échantillons de lait furent pris de la seule traite par animal réalisée le matin 24 heures après le traitement jusqu'au 14ème jour et ils ont été analysés par les méthodes microbiologiques BRT et Delvotest . Les résultats mettent en évidence que les cas positifs disparaissent le 10ème jour du traitement par les méthodes BRT et Delvotest et que le temps d'élimination de la solution dépend du niveau de production des animaux.*

Mots-clés : *Lait de brebis, résidus antibiotiques, temps d'attente, méthodes de détection.*

Introduction

The presence in raw milk of antimicrobial substances, such as antibiotic residues, may have serious toxicological and technical consequences (Moretain, 1986). The presence of chemical residues, particularly antibiotics, can delay (if not totally prevent) the bacteriological processes used in the manufacture of certain dairy products. It is therefore of fundamental importance to avoid the presence of these residues in raw milk in order to reduce problems during processing as well as to avoid their transmission to the consumers.

Treatment of various sheep diseases with pharmacological products is a standard practice. In many cases, antibiotic milk contamination is caused by an unnecessary treatment, done without veterinary prescription, or because of a limited knowledge about the dose, the way of administration and the depletion time of antibiotic solutions.

It has also to be mentioned that sometimes drug companies recommend withholding times too

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short to guarantee the elimination of antibiotic residues in milk, especially when the treatment is done at the end of lactation, when daily milk production is lower.

The detection of pharmacological residues derived from drugs used in the treatment of bovine mastitis has been poorly investigated (Moretain, 1981; Larocque and Neville, 1985, 1986), and even less data are available for goat (Zeng *et al*., 1996) and sheep milk (De Santis *et al.*, 1995).

The aim of this study was to analyse the time necessary to eliminate a drug mixture used in mastitis treatment of ewes at the end of the lactation period, using the Delvotest and BRT screening methods.

Material and methods

In this study milk samples from 12 Manchega ewes, at the end of their productive period, were analysed. Ten ml of Mames parenteral (125.000 UI colistine, 340.000 UI spiramycin and 0.125 mg Dexametason in 1 ml) were intramuscularly injected in two separated doses (5 ml) in 24 hour interval.

The 12 ewes were milked in the morning (once per day) and daily milk yield was recorded. Milk samples were collected after 24 hours from the drug treatment and during a period of 14 days. Milk samples were stored in disposable plastic tubes, to avoid external contamination, and were analysed with the BRT and Delvotest microbiological methods. Results were classified visually as either "negative", "doubtful" or "positive".

In order to test the effects of time from drugs treatment and of milk production on residues depletion time, measured by the BRT and Delvotest tests, the results were analysed using the multinomial logistic regression model (SAS , 1998). The following statistical model was used:

$$Y_{ijk} = \quad + T_i + P_j + \quad_{jjk}$$

where: Y_{ij} = the number of positive cases, = general mean, T_i = time of analysis (days), P_j = milk yield (ml/day) and $_{jjk}$ = residual error.

Results and discussion

Table 1 reports the absolute frequencies of visual classifications of the results obtained with the BRT and Delvotest methods, applied to milk samples, collected from 12 ewes treated with two intramuscular injection of antibiotic solution. The mean daily milk production of the 12 ewes was 228±114 ml.

Table 1. Absolute frequencies of visual classifications derived from BRT and Delvotest methods, used on milk obtained from 12 Manchega ewes, treated with antibiotic solution

Method	Result	Time after drug treatment (days)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
BRT	Negative	0	0	0	7	8	8	8	9	9	9	11	11	12	12
	Doubtful	0	0	4	4	3	3	3	3	3	3	1	1	0	0
	Positive	12	12	8	1	2	1	1	0	0	0	0	0	0	0
Delvotest	Negative	2	2	5	7	8	9	9	10	10	10	11	11	12	12
	Doubtful	0	3	3	1	1	2	2	1	1	1	0	0	0	0
	Positive	10	7	4	4	3	1	1	1	1	1	1	1	0	0

By applying the BRT test, the number of positive cases was high in the first three days after drug injections and fell down to 1 case after four days from drug treatment. However, it has to be noted that the number of doubtful cases was kept around 25% until 10 days after drug treatment.

On the other hand, the Delvotest seemed to be less sensitive in detecting colistine and spiramycin residues. The number of positives case in the first three days after the drug treatment was lower compared to the samples found to be positive with the BRT test.

Altogether these results suggest that, when considering only the positive cases, the depletion time of colistine and spiramycin residues in the sheep milk at the end of lactation is 4 days. However, when both positive and doubtful cases were taken into account, the depletion time increase up to 10 days.

Milk samples were also analysed for the presence of antibiotic residues on the basis of the ewe daily milk production. The statistical analysis, reported in Table 2, showed a clear high significant effect of milk yield on the depletion time of drug solution.

Table 2. The Chi-square values and significance levels of the factors which influence the visual classification of the BRT and Delvotest methods

Method	Factor	Chi-square (^{2})	P>chi-cuadrado
BRT	Time	64.196	0.0001
	Milk yield	28.502	0.0001
Delvotest	Time	48.432	0.0001
	Milk yield	15.823	0.0001

Finally, we attempted to make a prediction model for each of the screening methods we used for calculating the frequencies of positive and doubtful samples, taking into account the time from drug treatment (T) and the daily milk production (P):

BRT : $Y = 11.289\ X_1 + 13.784\ X_2 - 1.027\ T - 0.0307\ P$

Delvotest : $Y = 3.625\ X_1 + 13.784\ X_2 - 1.027\ T - 0.0307\ P$

where: X_1 = "1" to estimate the frequency of positive cases divided by the sum of the frequency of doubtful and negative cases; X_2 = "1" to estimate the sum of the frequency of positive and doubtful cases divided by the frequency of negative cases; T = time in days; P = milk production (ml/day).

When applied to our results (Figs 1 and 2), this mathematical prediction model highlights a clear effect of the milk yield on the number of positive cases, which is always higher for milk samples taken from ewes with a lower daily milk production.

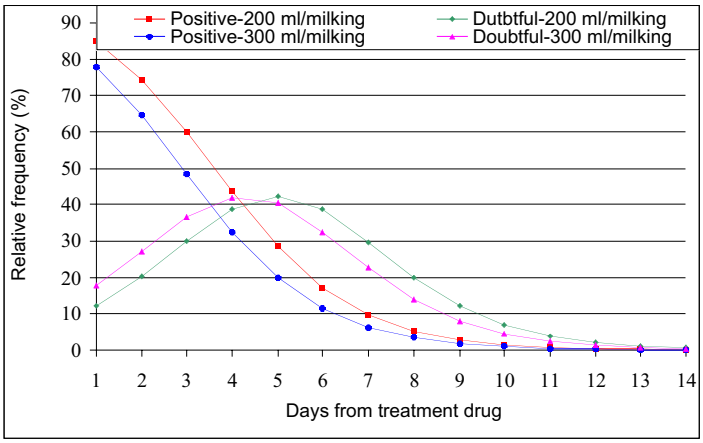

Fig. 1. Variation over time of the frequency of positive and doubtful cases resulting from the BRT .

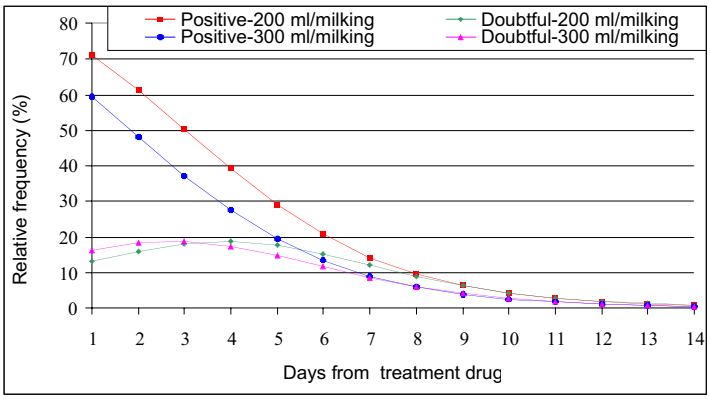

Fig. 2. Variation over time of the frequency of positive and doubtful cases resulting from the Delvotest .

Conclusions

A better knowledge on the depletion time of different drug products used in sheep production is quite important to limit residues which can affect the quality of the dairy products.

On the basis of our preliminary results, 10 days seems to be the time which guarantees the elimination of colistine and spiramycin residues in the milk of ewes treated for mastitis at the end of the lactation period (5th month). However, it has to be considered that the daily milk yield at the end of the lactation is often so low that it could be more convenient to dry off the ewes treated against mastitis so as to avoid the risk of finding high levels of antibiotic residues in the milk sent to the industries.

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