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Persistence of gentamicin residues in milk after the intramammary treatment of lactating cows for mastitis*

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Abstract: This study was designed to investigate persistence of gentamicin residues in milk after the intramammary treatment of lactating cows for mastitis. Milk samples were collected at a 1-d interval after the last administration from 34 individual cows that had received intramammary infusions of gentamicin. The doses and treatment times evaluated in this study represented those that have been applied by veterinarians in practice. The tetrazolium chloride assay was used to determine whether there were significant residues of the antibiotic in the samples. Persistence of detectable drug residues in milk from 33 cows (28 cows, ≤6 infusions at ≤0.7 g gentamicin; and 5 cows, 2 infusions at 0.8 g gentamicin) did not exceed 5 d; but 1 cow (5 infusions at 0.8 g gentamicin) had detectable residues in its milk for 9 d. Our results suggest that a 5-d milk withdrawal period might be insufficient to secure the clearance of the contamination of gentamicin, because treatment times and dosages contribute to the antibiotic clearance. A larger scale of samples are needed for further investigations.

Key words: Gentamicin, Mastitis, Intramammary infusion, Residue
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INTRODUCTION

Antibiotic residues in milk are of great concern to dairy farmers, milk processors, regulatory agencies, and consumers. The presence of antimicrobial drug residues in milk can provoke allergic reactions in some hypersensitive individuals (Dewdney *et al*., 1991; Dayan, 1993) and may induce resistant populations of bacteria that do not respond to treatments commonly used for human illnesses (Nijsten *et al*., 1996; van den Bogaard *et al*., 2001). Drug residues also alter the processing qualities of raw milk by inhibiting starter cultures used in the preparation of cheese and other fermented dairy products (Brady and Katz, 1988). Pasteurization and other forms of heat treatment eliminate pathogenic microorganisms but have limited or variable effects on drug residues (Moats, 1988).

In order to keep antibiotic residues out of the food chain of humans, regulatory authorities have established the withdrawal period for antibiotics that must be observed by producers before the milk from the treated cows can be sent to market. In China, the withdrawal period for all antibiotics in lactating cows is 5 d (Tan *et al*., 2007). Nevertheless, drug residues in marketed milk continue to occur (Wu *et al*., 2002; Deng *et al*., 2004; Bai *et al*., 2005; He and Wang, 2007). Other than the report by Tan *et al*.(2007), very little empirical work has been done in China to verify whether the present 5-d withdrawal period can be used as a reliable measure to prevent milk from being significantly contaminated with approved antibiotics, let alone with drugs that are not approved for use in dairy cows but commonly used on dairy farms.

Worldwide, mastitis is the most common disease in dairy cattle. The use of antibiotic therapy to treat and prevent udder infections in cows is a key

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component of mastitis control (Hillerton *et al*., 1999). However, intramammary infusion of antibiotics for mastitis therapy was cited as a major reason for milk contamination (McEwen *et al*., 1991; Wilson *et al*., 1998). Gentamicin, an aminoglycoside antibiotic known to be useful in the treatment of coliform and other gram-negative pathogens (Burrows *et al*., 1987), is extralabely applied for the treatment of mastitis by intramammary route on some Chinese farms. Although the withholding time for gentamicin after intramammary administration has not been officially recommended, farmers and veterinarians often implement milk discard time of 5 d, as mentioned above for approved drugs. This study was conducted to investigate if there were significant residues of this drug in milk over a 5-d withdrawal period after intramammary infusions.

MATERIALS AND METHODS

Animals

Thirty-four mastitic cows in a commercial herd in a suburb of Hangzhou City, China were used in this study. A case of clinical mastitis was defined as that a cow had one quarter that was enlarged or reddened and milk from this quarter was visibly abnormal. The selected cows were all lactating Holsteins in their 3rd~4th lactation cycles. They were milked thrice a day into individual buckets, at an 8-h interval (at 06:00, 14:00, 22:00), through the duration of the study. These cows varied considerably in their daily milk production at the time of this experiment (kg milk/d on the day of enrollment, mean=14.4, ranging 9.2~19.7).

Treatment and milk sampling

Commercial gentamicin sulfate infusion (80 mg/2 ml, with sodium carbonate and natrium bisulfurosum as adjuvant) was infused into the affected quarter of each cow once daily following morning milking, with a frequency from 2 to 6 infusions at a dose range of 0.3 to 0.8 g (Table 1). The doses and treatment times evaluated in this study represented those that have been applied by veterinarians in practice. The animals had not been treated with any antibiotic at least 30 d prior to gentamicin administration. Milk samples were taken from each whole milking from each cow at a 1-d interval, starting 24 h after the last dose of antibiotic and continuing until no gentamicin could be detected.

Assay

The gentamicin was detected by the tetrazolium chloride assay, a microbiological screening test approved in China for the evaluation of antibiotic residues in milk (Ministry of Health of the People's Republic of China, Standardization Administration of the People's Republic of China, 2003). The antibiotic-free milk was supplied by Hangzhou Meilijian Dairy Company. The tetrazolium chloride was purchased from Sinopharm Chemical Reagent Company (Beijing, China) and the standard gentamicin from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Milk samples (9 ml each) were heated in water at 80 °C for 5 min, cooled to 37 °C, and then 1 ml of culture of *Streptococcus thermophilus* diluted 1:1 (v/v) with skimmed milk was added to each sample. The samples were incubated in water at 36 °C for another 2 h, then 0.3 ml of 4% (v/v) tetrazolium chloride was added to each sample, and they were incubated again at 36 °C. The color change of each sample was viewed after 15 min; samples that turned red were considered as negative for antibiotic residues, and those that did not change color were considered as positive. Antibiotic-free milk was analyzed along with the milk samples to verify test accuracy. Preliminary experiments showed that the minimum limit of this assay for detection of standard gentamicin was 200 μg/kg milk, which meets the standard for the maximum residual concentration approved by the Ministry of Agriculture of China (Bureau of Husbandry and Veterinary, Ministry of Agriculture of the People's Republic of China, 2003).

RESULTS

The results are shown in Table 1. All the cows had milk with gentamicin residues in the first milking after their last treatment. The milk samples from the 2 cows treated 6 times at dose of 0.3 g were gentamicin-positive for 1 d after the last dose. Gentamicin persisted for 1~5 d in the milk samples from the 26 cows that had received 2 to 6 infusions at doses ranging

from 0.4 to 0.7 g. Among the 6 cows given 0.8 g gentamicin per dose, the 5 treated twice required 1 to 5 d for their milk to become antibiotic-negative; however, the one treated 5 times did not produce gentamicin-negative milk until 9 d after the last administration.

Table 1 Persistence of tetrazolium chloride (TTC)-positive milk following the intramammary infusion of gentamicin for mastitis therapy

Gentamicin (g/dose)	n_i	n_c	t (d)
0.3	6	2	1
0.4	2	4	1~4
	4	2	1~2
0.5	3	2	1
	4	4	1~5
	5	2	2~3
	6	8	1~5
0.6	5	1	3
0.7	6	3	1~5
0.8	2	5	1~5
	5	1	9

n_i: number of intramammary infusions; n_c: number of cows; t: period of persistence of TTC-positive in milk (days shortest to longest)

With regard to the longest days of detectable gentamicin residues in response to different therapy regimens, our results show that the persistence of residues in milk tended to be extended with increased treatment times at a given dose, with an exception of milk from the cows treated with 0.4 g gentamicin per dose. Similarly, cows received elevated doses of gentamicin at the same infusions seemed to have prolonged drug residues in milk.

DISCUSSION

Gentamicin is potentially ototoxic and nephrotoxic and is known to cause immune deficiencies leading to drug resistant bacteria in animals and humans (Ramsden *et al*., 1980; Frazier *et al*., 1988; Garg *et al*., 1991; Thibault *et al*., 1994; Weir and Mdzumdar, 1994). Therefore, its residues in animal-originated foods are of particular public concern. In many countries such as the US, the drug is not approved for use in dairy cattle and the extralabel use of this drug is not encouraged (Payne *et al*., 1999; Smith *et al*., 2005). In China, gentamicin has also been excluded from the approved drugs for dairy cows. However, extralabel use of this antibiotic is in fact very common. With the extensive use of gentamicin in dairy cows, some mastitis pathogens showed resistance to this drug (Wang *et al*., 2006). In order to improve its therapeutic effect on mastitis, many veterinarians often administer larger doses and more frequent treatments, a practice that is likely to increase the risk of drug residues in cow's milk.

In the present study, the periods for which the milk of individual cows contained detectable residues of gentamicin were wide variable. However, as reported in the study of Pedersoli *et al*.(1995), increased treatment times were prone to prolong milk residues when cows were treated at the same doses of gentamicin. This trend was particularly obvious in the cows that had received intramammary infusions of the antibiotic at doses of 0.5 and 0.8 g.

In addition to the treatment times, the dose of aminoglycoside antibiotics was also found to influence the elimination of drug residues in milk following intramamary infusions (Moretain and Boisseau, 1993). In the present study, increased doses tended to extend the persistence of drug residues as well. For example, the cows that were treated 5 times at doses of 0.5 g had gentamicin residues for 2~3 d. However, the cow receiving 5 treatments at a daily dose of 0.8 g required 9 d for its milk to become antibiotic-undetectable. Extended residue periods were also observed in milk from the cows treated 4 times as well as from those treated 6 times at increased doses of gentamicin.

In context, despite the fact that only 1 of 34 cows had detectable drug residues for more than 5 d, our results do not suggest that a 5-d withdrawal interval might be long enough to prevent milk from being contaminated with this antibiotic. Notably, although tetrazolium chloride assay may be reliable for testing drug residues in milk samples from individual mastitic cows (Tan *et al*., 2007), this microbial inhibitor test may be less sensitive or accurate than other tests such as liquid and gas chromatography (Schenck and Callery, 1998; Anderson *et al*., 1998; Popelka *et al*., 2003). It is thus reasonable to speculate that more milk samples would have been detected to have significant antibiotic residues after 5 d using more sensitive tests.

Overall, our results indicate that simply following a 5-d withdrawal period after extralabel use of gentamicin in lactating cows may not ensure the safety of milk products. To reduce the potential for significant antibiotic residues, an ideal method is to test milk samples from individual treated cows and then discard the milk with positive results. However, this would be impracticable on many farms in China. Therefore, veterinarians should be discouraged to use gentamicin in dairy cattle and be aware that there are other drugs to treat bovine mastitis that are less potential for residues.

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