Summary

Goat milk is primarily destined for the production of fermented products, in particular cheese. Therefore, the control of antibiotic residues in milk is of great importance, since these could have negative repercussions on technological properties of the milk as well as on the health of consumers.

In milk quality control programs, microbial inhibitor tests are widely applied to detect antibiotics during the screening stage. However, tests are non-specific and may be affected by substances other than antimicrobials which could inhibit the growth of the test micro-organism, causing false positive results.

The aim of this thesis was to evaluate the interference, related to the presence of different contaminants in goat milk, on the response of microbial inhibitor tests commonly used in Spain to detect antibiotics (BRT MRL, Delvotest SP-NT MCS and Eclipse 100 tests). The influence of the physicochemical characteristics of goat milk on the false positive outcomes in microbial screening tests was also investigated.

The suitability of microbial inhibitor tests for screening antibiotics in colostrum secretions was studied by analysing antibiotic-free colostrum and milk samples from forty-three Murciano-Granadina goats, collected every 12 hours during the first week post-partum. Microbial inhibitor tests were not suitable for the analysis of goat colostrum because they presented a high percentage of doubtful and positive results (up 37.2% in the 36 hours after partum).

To evaluate the effect of caprine colostrum on the microbial test response, antimicrobial-free goat milk spiked with different concentrations of colostrum was analysed to calculate the inhibitory concentrations producing 5% of positive results. The highest interferences were obtained for the addition of colostrum from 12 to 24 hours post-partum and the colostrum concentrations producing 5% positive results were between 5.1 and 34.6%. The BRT MRL was the test the most affected.

In another study, the interference of detergents and disinfectants used for the cleaning of milking equipment and milk storage tanks of dairy farms was investigated. Antimicrobial-free goat milk was spiked with eight concentrations of different cleaning products (5 acid, 5 alkaline, 5 domestic washing-up liquids, and 1 disinfectant) and analysed using microbial screening tests. The presence of acid detergent and disinfectant based on sodium hypochlorite in goat milk did not affect the microbial test response. However, alkaline detergents at concentrations $\geq$ 1 ml/l could lead to false positive results in microbial inhibitor tests (up to 16.7%) and from 4 ml/l on 100%

positive results were obtained. Regarding the products used for home use, and those used on farms and small size dairies, washing-up liquid containing sodium laureth sulphate and ethanol had the greatest effects on microbial inhibitor tests, even starting from a relatively low concentration (1 ml/l). On the other hand, the presence of a relatively low concentration of detergents in goat milk (0.5 ml/l) slightly modified the detection capability of the microbial inhibitor tests for amoxicillin, ampicillin, benzylpenicillin, and cloxacillin, although the detection of these drugs at MRL (safe level) was not compromised.

Antiparasitic agent residues in goat milk could be another possible cause of false positive results in microbial screening tests. An *in vitro* study to evaluate the effect of seven parasiticides commonly used in dairy goats was carried out. Further two studies, where albendazole and ivermectin were applied to two groups of dairy goats in lactation were performed. It should be noted that the parasiticide ivermectin is banned for the treatment of animals producing milk for human consumption, although its inclusion in this study was considered interesting to understand the potential effect of their residues in milk, in the event the practice was performed illegally.

In the *in vitro* study, raw antibiotic-free milk from goats was spiked individually with eight different concentrations of albendazole, closantel, diclazuril, febendazole, levamisole, diazinon, and ivermectin. The microbial inhibitor test results showed a great variability according to the test and the drug under study. Of the tests considered, the BRT MRL test was the most sensitive to antiparasitic agents, with the lowest concentrations of antiparasitic agent causing 5, 10, and 50% of positive results. Generally, closantel and diazinon were the antiparasitic agents that produced higher interferences in all tests, since low concentrations already resulted in positive results, while only higher concentrations of diclazuril and ivermectin showed an inhibitory effect.

To evaluate the effect of albendazole residues on the microbial inhibitor test response, eighteen healthy Murciano-Granadina goats in mid-lactation were treated with a single oral administration of the commercially available albendazole registered for dairy sheep (7.5 mg/kg b.w. of active compound) with a withdrawal period of 4 days for milk production in ovine. Albendazole and its metabolite residues in goat milk after under cascade treatment were not detected above MRL from the third day post-administration. However, a high occurrence of non-compliant results was obtained for the BRT MRL test during the first six days after treatment, suggesting that factors related to the albendazole application other than the drug concentration are able to affect the microbial inhibitor test response in some cases.

Regarding the ivermectin study, twenty-eight Murciano-Granadina goats infested with *Sarcoptes scabiei* var. *caprae* were treated with a subcutaneous injection of ivermectin (200 µg/kg b.w.), with a second dose applied seven days after the first treatment. Drug residues in goat milk were recorded during the first fifteen days of the experiment with concentrations ranging from 8.13 to 24.25 ng/ml. In addition, all the microbial screening tests seem to be affected by the ivermectin treatment, with BRT MRL the most affected (20%) compared with Delvotest SP-NT MCS and Eclipse 100 (6.6 and 5.7%, respectively). These positive results cannot be associated with the ivermectin concentration in goat milk, as the concentrations measured were lower than the inhibitory concentrations as reported in a previous *in vitro* study for these microbial tests. Thus, as suggested by some authors, interferences could be related to changes or alterations caused by the application of the parasiticide agent or by the parasitic disease itself, which could affect the immune response of the animals favouring the presence of inhibitory substances in milk.

The study of the effect of the goat milk composition on the specificity (rate of false positive results) of microbial inhibitor tests for screening antibiotics was also considered. Thus, individual goat milk samples (n=200) were analysed by microbial inhibitor tests using both visual and instrumental classification of the test results. The highest specificity values were obtained for the instrumental interpretation of the test results (94-99% *vs* 90-96%) due to the occurrence of samples with intermediate colorations (green-yellow, yellow-blue) making the visual classification more difficult and subjective. A relation was found between positive results in BRT MRL and Eclipse 100 tests and an elevated fat content in the goat milk. Positive outcomes in Eclipse 100 were associated with the butyric acid concentration in the milk. Further, the Delvotest SP-NT MCS test response was affected by elevated pH values, high lactoferrrin and myristoleic acid concentrations in the goat milk. This percentage of positive results could be minimized by a pre-treatment prior to microbial inhibitor test analysis, such as fat removal by centrifugation (3,100 g for 10 min at 4 ºC) and/or heating (80 ºC for 10 min).

Undoubtedly, improvements on the specificity of the microbial inhibitor tests for screening antibiotics in goat milk are desirable to avoid the destruction of milk compliant for human due to the occurrence of false positive results. The related financial losses affect farmers and dairies. However, it should be noted that the presence of contaminants in goat milk could be avoided by applying good farming practices designed to ensure that milk is obtained from healthy animals under proper hygienic conditions so ensuring the food safety of goat milk and related dairy products.