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Innovation for Sustainability in Sheep and Goats

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Performance of Eclipse Farm test coupled with e-Reader for screening antibiotics in sheep and goat's milk

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Abstract. The presence of antibiotic residues in milk is a concern due to technological and health reasons. Although various methods have been developed to analyse antibiotic residues in cow milk, such methods do not always work correctly with sheep and goat's milk. Herein, we present a study of the performance of a new system for the screening of antimicrobial residues in sheep and goat's milk. The method combines a microbial inhibitor test (Eclipse Farm) and a device (e-Reader) that integrates incubation at 65ºC and continuous monitoring of the change in colour. The performance of the new system was validated according to European Commission Decision 2002/657/EC. Sensitivity of the new system was evaluated on 12 molecules from several families of antimicrobials. The detection limits were close to European maximum residue limits (MRL). Detection capabilities (CCβ) were also determined for 7 molecules representing the main antimicrobial groups used in dairy husbandry (penicillins, cephalosporins, aminoglycosides, tetracyclins, sulphonamides and macrolides). Most of the molecules were detected at MRL level. The Eclipse Farm test coupled to e-Reader has shown to be a valuable tool for screening a broad-spectrum of antimicrobial residues in sheep and goat milk.

Keywords. Antimicrobial residues – Screening test – Sheep milk – Goat milk.

Evaluation de la performance du test Eclipse Farm 3G couplé avec le lecteur e-Reader pour le dépistage d'antimicrobiens dans le lait de brebis et de chèvre

Résumé. *La présence de résidus d'antimicrobiens dans le lait représente un problème technologique pour la filière laitière et un risque pour la santé publique. De nombreuses méthodes ont été développées pour analyser les résidus d'antibiotiques dans le lait de vache, cependant ces méthodes ne fonctionnent pas toujours correctement avec le lait de brebis et de chèvre. Dans ce travail, nous présentons une évaluation sur la performance d'un nouveau système de dépistage de résidus d'antibiotiques dans le lait de brebis et de chèvre. Le nouveau système combine un test d'inhibition microbienne (Eclipse Farm) et un dispositif (e-Reader) qui intègre une incubation à 65ºC et une surveillance continue du changement de la couleur du test. La performance du test a été validée conformément à la décision 2002/657/CE de la Commission Européenne. La sensibilité a été évaluée sur 12 molécules provenant de plusieurs familles d'antimicrobiens. Dans tous les cas, les limites de détection calculées étaient proches aux limites autorisées (Limite Maximale de Résidus). Les capacités de détection (CCβ) ont été également déterminées pour 7 molécules représentant les principaux groupes d'antimicrobiens utilisés dans l'élevage laitier (pénicillines, céphalosporines, aminoglycosides, tétracyclines, sulfamides et macrolides). La plupart des molécules ont été détectées au niveau de la LMR. L'Eclipse Farm combiné avec le e-Reader est donc un test à large spectre, qui permet de couvrir la détection d'un nombre important de molécules d'antimicrobiens dans le lait de brebis et de chèvre.*

Mots-clés. *Résidus des antimicrobiens – Test de dépistage – Lait de brebis – Lait de chèvre.*

I – Introduction

The use of antimicrobials in the context of preventive or curative treatments is a common practice in dairy animals. The administration of these drugs may lead to the presence of inadmissible levels of antimicrobial residues in milk. As a consequence, the presence of such residues in milk could cause interference with the manufacture of dairy products and some direct health implications. However, the selection of resistant bacteria is generally considered the main risk derived from the use of antimicrobials in farm animals. To protect consumers from the harmful effects of the antibiotic, the European Union established a legal framework setting of maximum residue limits (MRL).

Screening microbiological inhibition tests are most commonly used at the control first step since they are ready-to-use, cheap, easy to perform and have a broad spectrum of sensitivity. Commercial microbial tests in ampoule format are generally used in dairy farms. Moreover, a new portable device (e-Reader) has recently been validated for the automatic and objective detection of antibiotics in bovine raw milk in combination with the Eclipse Farm test (Mata *et al*., 2016).

In some Mediterranean countries, sheep and goat's milk production plays a prominent role and it is mainly destined for the elaboration of specific high quality cheeses. Thus, it is desirable to have analytical methods available to detect the most frequent drugs used in treatment in such species.

The aim of this work was to study the performance and applicability of the Eclipse Farm test in combination with the e-Reader for detection of antimicrobials in sheep and goat's raw milk.

II – Material and methods

1. Milk samples

To set the cut-off values of the system and to establish the preliminary limit of detection, ewe and goat's bulk milk samples were obtained from several milk control laboratories from different Spanish regions. All samples were previously tested with the Eclipse 100 (ZEULAB, Zaragoza, Spain) and TwinSensor (UNISENSOR, Wandre, Belgium) to confirm they were antibiotics-free. Samples were stored at 2–8°C and analysed for antimicrobials within 24 h after arrival. Milk composition, somatic cell counts (SCC) and bacterial count were analyzed by using MilkoScan 6000, Fossomatic 5000 and Bactoscan FC (Foss, Hillerød, Denmark). pH value was measured with a conventional pH-meter (Crison, Barcelona, Spain). Spiked samples with different antimicrobials were prepared as previously described by Mata *et al*. (2016).

2. Performance of Eclipse Farm Test and e-Reader

Eclipse Farm (ZEULAB, Zaragoza, Spain) is a microbial inhibitor tube test for the detection of antimicrobials in milk. The test procedure was carried out following manufacturer recommendations. Briefly, the test procedure includes addition of 100 µl of milk sample, a pre-incubation at room temperature for 1 h followed by a washing step and incubation in e-Reader.

According to Commission Decision 2002/657/EC, the false-positive rate that was calculated by analysing 250 ewe and 150 goat's individual milk samples. The determination of detection capabilities were performed as described in CRL guidelines for the validation of screening methods.

III – Results and discussion

1. Reading and interpretation of results

Cut-off level for Eclipse Farm coupled to e-Reader was initially set. For this purpose, 180 ewe and 123 goat tank raw milk samples were analysed (Fig. 1). Mean (42.6, 40.1) and standard deviation (7.8, 7.7) values were obtained for ewe and goat samples at the end point of the assays, respectively. Thus, a cut-off level of 63.4 and 63.2 was calculated (mean of the negative samples plus 3 times the standard deviation). For practical reasons, these figures were rounded to 65.

2. False-positive rate

Composition of ewe and goat milk presented a wide range of variation in quality. Fat ranged from 3.1 to 4.9% and from 3.1 to 8.3% in goat and ewe samples. Protein was 3.1 to 3.8 for goat and 4.8 to 7.1% for ewe samples. SCC (log) were between 1.2 and 3.8 for goat and 1.4 and 3.3 for ewe samples. Bacterial counts (log) reach to 2.7 and 2.4 for goat and ewe samples, respectively.

Very few or no false-positive results were found both for ewe (0.5%) and goat's (0%) milk samples. This result was coherent with those from other complementary tests (Eclipse 100 and TwinSensor).

3. Sensitivity and detection capabilities

Limits of detection in ewe and goat's milk for 12 antimicrobials are summarized in Table 1.

Table 1. Detection limits (µg L-1) of Eclipse Farm coupled to e-Reader in ewe and goat's raw milk

Antimicrobial	MRL	Detection limit[a]		e-Reader value[b]	
		Ewe	Goat	Ewe	Goat
Amoxicillin	4	4	4	77	111
Benzilpenicillin	4	3	3	132	144
Cephalexin	100	100	100	151	160
Cloxacillin	30	>30	30	68	101
Gentamycin	100	250	>250	78	68
Neomycin	1,500	600	600	91	82
Lincomycin	150	150	150	74	80
Tylosin	50	25	25	75	100
Sulfathiazole	100	80	80	130	121
Sulphametazine	100	100	100	106	101
Oxytetracycline	100	150	150	76	74
Tetracycline	100	200	150	91	93

[a] Positive results are defined as an e-Reader value higher than 65. [b] Mean value (n = 6).

Most of the antimicrobials showed detection limits at or below the MRL. In the case of the goat's milk, for benzylpenillin, neomycin, tylosin and sulfhiazole, the detection limits were lower than EU-MRL (Table 1), indicating a high sensitivity to detection of betalactams, amynoglicosides, macrolides and sulphonamides in this matrix. In this way, the detection of antibiotics in ewe's milk was also below their legal limits for the same substances, with the exception of cloxacillin. However, this test could neither detect gentamycin or tetracyclines at EU-MRL in both species.

Detection capabilities were determined for seven molecules that represent every family of antimicrobial included in this study. Results are showed for ewe (Table 2) and goat's milk (Table 3).

Table 2. Detection capability (µg/kg) of Eclipse Farm coupled to e-Reader in sheep milk

Antimicrobial	MRL	Detection capability[a]	No. positive[b]/no. samples	E-Reader value[b]
Amoxicillin	4	4	58/60	70.3 ± 9.2
Benzilpenicillin	4	4	59/60	90.1 ± 12.0
Cephalexin	100	100	60/60	140.1 ± 10.0
Gentamycin	100	250	20/20	83.8 ± 9.7
Oxytetracycline	100	300	20/20	100.4 ± 10.1
Sulfathiazole	100	80	40/40	103.9 ± 16.2
Tylosin	50	35	40/40	89.1 ± 14.1

[a] Positive results are defined as an e-Reader value higher than 65; [b] Mean ± SD.

Table 3. Detection capability (µg/kg) of Eclipse Farm coupled to e-Reader in goat's milk

Antimicrobial	MRL	Detection capability[a]	No. positive[b]/no. samples	E-Reader value[b]
Amoxicillin	4	4	60/60	106.6 ± 19.8
Benzilpenicillin	4	3	40/40	119.9 ± 13.6
Cephalexin	100	100	60/60	137.7 ± 14.5
Gentamycin	100	300	20/20	102.2 ± 10.0
Oxytetracycline	100	250	20/20	84.8 ± 8.7
Sulfathiazole	100	80	40/40	106.2 ± 8.1
Tylosin	50	35	40/40	87.4 ± 9.8

[a] Positive results are defined as an e-Reader value higher than 65; [b] Mean ± SD.

In the case of sheep milk, all betalactams included in the study were detected to levels equal to their EU-MRL, and for macrolides and sulphonamides the results were even lower than MRL. However, the detection of gentamycin and oxytetracycline was greater than the values of MRL.

For goat's milk, results were quite similar than for sheep. However, results were different to those obtained by Beltrán *et al.* (2015) using different microbial tests to detect betalactams and non-betalactams antibiotics in sheep and goat's milk. For sheep milk, they obtained CCβ lower than the calculated in this study with exception of gentamycin, whose CCβ was similar. On the contrary, in the case of goat's milk, both amoxicillin and gentamycin were also similar, but benzilpenicillin and cephalexin were below the results obtained. The CCβ of tylosin was higher than the obtained in this study. As in the present work, no microbial test used by these authors was able to detect oxytetracycline at MRL level. Comparing these results with those reported in cow milk (Mata *et al.,* 2016) by using Eclipse Farm coupled to e-Reader, detection capabilities were lower than the results of our work for all molecules studied, except for amoxicillin, whose CCβ was the same to LMR.

IV – Conclusions

The Eclipse Farm test coupled to e-Reader device presents a low percentage of false-positive for the detection of antibiotics in ewe and goat's milk and most of the detection capabilities were at or below the EU-MRL level. The great performance of the Eclipse Farm coupled to e-Reader test makes it suitable to be included in ewe and goat's milk quality programs on farms and dairies.

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Innovation for Sustainability in Sheep and Goats

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Sheep and goat farming systems face a harsh present and an uncertain future, apparently compromised by a general lack of competitiveness stemming from poor technical and economic results, but also due to severe social and environmental challenges. Innovative solutions are needed to make the sheep and goat value chain more efficient, profitable and sustainable, but also more appealing for society, particularly to guarantee generational turnover for farms. Such innovations should be aimed at improving production techniques, labour organisation, equipment and infrastructures and developing collective programmes for selection or health campaigns. Innovation should also contribute to strengthening social forms of organisation such as product quality schemes or communal areas management. Also, innovative feeding strategies coupled with precision flock management practices that reduce gaps in production and adjust to the environmental challenges, hold promise to tackle the above mentioned objectives.

This publication compiles 81 contributions presented at the **joint Seminar of the FAO-CIHEAM Sub-Networks on Production Systems and Nutrition on Sheep and Goats, held in Vitoria-Gasteiz, Spain, in October 2017.** The Seminar was co-organised between the Department of Animal Production of Neiker-Tecnalia (the Basque Institute for Agricultural Research and Development) and the Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM), with collaboration of the H2020 Project iSAGE (Innovation for Sustainable Sheep and Goat Production in Europe), the FAO, and support of the Department for Economic Development and Infrastructures of the Basque Government, the Municipality of Vitoria-Gasteiz, the Diputación Foral de Alava and the Idiazabal Denomination of Origin.

The articles are grouped into the four thematic sessions of the Seminar: (i) Innovation's conceptual and practical framework: application to the agro-food sector; (ii) Innovations to adapt sheep and goat feeding and production systems and industry to new societal demands; (iii) Precision farming and other technical innovations for increasing efficiency in sheep and goats; (iv) Success stories of innovations in the sheep and goat industry, with special focus on increasing consumption and adding value to products.

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