

*Original Research***Effectiveness of Delvotest *Bacillus stearothermophilus* var *calidolactis* in the Detection of Antibiotics Residues in Bovine Milk in Biskra Governorate, Algeria****Mammeri Adel ^{1*}, Kayoueche Fatima Zohra and Benmakhlof Abdelmalek**

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Rec. Date:	Jul 07, 2017 09:06
Accept Date:	Nov 11, 2017 16:17
Published Online:	April 25, 2018
DOI	10.5455/ijlr.20170707090625

Abstract

This study aimed to examine the efficacy of Delvotest to detect antibiotics in Biskra governorate. 34 samples of raw bovine milk were collected from the 4 quarters, while 3 samples were taken from a milk collection unit. The results revealed that 2/37 (5.40%) of the samples were clearly negative. Also, 35/37 (94.60%) of the samples showed a concentration lower than or equal to the limit of detection, while no sample was clearly positive. The high rate of doubtful samples (94.60%) is indicative of either an insufficiency of the detectability threshold of Delvotest for some (False positives), as well as inhibitors and antibacterial substances naturally produced in milk during clinical and/or subclinical mastitis, rather with infinitesimal quantities (giving false positives). It is essential to use advanced, efficient and more reliable technologies, in conjunction with the Delvotest, in order to ensure better safety of dairy products without sanctioning Algerian animal production, indiscriminately.

Key words: Antibiotics, Delvotest Sensitivity, Consumer Safety, Mastitis, Milk Inhibitors

How to cite: Adel, M., Zohra, K., & Abdelmalek, B. (2018). Effectiveness of Delvotest *Bacillus stearothermophilus* var *calidolactis* in the Detection of Antibiotics Residues in Bovine Milk in Biskra Governorate, Algeria. International Journal of Livestock Research, 8(4), 45-49. <http://dx.doi.org/10.5455/ijlr.20170707090625>

Introduction

Injudicious use of antibiotics in veterinary practice and non-compliance with specific waiting times would lead to the presence of residues that are harmful to the consumer and also compromise the industrial future of milk (Codex alimentarius, 2004; Mitchell, 2005; Stora, 2010). The quality of raw milk is a very important factor in its industrial valorisation, especially in terms of residues of antibiotics and inhibitors

(Vignola, 2010). On the other hand, among other things, mammary infections are a major scourge of dairy cattle breeding in Algeria (Ghazi and Niar, 2011; Boufaïda *et al.*, 2012).

For the consumer, antibiotic residues in milk can cause allergies, especially those of beta-lactams (Châtaigner and Stevens, 2005; Fabre *et al.*, 2006), in the form of urticaria, dermatoses, pruritus, shock or other forms (Federicci, 2000). Also, knowing that in the digestive tract live billions of saprophytic and commensal bacteria, especially anaerobic bacteria: bacteroides, fusobacterium (Belitz, 2009), consumption of products containing antibiotic residues, can disturb this flora by modifying its composition by selective inhibition (Abidi, 2004; Broutin, 2005).

In addition, resistant bacteria are potentially transmissible to humans via foodstuffs (Sanders, 2005). The development of this resistance may be linked to poor treatment practices (inadequate dosage, frequency of administration, non-compliance with the prescription) (Aghuin-Rogister *et al.*, 2001; Fabre *et al.*, 2006) or to the use of antibiotics as growth factors in the form of food additives (Châtaigner and Stevens, 2005). In food technology, residues are a real problem for dairy transformations because of their detrimental effects on lactic fermentations (Brouillet, 2002) and are the major problem of manufacturing accidents (Quellette, 2004), particularly in the manufacture of cheese, yogurt and other milk fermentation products (Broutin, 2005). The most common accidents are milk clotting defects, insufficient drainage and the uncontrolled proliferation of germs which are insensitive to antibiotics such as Coliformes, Bacillus, Clostridium, Aerobacter (Zinedine *et al.*, 2007). Thus, Thomas and Panes (1955 cited by Ryckaert, 2003) studied the manufacture of different cheeses with cow's milk and found that the presence of 0.04 to 0.15 IU of penicillin /ml cause a lower quality than controls with abnormal acidity, high humidity, spongy texture and sometimes bitter or sweet taste (Ryckaert, 2003).

Residues are, therefore, responsible for large financial losses that have repercussions throughout the dairy chain (Mitchell, 2005). The importance of this health and technological problem has incited us to carry out this study in cattle of Biskra governorate using the Delvotest Bacillus *stearothermophilus var calidolactis* which is a broad-spectrum test and is recommended in U.E countries.

Materials and Methods

This method makes possible to detect qualitatively higher levels of penicillin or other antibiotic residues in cow's raw milk. Delvotest is a broad spectrum microbiological test that detects the presence of antibiotics through a bacterial strain (*Bacillus stearothermophilus*) which is inhibited in the presence of an antibiotic in the milk tested. It can be used for dubious tank control before delivery for example. Delvotest SP NT MINI / 100 is in the form of ampoules which contain culture medium and *Bacillus stearothermophilus* spores. In the presence of milk which brings nutrients to the bacteria after incubation

(the optimum temperature of multiplication of this germ is 64 ° C) the bacteria can germinate and multiply. Only the presence of antibiotic residues in the milk prevents this multiplication.

The violet jelly is seeded with test organism spores (*Bacillus strearothermophilus var calidolactis*). These spores are stable for several months. They can germinate only if 0.1 ml of milk (without inhibitor) is added to the ampoule and after incubation of the ampoules for 3 hours at 64 ° C.

Sampling and Analyzes

A total of 37 samples of milk; 34 samples were taken from different herds distributed in 4 municipalities. While three samples were taken from a milk collection unit in Ourelal municipality (Table 1).

Table 1: Distribution of milk samples in the municipalities of Biskra governorate

Municipality	Tolga	Foughala	Leghrous	Bordj Ben Azzouz	Ourelal (collection unit)	Total
Number of samples	13	10	5	6	3	37

In order to not impair the reliability of the analyzes, there was no disinfection of teats prior to milk collection. Each sample of milk was instantly identified and stored at 4 ° C, protected from light until analyzed. Analyzes were carried out at the P.A.G.R laboratory (Animal Pathology and Reproductive Management) in El 'Khroub Veterinary Institute, University of Constantine1.

Results Interpretation

By observing the color changes of violet jelly-containing ampoules-

- a) A yellow color: negative test.
- b) A yellow / violet coloration: at the limit of detection.
- c) A violet coloration: positive test.

Results

Among 37 analyzed samples, 35 (94.59%) were found at the limit of detection, as they showed a yellow / violet coloration. While, 2 samples (5.40%) gave a negative test with a yellow coloration (Table 2).

Table 2: Results of the Delvotest in the municipalities of Biskra governorate

Commune	Negative	At the limit of detection	Positive
Tolga	0	13	0
Foughala	0	10	0
Bordj Ben Azzouz	0	5	0
Leghrous	0	6	0
Ourelal (unité de collecte)	2	1	0
Percentage	(2/37= 5.40 %)	(35/37=94.60 %)	0%

Discussion

The use of *Bacillus stearothermophilus var calidolactis* as a test microorganism is characterized by an antibiotic detection threshold closer to Maximal Residues Limits (MRLs) of antibiotics most commonly used in the treatment of dairy cattle, while retaining a broad spectrum of antibiotics such as: penicillins, chloramphenicol and tetracyclines. However, the Delvotest technique did not reveal any positive (0%) samples with violet coloration from a total of 37 samples in the various municipalities of the Biskra governorate. Samples showing a yellow/violet coloration which indicates the limit of detection and the presence of antibacterial substances at a concentration equal to or above the detection threshold remain doubtful. It should be pointed out that disinfection by antiseptics, of utensils and milking machine sleeves, or even milk collection and refrigeration tanks, could give a false positive result during the Delvotest (Hunter, 2006). On the other hand, inhibitors and antibacterial substances, naturally produced in milk during clinical and subclinical mastitis, would also give false positives during Delvotest (Vignola, 2010). Thus, to detect antibiotics, microbiological tests have the advantage of having a broad spectrum, nevertheless they have disadvantages such as the lack of sensitivity to certain antibiotics and the possible sensitivity to natural inhibitors (Fabre *et al.*, 2002).

It is evident that the Delvotest *Bacillus stearothermophilus var calidolactis* is not sufficiently reliable either to protect the consumer or to guarantee a prosperous industrial future of milk. In this context, Aghuin-Rogister (2005) recommends that screening tests should address the following criteria: sufficient sensitivity, no false negatives and few false positives. Abidi (2004) and Belitz (2009) suggest combining Delvotest with enzyme immunoassays such as; Delvo x Press; Penzym Test; Snap Test and ELISA.

Conclusion

The use of Delvotest *Bacillus stearothermophilus var calidolactis* lacks practical reliability. Under such circumstances, the possible risks of antibiotics - especially beta-lactam antibiotics - on consumers, and whose toxicological status (MRLs) remains unknown in foods of animal origin, cannot be ruled out in Biskra governorate. Thus, the detection of antibiotic residues in milk should be based on the use of more accurate, reliable and rapid methods such as enzyme immunoassays. However, their relatively prohibitive cost, increasingly requires, that they be taken care of by the Algerian authorities. Pharmacovigilance networks should therefore be reactivated throughout the national territory, especially as we are currently seeing an increasingly abusive and anarchic use of antibiotics in veterinary practice. This is mainly due to non-compliance with the specific withdrawal period for each drug and therapeutic doses, also the lack of control over authorized MRLs in foodstuffs of animal origin.

Therefore, the reliability of methods of detecting antibiotic residues in milk must be a concern of the competent authorities, in order to not disturb national animal production indiscriminately, nor to abuse the health of consumers.

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