Research Note

Incidence of False-Positive Results for Assays Used To Detect Antibiotics in Milk

LISA W. HALBERT,1 RONALD J. ERSKINE,1,* PAUL C. BARTLETT,1 and GILBERT L. JOHNSON II2

1Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824; and 2Michigan Milk Producers Association, Novi, Michigan 48376-8002, USA

(MS# 95-129: Received 7 June 1995/Accepted 20 December 1995)

ABSTRACT

The incidence of false-positive results from milk assays for antimicrobial agents was determined for composite milk samples collected from 407 lactating dairy cows with a history of no antibiotic treatment for a minimum of 30 days. Milk samples were also cultured for bacteria and analyzed for somatic cell count. Mean herd prevalence of intramammary infections (±SEM) caused by Streptococcus agalactiae and Staphylococcus aureus was 3.3 ± 2.8 and 20.2 ± 9.5% of lactating cows, respectively.

All 407 milk samples were assayed for antibiotics with three commercial tests; a fourth test was used to assay 391 samples. Samples were assayed twice with each test, and if the results from these repetitions did not agree, a third assay was performed and the result obtained in two of the three repetitions was reported. Because samples were only collected from cows with no antimicrobial treatment for a minimum of 30 days, positive assays were considered to be false-positive results. Three test kits did not yield any false-positive results, one test kit had 5 false-positive results of 407 samples collected (specificity, 98.8%). Although there was a trend for false-positive samples to have a higher somatic cell count than negative samples, the low incidence of false-positive results did not allow a meaningful comparison.

We conclude that the incidence of false-positive results is very low when testing milk from cows that have a history of no clinical mastitis or antimicrobial treatment.

Key words: Milk, antibiotics, assays, false-positive results

Antimicrobial drug residues in milk have become a consumer concern (5, 6), and as dairy producers comply with guidelines set out by dairy quality assurance programs, questions have arisen regarding the reliability of antibiotic residue testing kits (2, 4). Several commercial kits are available for screening various antimicrobial agents in milk; however, they were developed for assay of bulk tank milk. When applied to milk samples from individual cows, false-positive test results have been reported with pretreat-

* Author for correspondence.
with 7% sheep blood. Colonies identified as gram-positive cocci that were catalase positive were then submitted to tube coagulase testing. Isolates that were coagulase positive after 24 h were presumptively identified as \textit{S. aureus}. To isolate \textit{S. agalactiae} from milk samples, an additional 20 µl of each milk sample was plated on a plate containing tryptic soy agar with 7% sheep blood, with thallium acetate and crystal violet as selective reagents and β-hemolysin and esculin as diagnostic reagents. Colonies that were esculin-negative and camp-positive were diagnosed presumptively as \textit{S. agalactiae}. A representative number of isolates from each herd was confirmed as \textit{S. agalactiae} using a commercial latex agglutination test (Rapid Mastitis Test, Immucell, Portland, ME).

Milk samples were assayed for antimicrobials with four commercial tests; test A measured the presence of inhibitors by inhibition of growth of \textit{Bacillus stearothermophilus} var. \textit{calidolactis} (Delvo Mini-SP, Gist Brocades Food Ingredients Inc, King of Prussia, PA), test B relied on enzyme-linked immunosorbent assay ELISA technology (Delvo Express, Gist Brocades Food Ingredients Inc, King of Prussia, PA) and tests C (Snap β-lactam, IDEXX Corp, Portland, ME) and D (Penzyme, SmithKline Beecham Animal Health, Exton, PA) were based on the interaction of competitive binding of antibiotic to an enzyme-linked receptor. Due to a lack of availability of test kit C, only 62 of 78 samples collected from one of the five herds were assayed with this test. Thus a total of 407 assays were performed with tests A, B, and D, and 391 assayed with test C. Samples were assayed twice and if the results (positive or negative) did not agree, a third repetition was performed, and the result obtained in two of the three repetitions was reported. All positive results were considered to be false positives because of the history of no previous antibiotic treatment.

**RESULTS**

Mean prevalence (±SEM) of cows with intramammary infections caused by \textit{Streptococcus agalactiae} and \textit{Staphylococcus aureus} were 3.3% ± 2.8% and 20.2% ± 9.5%, respectively. Three test kits (A, B, and C) did not have any false-positive results recorded, test kit D (Penzyme) had five false-positive results of 407 samples collected, for a specificity of 98.8%. The mean log SCC of the 5 samples with false-positive results (5.59 ± 0.49 cells per ml) was similar to that of the samples with negative results (5.02 ± 0.3 cell per ml). The non-normal distribution of SCC and the existence of only 5 false-positive samples precluded meaningful statistical analysis. The distribution of log SCC obtained for all samples was plotted (Figure 1). No significant correlation between cow infection status and antimicrobial test result was determined by Fisher’s exact test.

**DISCUSSION**

The overall incidence of false-positive results in this trial was very low. This is in disagreement with several previous studies that have determined that false-positive results can be a common occurrence when antimicrobial residue tests are conducted on milk (8–11). However, previous reports have found false-positive results to be associated with milk from cows with a recent history of clinical mastitis (1, 3, 4, 7, 10, 11). Due to the high degree of inflammation that is present in a mammary gland displaying clinical signs of infection, it is reasonable to assume that false-positive results could occur as a result of endogenous immune modulators (1, 3, 11). This is especially true of tests that rely on bacterial inhibition or nonspecific binding to antibacterial substances to detect antimicrobial drug residues (1, 2, 8, 10). Our survey suggests that milk from nonmastitic cows is unlikely to yield false-positive results when tested for antimicrobial residues. However, our study found no relationship between SCC and false-positive results. This is in disagreement with a previous report that suggested that the probability of false-positive results increases as SCC increases (8).

We conclude that the incidence of false-positive results is very low in tests of milk from cows that have a history of no clinical mastitis or antimicrobial treatment, and that the relationship between SCC and the incidence of false antimicrobial positive results requires further research.

**ACKNOWLEDGMENTS**

The authors express their sincere gratitude to Dave Brady, Steve Lehman, and Mike Marvin for sample collection and maintenance of treatment records.

**REFERENCES**