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Specificity of assays used by regulatory agencies to detect antibiotic residues in tissues of culled dairy cows.

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OBJECTIVE: To determine percentage of false-positive test results for assays used by regulatory agencies to detect antibiotic residues in tissues. **DESIGN:** Prospective study. **ANIMALS:** 426 dairy cows. **PROCEDURE:** Dairy cows scheduled for culling that were identified as being unlikely to have antibiotic residues in tissues on the basis of strict inclusion criteria were used. A sample of kidney obtained from each cow at slaughter was tested on-site, using the swab test on premises (STOP; 97 samples) or the fast antibiotic screening test (FAST; 329 samples). Frozen samples (n = 1,278) of liver, muscle, and kidney were thawed and retested at a federal laboratory, using the same screening assays. Kidney and liver samples (n = 852) were also tested using the 7-plate bioassay confirmation test used for confirmation and identification of antibiotic residues. **RESULTS:** Results of screening assays performed onsite were negative. When frozen samples were retested, 20 (12 liver, 7 kidney, and 1 muscle) had positive FAST results, but none had positive STOP results. Of the samples tested with the 7-plate bioassay confirmation test, 4 liver samples had results indicating a tetracycline (n = 3) or an unidentified microbial inhibitor (1) as a residue. **CLINICAL IMPLICATIONS:** Results suggest it is unlikely that regulatory action will be taken against producers sending untreated cattle to market. However, because results of the FAST and 7-plate bioassay confirmation test were positive when applied to frozen tissue, use of assays based on microbial inhibition may not be valid for confirmation of residues.

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An evaluation of the tissue expression of a bovine dodecapeptide batenecin in the adult and fetal animal.

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Various antimicrobial cationic peptides have been isolated from the granules of bovine neutrophils. One of these peptides is only 12 amino acids in length and is called a dodecapeptide batenecin. A monoclonal antibody to the synthetic bovine dodecapeptide batenecin was produced, characterised and used in an immunofluorescent assay to assess the peptide's expression. When adult bovine peripheral blood and both adult and fetal bone marrow, tracheal lining, liver, spleen and intestinal tissues were examined, the peptide was found only within neutrophils and cells within the bone marrow. These findings both confirm and supplement

another report in the literature (Storici et al. 1992), however this is the first report of a bovine antimicrobial peptide detected within the bone marrow of fetal mammals. Our results also suggest the use of immuno- fluorescence assays to be especially well suited for the quick screening of antimicrobial peptide tissue expression.

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Cloning and expression of bovine neutrophil beta-defensins. Biosynthetic profile during neutrophilic maturation and localization of mature peptide to novel cytoplasmic dense granules.

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beta-Defensins are microbicidal peptides implicated in host defense functions of phagocytic leukocytes and certain surface epithelial cells. Here we investigated the genetic structures and cellular expression of BNBD-4, -12, and -13, three prototypic bovine neutrophil beta-defensins. Characterization of the corresponding cDNAs indicated that BNBD-4 (41 residues) derives from a 63-amino acid prepropeptide and that BNBD-12 (38 residues) and BNBD-13 (42 residues) derive from a common 60-amino acid precursor (BNBD-12/13). The peptides were found to be encoded by two-exon genes that are closely related to bovine epithelial beta-defensin genes. BNBD-4 and BNBD-12/13 mRNAs were most abundant in bone marrow, but were expressed differentially in certain non-myeloid tissues. In situ hybridization and immunohistochemical studies demonstrated that BNBD-4 synthesis is completed early in myelopoiesis. BNBD-12 was localized exclusively to the novel dense granules, organelles that also contain precursors of cathelicidins, antimicrobial peptides that undergo proteolytic processing during phagocytosis. In contrast to cathelicidins, Western blot analyses revealed that mature beta-defensins are the predominant organellar form in myeloid cells. Stimulation of neutrophils with phorbol myristate acetate induced secretion of BNBD-12, indicating that it is co-secreted with pro-cathelicidins. The exocytosis of BNBD-12 by activated neutrophils reveals different mobilization pathways for myeloid alpha- and beta-defensins.

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Evaluation of the Delvo-X-Press Assay for Detecting Antibiotic Residues in Milk Samples from Individual Cows

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Performance of the Delvo-X-Press -lactam antibiotic assay was examined using bulk-tank milk samples and milk samples from individual cows. Bulk-tank milk samples fortified with bovine lactoferrin at a concentration of 1 mg/ml or more consistently tested positive. False-positive results were also obtained from bulk-tank milk samples fortified with bovine plasma at concentrations of 20 and 40%. The assay yielded positive results for milk with antibiotic concentrations as low as 2 ppb. Individual milk samples were collected from 144 healthy lactating cows and from 34 cows with chronic *Staphylococcus aureus* mastitis. Specificity estimates for samples from healthy and mastitic cows were 0.88 (95% confidence interval [CI], 0.82, 0.93) and 0.94 (95% CI, 0.86, 1.00), respectively. Individual milk samples were collected from three cows with experimentally induced mastitis for 21 consecutive days. False-positive results occurred as late as 12 days postchallenge. A moderate but significant ($P < 0.01$) positive linear correlation ($r = 0.61$) was observed between test result and somatic cell count (SCC) values in milk samples with SCCs of $>106/\text{ml}$.
