Seroprevalence of *Mycobacterium avium* subsp *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity

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**Objective**—To estimate seroprevalence of *Mycobacterium avium* subsp *paratuberculosis* (MAP) infection among adult dairy cows in Colorado and determine herd-level factors associated with the risk that individual cows would be seropositive.

**Design**—Cross-sectional observational study.

**Animals**—10,280 adult (≥ 2 years old) dairy cows in 15 herds in Colorado.

**Procedure**—Serum samples were tested with a commercial ELISA. A herd was considered to be infected with MAP if results of mycobacterial culture of ≥ 1 individual cow fecal sample were positive or if ≥ 1 culled cow had histologic evidence of MAP infection.

**Results**—424 of the 10,280 (4.12%) cows were seropositive. Within-herd prevalence of seropositive cows ranged from 0% to 782% (mean, 2.6%). Infection was confirmed in 11 dairies. Cows in herds that had imported ≥ 8% of their current herd size annually during the preceding 5 years were 3.28 times as likely to be seropositive as were cows in herds that imported < 8%. Cows in herds with ≥ 600 lactating cows were 3.12 times as likely to be seropositive as were cows in herds with < 600 lactating cows. Cows in herds with a history of clinical signs of MAP infection were 2.27 times as likely to be seropositive as were cows in herds without clinical signs.

**Conclusions and Clinical Relevance**—Annual importation rate, herd size, and whether cows in the herd had clinical signs typical of MAP infection were associated with the risk that individual cows would be seropositive for MAP infection. (J Am Vet Med Assoc 2004;225:97–101)

In cattle, infection with *Mycobacterium avium* subsp *paratuberculosis* (MAP), commonly known as Johne’s disease, has been associated with substantial economic losses as a result of decreased milk production, decreased value of culled cows, increased time to pregnancy, and increased replacement costs. Estimates of the prevalence of MAP infection vary depending on the methods used to select cattle for testing and to verify infection (eg, detection of serum antibodies vs culture of the organism). For herds in the Midwest and Eastern United States, reported within-herd prevalences of MAP infection, determined by means of an ELISA, range from 6.9% to 17.1%. During the past 2 decades, the dairy cattle population in the Midwest and Eastern United States has been decreasing, while the population in the Western United States has been increasing, as dairies have been purchasing cattle from various sources in the United States. During 2001, the dairy cow population in the Western United States increased by 72,000 head, while the dairy cow population in the rest of the country decreased by 163,000 head.

Purchase of MAP-infected cattle is the most common method by which the organism is introduced into susceptible herds and has been documented as the cause of various outbreaks of MAP infection. Large dairies in the Western United States now represent a composite of cattle from Midwest and Eastern US dairies as a result of herd dispersal sales from these regions. The National Animal Health Monitoring System study conducted in 1996 reported that 44% of dairies brought cattle into their herd during 1995 and that 65.5% of dairies consisting of ≥ 200 milk cows purchased cattle from an outside source. However, only 9% of all dairy producers that purchased replacement animals in 1995 required testing of source herds for MAP infection.

The National Animal Health Monitoring System study estimated that in 23% to 40% of herds in the Western United States, ≥ 10% of animals were infected with MAP. However, this was 1 of only 2 studies to date that have reported data on prevalence of MAP infection among dairy cattle in the Western United States and was designed to detect a predefined level of disease, not to establish within-herd prevalence. In addition, Colorado was not included in this study, and statewide testing for MAP infection has not previously been undertaken. The purpose of the study reported here, therefore, was to estimate prevalence of MAP infection among adult dairy cattle in Colorado by means of serologic testing with a commercially available ELISA and to determine herd-level factors associated with the risk that individual cows would be seropositive. Herd-level factors that were evaluated were herd risk factors.
size, rate of introduction of new cattle (importation rate), and whether cows in the herd had clinical signs typical of MAP infection. Mycobacterial culture of fecal samples and necropsy examination of seropositive cattle were used to document MAP infection in the herds.

Materials and Methods

Herds—For 8 months prior to initiation of the study, educational seminars on MAP infection were offered to dairy producers by Colorado State University faculty. Approximately 30% of Colorado dairy producers (70 of the estimated 220 dairy producers in Colorado during 1999 and 2000) attended these seminars. Attendees were asked to volunteer to participate in the study, and 15 Colorado dairies were eventually enrolled in the study.

For each dairy enrolled in the study, blood samples were collected from all cattle ≥ 2 years old, except that blood samples were not collected from 2-year-old cattle that had not yet given birth to a calf. Blood samples were collected from the tail vein into 10-mL evacuated tubes with 1-inch, 18-gauge needles. For 13 of the 15 herds, all blood samples were collected over a 1- or 2-day period. For the remaining 2 herds, which were the largest herds, blood samples were collected over a 3-day period. Blood samples were collected during 1999 and 2000; details of the sampling procedure have been described.

Serologic testing—Blood samples were centrifuged, and serum was separated on the day of blood collection. Serum samples were tested with a commercially available ELISA for antibodies against MAP according to the manufacturer's protocol, except that samples were not run in duplicate to reduce cost to the dairy producers. All samples were tested in a single laboratory by 1 individual.

Results for the ELISA were expressed as the optical density for the test sample divided by the optical density for a positive control sample (test sample to positive-control sample [S:P] ratio). Samples with extremely high (≥ 2.0) S:P ratios were retested to verify results. The manufacturer of the ELISA recommends that an S:P ratio ≥ 0.25 be classified as a positive result and an S:P ratio < 0.25 be classified as a negative result. For purposes of the present study, an S:P ratio ≤ 0.09 was classified as a negative result, an S:P ratio ≥ 0.25 but ≤ 0.29 was classified as a suspect result, and an S:P ratio ≥ 0.30 was classified as a positive result. These cutoffs were selected on the basis of results of a previous study in which cows with S:P ratios in the suspect range were more likely to be infected with MAP than cows with S:P ratios in the negative range.

Mycobacterial culture of fecal samples—After results of serologic testing were available, participating producers were encouraged to submit fecal samples from cows with positive or suspect ELISA results for mycobacterial culture. The number of cows in each herd from which fecal samples were collected varied depending on economics and producer-perceived value of mycobacterial culture of feces.

A modified National Veterinary Services Laboratory procedure was used for mycobacterial culture of feces. Briefly, 1 g of feces was mixed with 35 mL of hexadecylpyridinium chloride. This mixture was allowed to settle for 3 hours, and the supernatant was drawn from approximately 2 cm above the sediment and allowed to stand overnight. Approximately 0.1 mL of the resulting sediment was directly inoculated onto 1 tube each of Herrold's egg yolk medium (HEYM) with mycobactin J, HEYM with mycobactin J and sodium pyruvate, and HEYM without additives.

Cultures were held at 35°C for 16 weeks before a final determination of results was made. Cultures were examined every other week for the first 8 weeks for signs of gross contamination, and contaminated samples were recultured. For samples with positive culture results, results were reported as number of colonies per tube of HEYM. For samples with negative culture results, results were reported as no growth of MAP. For samples classified as contaminated that were still contaminated after a single attempt at reculture, results were reported as contaminated.

Necropsy of cows with clinical disease—Participating producers were asked to submit cows culled with clinical signs of MAP infection to the Colorado State University Veterinary Diagnostic Laboratory for necropsy and histologic examination of tissues to confirm the diagnosis of MAP infection. In addition, a few seropositive cows that did not have clinical signs of disease but were being removed from the herd for other reasons were purchased and submitted for necropsy and histologic examination. Blood and feces were obtained from the cows prior to euthanasia and tested by means of the ELISA and mycobacterial culture, respectively. Cows were confirmed to be infected with MAP if they had histologic evidence of infection or if results of mycobacterial culture of the fecal sample collected prior to euthanasia were positive.

Collection of herd management data—Specific herd management factors were targeted as risk factors on the basis of clinical impressions of the authors. Factors that were examined included herd size, importation rate, whether cows in the herd had clinical signs typical of MAP infection, and culling rate.

The 5-year importation rate was calculated by dividing the number of cattle ≥ 20 months old imported to the dairy from an outside source during the 3 years preceding whole-herd serologic testing by the number of adult cattle (ie, cattle ≥ 2 years old that had calved at least once) in the herd at the time of testing. The 5-year importation rate was calculated because it was thought to give a better measurement of herd status than a single year of importation data. Theoretically, if a dairy producer had bought 20-month-old infected heifers 5 years earlier, he or she could have expected to see clinical disease among a subset of those cows during this 5-year period, with dairies that imported more animals more likely to have cows with clinical signs of MAP infection than dairies that imported fewer animals. To provide some estimate of the annual importation rate, the 5-year importation rate for each herd was divided by 5. This was not a true annual rate because the only value for herd size used in this calculation was herd size at the time of serologic testing in 1999 and 2000.

To determine whether any cows in the herd had had clinical signs typical of MAP infection, dairy owners and managers were asked to recall whether they had noticed cows with such signs during the preceding 5 years. Specific clinical signs included a combination of chronic, unresponsive, normal-colored diarrhea; weight loss; and decreased milk production with maintenance of appetite and absence of fever. If dairy personnel had recorded or could recall having observed cows with these clinical signs during the 5 years prior to whole-herd testing, the dairy was classified as having cows with clinical signs of MAP infection. If dairy personnel did not record or recall seeing cows with clinical signs of MAP infection during this period, the dairy was classified as not having cows with clinical signs of MAP infection.

The culling rate was taken from the Dairy Herd Improvement Association herd summary sheet on the month of whole-herd serologic testing (ie, percentage of cows leaving the herd for any reason). Three herds were not participating in the Dairy Herd Improvement Association, so dairy production records were used to calculate the percentage of cows leaving the herd for any reason during the 12 months prior to whole-herd serologic test. Calculation of the culling rate did not include cattle that died on the dairy; thus, culling rate...
may not have been valid because of variations in culling decisions between farms. For this reason, mean culling rates were calculated but not used in any analyses.

Data analysis—Results of mycobacterial culture of fecal samples and necropsy of culled cows were used to classify herds as infected or not infected with MAP. A herd was considered to be infected with MAP if results of mycobacterial culture of ≥1 fecal sample were positive or if ≥1 culled cow had histologic evidence of MAP infection.

Descriptive statistics were calculated for all data. For continuous variables, the Shapiro-Wilk test was used to determine whether values were normally distributed. Within-herd estimates of prevalence of positive and suspect ELISA results were calculated by dividing the number of cows with positive or suspect results by the number of cows tested. Pearson correlation coefficients were calculated for pairwise comparisons of herd size, prevalence of seropositive cattle, and annual importation rate.

Logistic regression was used to determine whether herd size and annual importation rate were associated with serologic status (ie, seropositive vs not seropositive) or whether cows in the herd had clinical signs of MAP infection (yes vs no). Cows with suspect ELISA results were grouped with seronegative cows for this analysis. To simplify these analyses, annual importation rate (<8% vs ≥8%) and herd size (<600 vs ≥600 lactating cows in herd) were dichotomized on the basis of the median importation rate for this data set (7.8%) and mean herd size in Colorado (600 cows) at the time of the study. Generalized estimating equations were used to control for the lack of independence (clustering) among outcomes and exposures within any herd. Odds ratios and 95% likelihood-ratio-based confidence intervals (CIs) were obtained from the outcomes of logistic regression models.

Results

Seroprevalence of MAP infection—During the study period, blood samples from 10,280 cows were submitted for serologic testing. This represented approximately 12.3% of all adult dairy cattle in the state of Colorado at the time of the study. Overall, results of the ELISA were positive for 424 of the 10,280 (4.12%) cows that were tested (Table 1). Mean ± SD within-herd prevalence of seropositive cows, as determined by use of the commercial ELISA, was 2.6 ± 1.94% (range, 0% to 7.82%). For 8 of the 15 dairies, <2.0% of cows were seropositive. Mean within-herd prevalence of cows with suspect ELISA results was 6.5 ± 3.49% (range, 2.9% to 14.7%).

Herd-level factors—There were positive correlations between herd size and annual importation rate (r = 0.91), between importation rate and prevalence of seropositive cattle (r = 0.87), and between herd size and prevalence of seropositive cattle (r = 0.82). Neither herd size nor importation rate was significantly associated with percentage of seropositive cattle in a multivariate logistic regression model.

Mean ± SD annual importation rate was 7.4 ± 7.36% (range, 0% to 27.2%). Three herds did not import cattle into the herd during the 5 years prior to whole-herd serologic testing. Cows in herds that had imported ≥8% of their current herd size annually during the preceding 5 years were 3.28 times as likely (95% CI, 2.15 to 5.03; P = 0.03) to be seropositive for MAP infection as were cows from herds that had imported <8% of their current herd size annually during the preceding 5 years. Cattle were purchased from Colorado, Alabama, Connecticut, Florida, Idaho, Iowa, Kansas, Louisiana, Minnesota, Nebraska, New Hampshire, New York, Ohio, Pennsylvania, South Dakota, Texas, Vermont, Virginia, Washington, Wisconsin, and Wyoming.

Mean herd size was 685 cows (range, 79 to 2,519 cows), which was similar to mean herd size for all MAP-positive herds in a previous study in Colorado. These results were published by a group of Colorado dairy owners and managers and are similar to mean herd size for all MAP-positive herds in previous studies. The Colorado dairy herd was approximately 30,000 cows located in 15 counties, with an estimated 100,000 dairy cows in the state of Colorado.

Table 1—Results of a serologic survey for Mycobacterium avium subsp paratuberculosis (MAP) infection among adult dairy cows in Colorado.

<table>
<thead>
<tr>
<th>Dairy</th>
<th>No. of seropositive cows (%)</th>
<th>No. of cows tested</th>
<th>Clinical signs of disease</th>
<th>Culture of feces (No. positive/No. tested)</th>
<th>Infection confirmed</th>
<th>Mean annual importation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 (0)</td>
<td>79</td>
<td>No</td>
<td>1/3</td>
<td>Yes</td>
<td>11.1</td>
</tr>
<tr>
<td>B</td>
<td>6 (1.22)</td>
<td>493</td>
<td>No</td>
<td>0/6</td>
<td>No</td>
<td>0.0</td>
</tr>
<tr>
<td>C</td>
<td>21 (3.31)</td>
<td>153</td>
<td>No</td>
<td>1/8</td>
<td>Yes</td>
<td>7.8</td>
</tr>
<tr>
<td>D</td>
<td>8 (1.47)</td>
<td>543</td>
<td>No</td>
<td>4/28</td>
<td>Yes</td>
<td>0.8</td>
</tr>
<tr>
<td>E</td>
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<td>386</td>
<td>No</td>
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<td>No</td>
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<tr>
<td>F</td>
<td>6 (1.58)</td>
<td>380</td>
<td>No</td>
<td>0/27</td>
<td>No</td>
<td>3.3</td>
</tr>
<tr>
<td>G</td>
<td>6 (1.76)</td>
<td>340</td>
<td>Yes</td>
<td>1/199</td>
<td>Yes</td>
<td>2.4</td>
</tr>
<tr>
<td>H</td>
<td>19 (3.94)</td>
<td>979</td>
<td>No</td>
<td>3/52</td>
<td>Yes</td>
<td>0.0</td>
</tr>
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<td>14 (2.09)</td>
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<td>3/51</td>
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<tr>
<td>J</td>
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<td>655</td>
<td>Yes</td>
<td>0/30</td>
<td>No</td>
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<td>K</td>
<td>35 (3.41)</td>
<td>1,025</td>
<td>Yes</td>
<td>6/16</td>
<td>Yes</td>
<td>8.6</td>
</tr>
<tr>
<td>L</td>
<td>4 (1.57)</td>
<td>112</td>
<td>No</td>
<td>3/15</td>
<td>Yes</td>
<td>0.0</td>
</tr>
<tr>
<td>M</td>
<td>8 (1.81)</td>
<td>166</td>
<td>No</td>
<td>0/5</td>
<td>No</td>
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</tr>
<tr>
<td>N</td>
<td>93 (5.22)</td>
<td>1,781</td>
<td>Yes</td>
<td>0/0</td>
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<td>16.7</td>
</tr>
<tr>
<td>O</td>
<td>197 (7.82)</td>
<td>2,519</td>
<td>Yes</td>
<td>0/0</td>
<td>Yes</td>
<td>27.2</td>
</tr>
</tbody>
</table>

*In each herd, all adult cows ≥2 years old that had produced at least 1 calf were tested with a commercial ELISA. 10 Dairy owners and managers were asked to recall whether they had noticed cows with signs of MAP infection during the preceding 5 years. In all herds except herd C, fecal samples were collected only from cows with positive or suspect ELISA results. 1A herd was considered to be infected with MAP if results of mycobacterial culture of ≥1 fecal sample were positive or if ≥1 culled cow had histologic evidence of MAP infection. 1B Five-year importation rate was calculated by dividing the number of cows ≥20 months old imported to the dairy from an outside source during the 5 years preceding whole-herd serologic testing by the number of adult cows in the herd at the time of testing; annual importation rate was calculated by dividing the 5-year importation rate by 5.
dairies in Colorado during the study period. Cows in herds of 600 lactating cows were 3.12 times as likely (95% CI, 1.65 to 3.12; \( P < 0.001 \)) to be seropositive as were cows from herds with < 600 lactating cows.

On the basis of dairy records and farm manager and owner recollection, 9 dairies were classified as not having cows with clinical signs of MAP infection during the preceding 5 years and 6 were classified as having cows with clinical signs of MAP infection during this period. Cows from herds with a history of clinical signs of MAP infection were 2.27 times as likely (95% CI, 1.65 to 3.12; \( P < 0.001 \)) to be seropositive as were cows from herds without clinical signs of MAP infection, after controlling for herd size in the analysis. For 4 of the 6 herds with clinical signs of MAP infection, percentage of seropositive cows ranged from 1.76% to 3.41%. For the remaining 2 herds, percentages of seropositive cows were 5.22% and 7.82%. For 7 of the 9 herds without clinical signs of MAP infection, percentage of seropositive cows was < 2%. For the remaining 2 herds, percentages of seropositive cows were 3.37% and 3.61%. However, mean percentage of seropositive cows in herds with clinical signs of disease (mean ± SD, 2.9 ± 1.94%) was not significantly (\( P = 0.57 \)) different from mean percentage of seropositive cows in herds without clinical signs of disease (2.5 ± 1.55%), after adjusting for herd size.

Mean ± SD culling rate for the year preceding whole-herd serologic testing was 25.6 ± 8.52% (range, 6.5% to 37.6%).

**Confirmation of MAP infection**—Nine of the 15 dairies submitted at least 1 cow for necropsy, and 12 submitted fecal samples from cows with positive or suspect ELISA results for mycobacterial culture. Overall, 11 of the 15 dairies were confirmed to be infected with MAP. Results of mycobacterial culture were positive for at least 1 cow from 8 of the 12 dairies that submitted fecal samples for testing; whereas for the remaining 4 dairies, results of mycobacterial culture of fecal samples from cows with positive or suspect ELISA results were negative. Cows for which results of mycobacterial culture were positive had S:P ratios ranging from 0.10 to 1.74.

The 3 dairies that did not submit fecal samples for mycobacterial culture were all determined to be infected with MAP on the basis of results of necropsy; with at least 1 cow from each of these dairies having histologic evidence of MAP infection or positive results for mycobacterial culture of a fecal sample collected at the time of euthanasia.

All 6 of the herds that were reported to have had cows with clinical signs of MAP infection during the preceding 5 years and 5 of the 9 herds without any history of cows having clinical signs of MAP infection during this period were confirmed to be infected with MAP.

**Discussion**

The overall seroprevalence of MAP infection among cattle in the present study was 4.12%, which is lower than the range (3% to 17%) that has been reported from other states in the United States. Important limitations of the present study are that only 15 of the approximately 220 dairy herds in Colorado were included and herds were not randomly selected; however, 12.3% of adult dairy cattle in Colorado were tested. On the one hand, producers who participated in the study may have been more progressive and more likely to institute management changes than were producers who did not participate. Thus, they may have been more conscientious and more likely to institute general biosecurity procedures than producers who did not participate, in which case seroprevalence in the present study may underestimate the true seroprevalence of MAP infection among all dairy herds in Colorado. On the other hand, producers who had already noticed cows with clinical signs of MAP infection may have been more likely to participate because of the reduced cost for testing their herds. In this case, seroprevalence in the present study may overestimate the true seroprevalence. However, most dairies in the present study had not noticed clinical signs of MAP infection in the years prior to the study.

Nine herds in the present study reported not having seen any cows with clinical signs of MAP infection during the preceding 5 years; however, MAP infection was documented in 5 of these 9 herds on the basis of results of necropsy or mycobacterial culture of fecal samples from cattle with positive or suspect ELISA results. It is possible that infection may have also been confirmed in some of the remaining herds if we had necropsied or cultured fecal samples from some of the seronegative cattle. However, funding was an issue, and we relied on the dairy producers to provide payment for mycobacterial culture of fecal samples. Regardless, our findings clearly demonstrate that even in herds without any history of compatible clinical signs, some cows may be infected with MAP.

An important finding of this study was that the ELISA was sensitive enough to identify some infected cows in herds with low seroprevalence whose owners had not recognized any cows with clinical signs suggestive of MAP infection. This suggests that initial whole-herd serologic testing with this ELISA could be an inexpensive way for producers to identify high-risk cows, even in herds without a history of clinical disease. Although the ELISA is not recommended for diagnosis of MAP infection in individual cows, it may be useful in identifying cows that should undergo additional testing, such as mycobacterial culture of feces or necropsy at culling, to document the presence of MAP infection in the herd. Once MAP infection is confirmed, the producer might then be more likely to implement control measures to decrease transmission within the herd.

In the present study, cows from herds with a history of compatible clinical signs were 2.27 times as likely to be seropositive for antibodies against MAP as were cows from herds without such a history, after controlling for herd size. This association has been reported previously and likely is related to the chronic nature of MAP infection, whereby clinical signs develop late in the course of disease when the ELISA is thought to be most sensitive. In general, herds without a history of compatible clinical signs had seroprevalence rates...
times as likely to be seropositive as were cows from herds with ≥ 600 lactating cows. Realizing that larger herds may be more likely to purchase cattle,17 we speculate that higher seroprevalence was related to importation of more infected cattle.

Finally, we found in the present study that cows from herds that had imported ≥ 8% of their current herd size annually during the preceding 5 years were 3.28 times as likely to be seropositive as were cows from herds that imported < 8%. In a previous study,16 dairy herds in which ≥ 25% of the cows were born on other dairies were 2.1 times as likely to be classified as positive for MAP infection as were herds in which none of the animals were born on other dairies. In contrast, other studies17,18 have not found a significant association between whether dairies purchased cattle and MAP infection status of the herds. This discrepancy may be attributable to differences in the sources of purchased cattle in each study,19 how importation data were analyzed, and how MAP infection status or prevalence was determined.

When herd size and importation rate were included in a multivariate logistic regression model in the present study, neither variable had a significant effect on percentage of seropositive cattle in herds. We did not attempt to interpret the parameter estimates from the multivariate model because of the close relationship between the 2 variables (r = 0.91).

Dairies in the Western United States have been expanding since 1980,20 but in our experience, few producers have tested cattle for MAP infection prior to purchase. In the present study, 12 of 15 herds had imported cattle in the preceding 5 years, but only 3 of these had tested cows prior to purchase. By comparison, only 9% of producers had tested cattle for MAP infection in a previous national study.21 Regardless, we strongly suggest that source herds be tested for MAP infection before purchasing animals from those herds.

The mean culling rate (25.6%) in the present study was similar to rates reported recently.12,15 In 1 of these studies,12 the mean ± SD US culling rate in 1995, as a percentage of the January 1996 dairy cow inventory, was 24 ± 0.4%. The proportion of cows slaughtered in federally inspected plants, as reported by the USDA as a percentage of the national herd, was 28% in 2001 and ranged from 28% to 31% between 1997 and 2000.18

References