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Occurrence of *Mycobacterium avium* subspecies *paratuberculosis* Infection in Cattle in North Dakota, 1995–2005

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Abstract

The objective of this study was to evaluate trends and risk factors for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) shedding in cattle in North Dakota. The North Dakota State University Veterinary Diagnostic Laboratory records of fecal culture-positive MAP cases diagnosed from 1995 to 2005 were examined. Epidemiological data on clinical history, age, sex, breed, herd size and county of origin were extracted. Additionally, data on producers enrolled in North Dakota's Voluntary Johne's Disease Control Program were obtained from the North Dakota State Veterinarian's office. Data were analyzed using Geographic Information Systems Arc Info 9.1 software, Epi Info version 6 and SAS version 9.2.

Of the 53 counties in North Dakota, 42 (79%) reported MAP infection (range 1 - 86, median 6) in both beef (n=204) and dairy cattle (n=175). Also, there was a correlation between distribution of cases by county and distribution of producers participating in the ND Johne's Disease Voluntary Control Program; participating counties had more MAP cases reported than counties without participants. The number of MAP cases reported increased during the study period with seasonal trends, as shedding was higher in winter and spring than summer and fall. Chi-square and logistic regression analyses indicated that large herds, female, beef animals and animals greater than four years of age were more likely to be categorized as high shedders of MAP than herds without these attributes.

Keywords: bovine, Johne's disease, MAP

Résumé

L'objectif de cette étude était d'évaluer les tendances et les facteurs de risque associés à l'excrétion de Mycobacterium avium sous-espèce paratuberculosis (MAP) chez les bovins du Dakota du Nord. Les cas de culture fécale positive pour le MAP diagnostiqués entre 1995 et 2005 et recueillis par le laboratoire de diagnostic vétérinaire de la North Dakota State University ont été utilisés. Des données épidémiologiques sur les antécédents cliniques, l'âge, le sexe, la race, la taille du troupeau et le comté d'origine ont été saisies. De plus, des données provenant de producteurs participants au programme volontaire de contrôle de la maladie de Johne au Dakota du Nord ont été recueillies à partir du bureau des vétérinaires de l'état du Dakota du Nord. Les données ont été analysées avec le logiciel Arc GIS version 9.1, Epi Info version 6 et SAS version 9.2.

Un total de 42 (79%) des 53 comtés du Dakota du Nord ont rapporté des infections au MAP (étendue 1-86, médiane 6) à la fois dans les productions de boucherie (n=204) et les productions laitières (n=175). De plus, il y avait une corrélation entre la distribution des cas par comté et la distribution des producteurs participants au programme volontaire de contrôle de la maladie de Johne au Dakota du Nord. En effet, les infections au MAP étaient plus fréquentes dans les comtés avec participants que dans les comtés sans participants. Le nombre de cas d'infection au MAP rapportés augmentait au courant de l'étude avec des fluctuations saisonnières car l'excrétion était plus fréquente l'hiver et au printemps que l'été et l'automne. Des analyses de chi-carré et de régression logistique indiquaient que l'excrétion de MAP était plus fréquente dans les troupeaux de grande taille, chez les femelles, chez les animaux de boucherie et chez les individus âgés de plus de quatre ans.

Introduction

Paratuberculosis, or Johne's disease, is a chronic, nontreatable granulomatous enteritis caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) that affects both domestic and wild ruminants.[7] In recent years, the incidence of Johne's disease is reportedly increasing in many countries worldwide, including the United States (US), with considerable economic losses primarily reported in the dairy herds.[12,20,22,25,27] Various studies have documented major economic losses in dairy and beef cattle herds resulting from decreased production, weight loss, lower slaughter value and premature culling of infected cattle.[14,20,23,25,27] Major obstacles to control of Johne's disease include identifying subclinically infected animals[7] and the wide host range in domestic and wild animals.[11,18,23,29,30,31,33] Studies have shown that only one in 20 infected animals show overt clinical signs of Johne's disease, thus making early detection and culling difficult.[26]

The negative economic impact caused by decreased animal productivity and welfare is often masked, and may appear insufficient to justify large investments in control programs by individual farmers, livestock industries or government.[34] MAP has also become a public health concern[2,10,33] with some studies reporting that DNA from MAP has been found in up to 69% of patients with Crohn's disease.[10,33] Whether this association is causal or merely coincidental is not yet fully understood. While this association remains unproven and contentious, public perceptions of a causal link represents one of the most important economic risks to the milk and meat industries.[19]

In the US, one study[4] reported that up to 35% of herds were infected with MAP, causing an estimated $1.5 billion annual loss to the cattle industry.[24] Because of the economic importance and public health concerns about MAP, many states within the US have instituted Johne's disease certification programs to eradicate the disease. The USDA-US Voluntary Johne's Disease Herd Status Program (1998), and later USDA-Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Programs (2006),[32] were established to standardize control programs in the US. The North Dakota Voluntary Johne's Disease Control Program (CVJDCP) is a sequel of the initial effort started in 2001 to screen the state cattle herds. Farmers participating in the CVJDCP routinely submit fecal samples along with a disease history form to state diagnostic laboratories (SDL) for diagnosis of MAP.[3] The compilation and dissemination of information from SDL provides information from which technical decisions are made for control programs. This information is useful to monitor the effectiveness of the control program, and is the basis for making adjustments to the Voluntary Johne's Disease Control Program.

In general, examination of laboratory data and relating it to epidemiological information from the field is essential for strengthening national disease monitoring and surveillance systems (MOSS). Review of laboratory information is helpful for explaining changes in the disease seen in the field. The objective of this study was to evaluate trends and risk factors of MAP shedding in North Dakota (ND) cattle from 1995 to 2005, using data from the North Dakota State University Veterinary Diagnostic Laboratory (NDSU-VDL) in Fargo, ND.

Materials and Methods

Study Design

A retrospective case series of veterinary medical records of fecal culture-positive MAP infections in cattle diagnosed at the NDSU-VDL from 1995 to 2005 were examined. Suspected fecal samples were processed as follows: conventional fecal culture on Herrold's egg yolk (HEY) agar slants was performed as previously described,[37] with brief modifications. Briefly, one gram of feces was added to 20 mL of sterile distilled water, tubes were shaken for 30 minutes and then allowed to stand undisturbed for 30 minutes. Five mL of supernatant were added to a decontaminant mixture containing 25 mL of 0.9% hexadecylpyridinium chloride monohydrate[a] (HPC) and 30 mL of brain heart infusion (BHI) broth, and the samples were allowed to decontaminate overnight at 98.6°F (37°C). Samples were centrifuged at 900 xg for 30 minutes and the supernatant discarded. Pellets were re-suspended in 1 mL of antibiotic brew containing 50% BHI broth with 100 µg/mL vancomycin, 100 µg/mL nalidixic acid and 50 µg/mL amphotericin B. Tubes were vortexed and incubated overnight at 98.6°F. HEY slopes were inoculated with 0.25 mL of the suspension. Each sample was cultivated in duplicate on HEY with mycobactin J and one tube without mycobactin as the culture medium. Tubes were incubated at 98.6°F and observed at two-week intervals for 16 weeks. Isolation of a slow-growing, acid-fast organism with colonial morphology typical of MAP on HEY with mycobactin, but not on HEY without mycobactin, was considered a positive culture. Animals were classified as high or low shedders if MAP colonies from cultured feces took less or more than two weeks, respectively.

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Epidemiological data on clinical history, age, sex, breed, shedder status, herd size, season and county of origin of each case were extracted. Additionally, data on producers enrolled in CVJDCP were obtained from the North Dakota State Veterinarian's office.

Data Analyses

Information presented in the individual animal data did not have complete entries. Only those with 65% or more of complete entries or those with variables of interest were used in the analysis. Descriptive statistics of cattle with MAP infection were summarized using SAS[b] and Epi Info version 6. The Geographic Information System (GIS) software[c] was used to display the spatial distribution of bovine Johne's disease cases reported in the state by county, and the distribution of producers enrolled in CVJDCP by county.

In the statistical analysis, the MAP shedding status of infected cattle was used as the outcome variable. The VDL laboratory protocol classifies an animal as a high or low fecal shedder based on whether MAP colonies took less or more than two weeks, respectively, to appear. Seasons were classified as winter (December to February), spring (March to May), summer (June to August) and fall (September to November). Age and herd size were dichotomized using their medians as the cut-off (above as high and below as low). Submission patterns were reviewed according to months, seasons, years and their geographical origin.

The cumulative incidence (CuI) was calculated from $CuI = 1 - e^{-I}$, where e is the natural logarithm, and *I* is incidence rate (*IR*). IR was calculated from the number of new positive cases diagnosed during each year divided by population at risk at the start of the year. The population at risk was calculated from simple moving averages of beef and dairy cattle census of 1992, 1997, 2002 and projections made for 2005.

All listed independent variables of biological relevance to epidemiology of MAP were individually assessed by univariate logistic regression analysis.[11] The variables assessed were age, sex, county of origin, breed, herd size, number affected and season. All variables with a *P*-value of ≤ 0.20 from the univariate analyses were offered to the multivariate logistic regression for model building. Multivariate logistic model with the MAP shedding status as outcome was constructed using forward stepwise selection procedure, while deviance was assessed using Akaike Information Criteria (AIC).[c] Interaction between key variables was assessed using the same strategy. The adjusted odds ratios were estimated with 95% confidence intervals (95% CI). The Wald and likelihood ratio test and the Hosmer and Lemeshow Goodness-of-Fit test were used to assess the model fit.

Results

In total, 562 of the 1,684 (33.4%) fecal samples from 42 of the 53 counties in ND submitted from 1995 to 2005 to NDSU-VDL, were diagnosed as culture-positive to MAP. Positive case reports varied from 1 to 86 (median=6) in the counties. Of the data with completed entries, 47.1% of the cases were dairy (n=197), while 52.9% (n=221) were beef breeds. Only the Holstein dairy breed was represented (100%; n=197). The beef breeds were Angus (49.3%; n=109); Gelbvieh (16.3%; n=36); cross breeds (9.0%; n=20); Shorthorn (5.4%; n=12); Limousin (4.1%; n=9); Simmental (4.1%; n=9); Brahman (1.8%, n=4); Charolais (1.8%; n=4); Hereford (1.8%; n=4); Black Maine, Corrient, Longhorn and Tarentaise-cross each represented 0.9% (n=2); and others constituted 3% (n=6). Breeds classified as "others" included: bucking bull (1), Normande (1) and Saler (1). Because of smaller numbers of individual cattle breeds involved, it was not possible to run analysis on their influence on fecal shedding; instead they grouped together according to their use (dairy or beef).

Figure 1 shows the spatial distribution of MAP cases per county in ND (n=467) from 1999 to 2005. The 1995-1998 (n=95) data were excluded due to missing county information in their entries. Figure 2 shows the distribution by county of producers participating in the CVJDCP as of March 22, 2007 (n=173). A total of 346 (91.8%) positive cases were females and 31 (8.2%) were males; 185 case records did not specify gender. Of the 562 culture-positive cattle, 225 (40%) had age information missing. Twenty-five (4.4%) were ≤2 years, 83

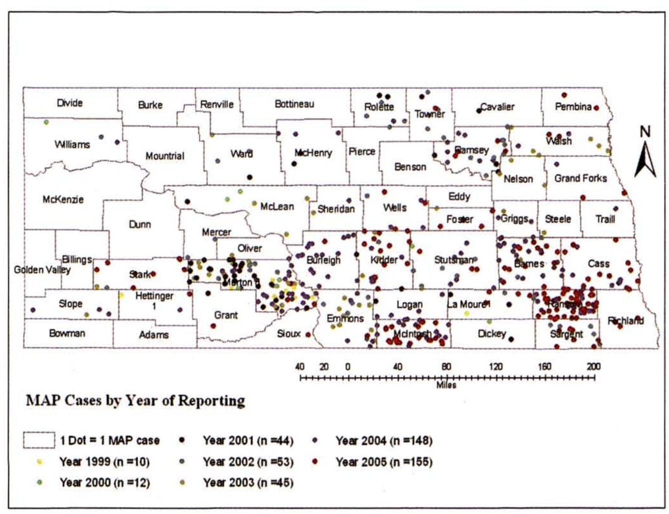

Figure 1. Spatial distribution by county of fecal culture-positive cases of *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle in North Dakota, 1999 – 2005 (n=467).

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(14.8%) were 3-4 years and 229 (40.7%) were recorded as adults.

Variations in the case reports by cattle type and year of reporting, 1995 to 2005, are given in Figure 3. As observed, the proportion of positive cases between the beef (52.9%, n=221/418) and dairy breeds (47.1%, n=197/418) were comparable although the breed entries for 144 other animals were missing. Eighty-eight percent (n=494/562) of MAP cases were reported from 2001 to 2005 following launch of the Voluntary Johne's Disease Control Program in 2001, while 58% of cases (n=327/562) were reported in 2004 and 2005, showing a very marked increase in the last two years.

Figure 4 shows the cattle type cumulative incidence of MAP per 10^6 cattle initially at risk in ND by year of reporting. Figure 5 shows a monthly relationship of culture-positive results to total submissions over the same period. Figure 6 describes the variation in the monthly trend in the mean number of culture-positive MAP cases reported from start of the program in 2000 to 2005 (n=508, detailed information missing [n=54] for 1995-1999). Trends show the highest mean number of

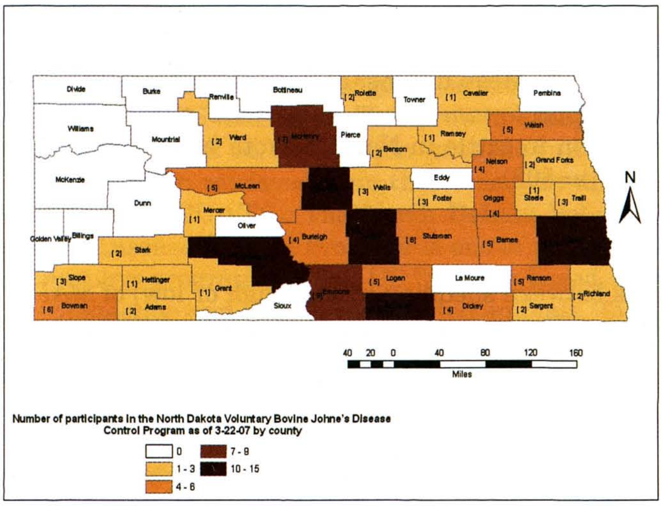

Figure 2. Distribution by county of producers participating in the North Dakota Voluntary Johne's Disease Control Program as of March 22, 2007 (n=173).

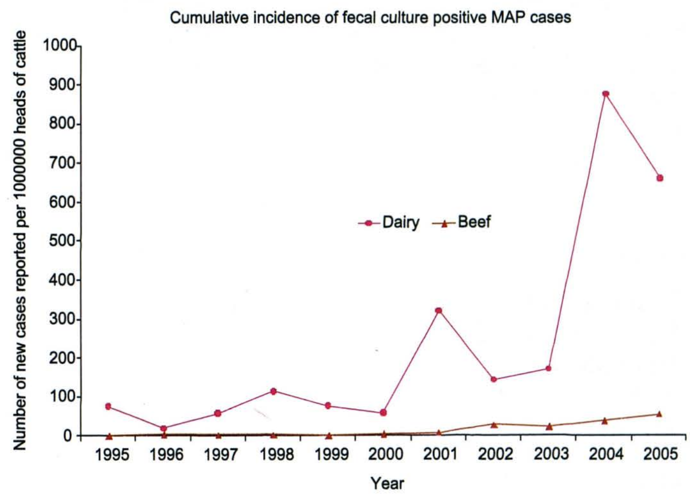

Figure 4. Distribution by breed and year of reporting, of fecal culture-positive cases of *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle in North Dakota, 1995 – 2005 (n=562).

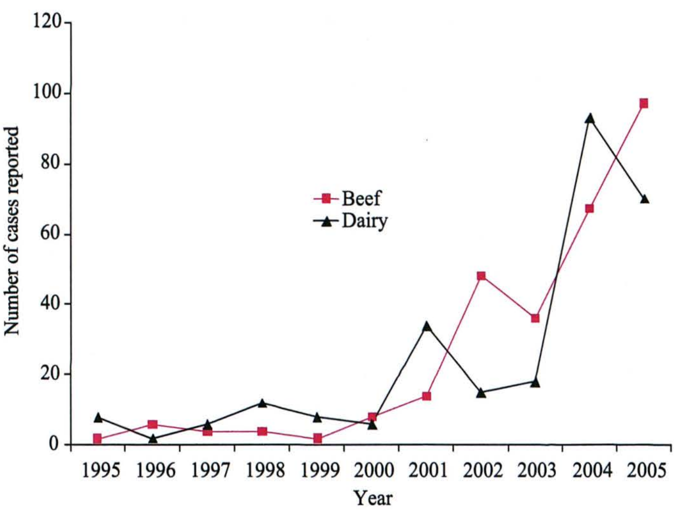

Figure 3. Spatial distribution by county and year of reporting of fecal culture-positive cases of *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle in North Dakota, 1999 – 2005 (n=467).

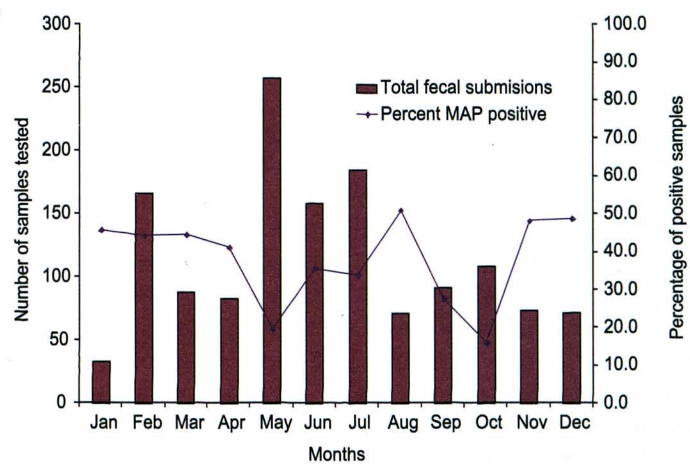

Figure 5. Cumulative incidence of fecal culture-positive cases of *Mycobacterium avium* subspecies *paratuberculosis* infection per 10^6 cattle initially at risk in North Dakota (2000-2005).

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positive MAP cases were diagnosed during early spring and late winter (38 and 25%, respectively) compared to summer and fall (19 and 18%, respectively; P=0.0091; Table 1). Further examination of submissions revealed higher fluctuations in the number of cases during May-July and November-December, showing yearly influence.

At the univariate level, shedding status among MAP-infected cattle was found to be significantly associated with herd size, gender, type (dairy/beef) and season (P<0.05; Table 1), while age category was not (P>0.05). Details of the results from the tabular and univariate logistic regression analyses are given in Table 1. The final multivariate logistic regression model retained only two of the original variables, cattle type (dairy/beef) and season. Of these variables, the interpretation of their parameter estimates and estimated odds ratios suggested a strong association with shedding of MAP. In the final model, beef cattle were associated more strongly

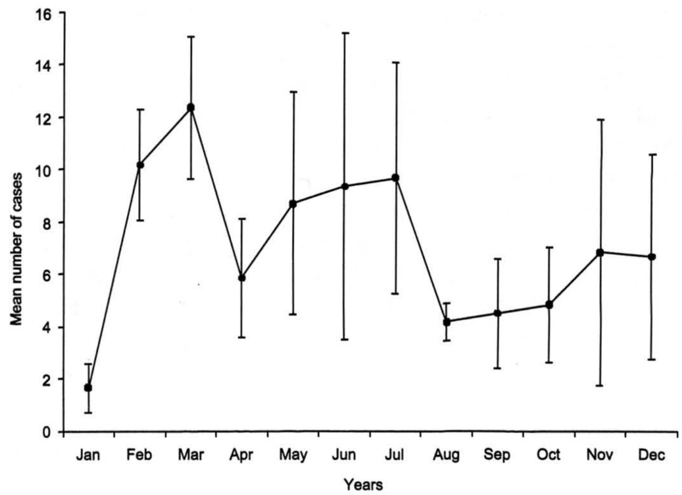

Figure 6. Mean number of fecal culture-positive cases of *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle in North Dakota by month, 2000 to 2005 (n=508).

Table 1. Univariate logistic regression analysis of associations between shedding status (high or low) of *MAP* infected cattle and various risk factors (herd size, gender, season, cattle use, age and number reported sick in herd), 1995-2005.

Variable	Category	No. tested	Percent high MAP shedder[1] (%)	*P*-value
Herd size[2]	Large (≥144)	46	52.2	0.006
	Small (<144)	42	23.8	
Gender	Female	317	23.3	0.043
	Male	24	41.7	
Within season variability	Winter	163	17.2	0.009
	Spring	133	33.8	
	Summer	87	24.1	
	Fall	80	21.3	
Comparison between seasons	Winter vs Fall			0.157
	Spring vs Fall			0.003
	Summer vs Fall			
			0.436	
Cattle use	Beef	195	29.7	0.003
	Dairy	169	16.6	
Age (years)	≤1	8	62.5	0.111
	2-3	61	27.9	
	≥4	156	28.2	
Number individuals reported sick in herd[3]	≤20	116	34.5	0.969
	≥20	76	34.2	

[1]High shedders: defined as high shedders if the MAP colonies from cultured feces took less than two weeks to show visible colonies.
[2]Herd size: herds with >144 heads of cattle considered large and <144 small.
[3]Number of individuals reported sick in herd: number farmer reported with similar signs at the time of diagnosis.

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with high shedding of MAP than dairy cattle (χ^2_{2df}=5.8, OR=2.13; 95% CI=1.3-3.5; P=0.016), despite higher case reports from the dairy breeds (Figure 2). Shedding variation was significantly different within season (χ^2_{3df}=11.6, OR=3.8; 95% CI=1.2-2.3; P=0.009). The total sample size used for this model was 215, concordance was relatively high at 61.5%, actual misclassification rate was 24.4% and the Hosmer and Lemeshow Goodness-of-Fit test for this model suggested that the model fit was adequate (χ^2=1.37, P=0.5029).

Discussion

Monitoring and Surveillance Systems (MOSS) are essential for determining the direction or effectiveness of disease control or eradication strategy. Review of laboratory data from an ongoing control program is one of the MOSS tools used. This present study evaluated the status of MAP infection in cattle in ND, using information retrieved from the veterinary medical records of NDSU-VDL. The North Dakota Voluntary Johne's Disease Control Program is one of many similar ongoing programs in the US,[32] and is based upon early detection and removal of infected animals. To our knowledge, this is the first study to use laboratory data to assess the progress of the bovine Johne's disease control program in ND.

This study gives insight into the quality of data captured and limitations of using such data for disease monitoring purposes. The observed results could have been biased by lack of standardization of sample submissions because it was voluntary. Furthermore, it could have been influenced by the continued improvements in the laboratory diagnostic protocols. Differences in cattle type (dairy/beef) submissions could have been influenced by other factors, such as variations in the farmers' interest. The sampling was open to both beef and dairy. The state informed producers that they would be compensated by the federal government for fecal and serologic testing if they enrolled, and were also informed of the herd health benefits of controlling Johne's disease. Despite this, it is known that dairy cattle are more likely to be closely monitored than beef breeds. Likewise, producers are more likely to collect and submit samples when cattle are gathered, hence a seasonality bias.

This study demonstrated widespread distribution (42 of 53 counties) of MAP infection in ND (Figure 1), suggesting existence of probable reservoirs and an endemic situation in the state. The appearance of an upward trend in the number of case reports could not be directly linked to increases in the rate of new infection without considering other factors, such as increased producer awareness and recruitment of new participants. Also, there was a correlation between distribution of cases by county (Figure 1) and distribution of producers participating in CVJDCP (Figure 2), with participating counties having more MAP cases reported than counties without participants, suggesting producer bias. In addition, it was evident that the lowest-percent positive submissions occurred in 2000, which was prior to the introduction of CVJDCP. The majority of cases (88%, n=494/562) were reported between 2001 and 2005, following introduction of the CVJDCP in 2001, suggesting that submissions changed with participation. This observation highlights the importance of CVJDCP participation as a way of increasing Johne's disease testing. From the available data, it is not possible to explain the appearance of an upward trend in the case reports. There are two possibilities: either there is a producer bias, where there is increased interest and participation, or simply that there is a true increase in the disease prevalence.

Early identification of MAP clinical shedders remains difficult,[36] partly due to low sensitivity of the tests used and the presence of subclinically infected cows in the herds, both of which may substantially increase risk of infection. Currently, available tests to detect MAP-infected animals produce many false negatives, particularly in subclinically infected or low-MAP fecal-shedding animals, thus making their interpretation and utilization in control programs challenging.[31] In the NDVJDCP serial testing was adopted, with either the whole herd or percentage of the herd being screened first with ELISA, and then followed by fecal culture of positive samples.

It has been suggested that the prolonged pre-clinical phase of lifelong MAP infections and the poor performance of diagnostic tests make identification of clinical shedders difficult. As a result, the susceptible population is exposed to subclinically infected cows, which are the main risk factor in the spread of the disease.[35]

The spatial-temporal distribution of MAP cases (not shown) showed cases clustered in the southwestern part of the state, while the more recent cases were reported mainly in southeastern ND. The direction of movement of the disease across the state is possibly linked to increased producer awareness and submissions from newly recruited participants. Interestingly, case reports and cumulative incidence of MAP infection in dairy breeds peaked every three years (1995, 1998, 2001 and 2004). This observation was more pronounced in dairy than beef cattle. It was not possible to establish whether this increment was an actual or an apparent increase in case-detection rate. However, from trends in our results, a three-year MAP infection cycle appears to exist in dairy herds in ND. Producers received incentives (testing) annually, and therefore monetary incentives should not have influenced the three-year trend.

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The main limitation of this study was lack of baseline information on the reference population. In addition, no information was provided on the sampling criteria, although we were told that any herd was eligible for entry into the program.

Because results of this study do not conclusively define whether new cases resulted from more farmers responding to the program or an actual increase in number of cases, an annual review of control strategies in ND is highly recommended. The comparable distribution of case reports between beef (52%) and dairy breeds (48%) in ND (Figures 3 and 4) suggest the two breed groups were equally susceptible; however, when considering the reference population, the MAP cumulative incidence was higher in dairy than beef breeds.

The occurrence of infection in both cattle production systems supports the notion that MAP can cause serious economic losses in both dairy and beef cattle.[1,12,27] However, the significantly higher MAP shedding rates in infected beef than dairy cattle (Table 1) could be linked to poor hygiene conditions of beef farms,[27] calves suckling infected cows, common beds or feeding.

In this study infection rates were higher in older animals (71%) than younger ones (29%). Also, larger herds were observed to have more heavy MAP shedders than smaller ones. This agrees with 1996 Dairy National Animal Health Monitoring System's (NAHMS) study (Johne's disease on U.S. dairy operations. USDA: APHIS:VS, CEAH, National Animal Health Monitoring System 1997; Fort Collins, Colorado), which reported that larger herds (>300 cows) were more likely to be infected than smaller herds (<50 cows). In the present study, the presence of high shedders in larger-sized herds could be due to increased close physical contact between individual animals, leading to more effective transfer of infection to non-infected animals in large herds. Also, sanitary practices to decrease the risk of new MAP infections are difficult to implement around beef cattle compared to dairy cattle.[8,13] While sanitary practices may be mandatory to control mastitis in dairy operations,[8,13] that is not the case for beef herds, making these particular groups more vulnerable to infection than dairy cattle.

Additionally, at a univariate level, there was a significant difference in the proportion of female cattle (93%) that were infected compared to males (7%), however, high shedding was observed more in males than females (P=0.043). This could be attributed to differences in the submission rates. There are fewer bulls than cows in both beef and dairy herds, and most dairies use artificial insemination so they have few bulls. This may account for the difference in proportion of infected females as compared to males.

Although in this study the shedding status was higher in animals four years of age or older, the difference was not statistically significant (P>0.05). This is contrary to several earlier studies in which high shedding was associated with increasing age. Several studies[5,22,35] reported that cattle infected with MAP have a long prepatent period in which no shedding of the organism occurs, which is followed by intermittent then continuous shedding, increasing in volume as the disease progresses.

Seasonality in the shedding pattern of MAP was also detected, with higher shedding in colder months (winter and spring) compared to warmer months (summer and fall; Figure 4 and Table 1), in spite of most samples being submitted in fall. This agrees with other studies that found higher MAP incidence during the winter months.[6,9] A likely explanation for the difference in incidence and shedding status (high or low) between spring and summer could be the effects of over-wintering on body condition and/or calving season stress and lactation. Stress compromises the immune system and results in higher levels of disease in many mammalian species in the breeding season.[21] Other researchers[14] have downplayed this winter and spring stress hypothesis, and attribute increased MAP case reports to increased case detection as a result of increased attention paid to cattle during spring rather than an actual increase in MAP prevalence. This could explain observations in this study. The high proportion of MAP infection in winter and spring is also in agreement with the seasonal variation of other enteric animal diseases.[28]

The main limitations of this study were lack of control of quality of data recorded (missing variables), limited access to management and ecological data and lack of information on the reference population. Access to these data could have provided vital information to better explain the host-environment-agent interactions during spring. This study could have been further limited by the low sensitivity (38%) of the fecal culture test used, as well as possible improvements in the laboratory protocols as the lab focused more on the program. Rapid and more sensitive procedures (with a sensitivity of 93 to 96%) using immunomagnetic bead separation and real-time PCR for diagnosis of clinical and subclinical Johne's disease (paratuberculosis) from milk and/or fecal samples from cattle and American bison have been reported.[16] These methods[16] have been reported to be very useful and cost-effective compared to bacteriological culture, which is constrained by time, labor, and expense under diagnostic laboratory conditions.

Conclusion

This study demonstrated widespread occurrence of MAP and documented some risk factors for MAP shedding in dairy and beef cattle in ND. Counties with

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producers participating in ND Johne's Disease Voluntary Control Program reported more MAP cases than counties without participants. The study also provided information on the progress of the control programs for bovine Johne's disease in ND, helping to identify some critical areas for further research and intervention. This study further justifies the retrospective examination of laboratory data to strengthen and add value to national disease monitoring and surveillance efforts. It also recognizes serious limitations of this data and recommends that new efforts be made to improve the value of data for state and national reporting.

Endnotes

[a]Sigma, USA, St. Louis, MO 63103

[b]SAS-Institute, Inc, Cary, NC 27513

[c]ArcInfo™ 8, ESRI, 380 New York Street, Redlands, CA 92373

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Dr. W. Ron DeHaven, AVMA executive vice president and CEO, and his wife, Nancy; Karen Hammer and Dr. Gregory S. Hammer, AVMA immediate past president; and Dr. Roger K. Mahr, 2006-2007 AVMA president, and his wife, Marilyn. Photo courtesy of Dr. Bob Smith, Stillwater, OK.