VETERINARSKI ARHIV 79 (4), 323-332, 2009

A retrospective longitudinal cohort study of Johne's disease prevalence in a dairy herd

Bačić Goran[1]*, Korana Stipetić[2], Ynte Hein Schukken[2], Chuck Guard[3], Gerdiene Van Schaik[4], and Hollis Nancy Erb[2]

[1]Clinic for Reproduction and Obstetrics, Faculty of Veterinary Medicine, Zagreb, Croatia

[2]Department of Population Medicine and Diagnostic Sciences, Veterinary College Cornell University, Ithaca, NY, USA

[3]Ambulatory and Production Medicine Clinic, Veterinary College Cornell University, Ithaca, NY, USA

[4]PhD, student at Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

BAČIĆ, G., K. STIPETIĆ, Y. H. SCHUKKEN, C. GUARD, G. VAN SCHAIK, H. N. ERB: A retrospective longitudinal cohort study of Johne's disease prevalence in a dairy herd. Vet. arhiv 79, 323-332, 2009.

ABSTRACT

The objective of this retrospective cohort study was to evaluate the efficacy of selected Johne's-disease control measures on a dairy farm. The Dairy Comp 305 archive files for 455 Holstein dairy cows born in 1996, 1997 and 1998 were used. The tested heifers were grouped into annual birth cohorts. Kinetic ELISA (KELA) results and fecal-culture results for solid- (HEYM) and liquid-media systems (ESP Culture System II) were described and compared between birth cohorts. Only one animal had a high KELA score. Nevertheless, the fecal-culture results showed that there were animals shedding in the highest category even in test year 2001 in all birth cohorts. The 1997 birth cohort showed a lower rate of fecal positive culture results compared to the 1996 and 1998 cohorts. Out of 455 cows in the study, 43 were culled because of Johne's disease clinical signs or high fecal-culture results. This cause-specific cull risk was 20% (21/105 animals) in 1996, 12% (12/100 animals) in 1997 and 10% (10/100 animals) in 1998. Survival analysis, where animals were classified according to the KELA and fecal-culture results for all animals tested as heifers, showed that heifers with the highest KELA value and/or the highest fecal-culture results had the shortest survival time in the herd. Analysis of birth cohorts led to an enhanced understanding of the response to the Johne's-control procedures. In this herd, early shedding led to short herd-survival.

Key words: Johne's disease, control, dairy farm, fecal culture

*Corresponding author:

Prof. Dr. Goran Bačić, DVM, Clinic for Reproduction and Obstetrics, Ambulatory Service, Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 322; Fax: +385 1 2390 230; E-mail: bacic@vef.hr

ISSN 0372-5480
Printed in Croatia

Introduction

Johne's disease (Paratuberculosis) is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and is a chronic, progressive enteric disease of ruminants. Cattle become infected with MAP as calves but usually do not develop clinical signs until 2 to 5 years of age (STABEL, 1998). The clinical disease is characterized by chronic or intermittent diarrhea, emaciation and sometimes death. MAP usually is introduced to dairy herds through the purchase of infected (though clinically normal) cattle (SWEENEY, 1996). Once MAP is present on a dairy farm, calves <6 months old are the most-susceptible animals; most infections are assumed to occur at this time. The primary method of transmission of infection is through fecal contamination of the calf's environment, including contamination of milk and feed. In addition, both clinically affected and normal-appearing infected cows can shed MAP in their colostrum and milk (SWEENEY et al., 1992; STREETER et al., 1995). On dairy farms, economic losses occur through premature culling, decreased milk production, and body-mass losses in slaughtered cattle. Milk-production losses alone ranged from 2 to 19% of milk yield in Johne's-positive cows compared to herdmates (NORDLUND et al., 1996). Although the economic impact of Johne's on the US cattle industry has not been determined, it is estimated to exceed $1.5 billion/ year. A Johne's-control program requires a herd plan specific to the operation (ROSSITER and BURHANS, 1996). It is a long-term strategy, because diagnosed cattle probably were infected at least 2 to 3 years before. Hence, analysis of the success of a control program in a dairy herd needs to take this disease feature into account. Because infection takes place at an early age with most clinical disease occurring later in life, an appropriate way to evaluate disease incidence is to perform birth-cohort analysis (JOHNSON et al., 2001). Birth cohorts re followed over time and their herd survival and test-scores are evaluated at set time intervals in their life-time.

We applied the birth cohort analysis on the last four years of a farm-specific Johne's program data. The farm tested all pregnant animals in mid-pregnancy by both KELA and fecal culture. "High" fecal-culture-positive animals (cows and heifers) were culled at the ends of their lactation. Also, Johne's-positive cows calved in a separate maternity pen from the Johne's-negative animals. Rubber pads were placed behind Johne's-positive animals at calving (to decrease calf contact with feces). Calves from those animals were removed immediately from their dams and fed colostrum from Johne's-negative animals. All clinical Johne's cows were separated from the herd and culled as soon as possible. However, this farmer decided not to cull fecal-culture "high shedders" immediately upon detection, and also continued to feed refusal feed from the milking herd to the young stock.

The objective of this paper is to show the birth-cohort evaluation of this farm's Johne's program.

Materials and methods

All Holstein heifers born in 1996, 1997, 1998 in a single farm and tested for MAP by kinetic ELISA (KELA) and fecal culture between January 1, 1998 and August 1, 2001 were included in the study. A computer search was performed on archive files in DairyComp 305 (Valley Agricultural Software) for eligible heifers. All eligible animals (regardless of testing results) were included in the study. The farm policy is to test all animals every year with both KELA and fecal culture at approximately 120 days of gestation. Animals that are "many" shedders in the fecal-culture results are culled at the end of their lactation. Their calves are delivered on rubber pads and immediately taken away.

At the beginning of investigation period total herd size were 485 animals, but the owner wished to increase the number of cows to 650 over 2-3 years. The last heifers were purchased in 1996/1997 from a herd not certified as Johne's free. Calves were housed separately from the cows in a calf facility, in individual cells. They were fed powdered milk replacer individually. Information was retrieved from the archive files for cow-identification number, birth dates, culling and Condition Affecting Record (CAR) records, KELA and fecal-test results and year of test. Animals were tested for MAP when they were two years old and then re-tested every year until exit from the herd. KELA results were grouped as: "low" (KELA 0-39), "medium" (KELA 40-54), "medium high" (KELA 55-164) and "high" (KELA>165) (JACOBSON et al., 1994). Fecal-culture results were grouped as: "negative" (0 colony-forming units (CFU) of feces), "few" (1-30 CFU/0.1g), "moderate" (31-299 CFU/0.1g) and "many" (≥300 CFU/0.1g or too numerous to count) shedders (SHIN, 1989). Animals were placed in birth-year cohorts, and those birth-year cohorts were monitored over time with regard to their Johne's infection and disease risks. Alternatively, heifers were monitored as cohorts based on the ages at testing: heifers (2-yr-olds), 3-yr-olds and so on. This was done because it is only useful to apply Johne's-disease diagnostics after a heifer is 2 years old (JUBB and GALVIN, 2000). The specificity (ability to identify an uninfected animal) of the KELA is estimated to be from 98.7% to 99.6% and sensitivity (ability to identify an infected animal) 18% to 32% when the probability of being a "moderate" or "heavy" fecal-shedder exceeds 50% as recorded by VAN SCHAIK et al., 2002.

Statistical analysis. The distributions of KELA and fecal categories were cross-tabulated by year in which the cow was born. KELA scores at age 2 years and then at age 3 years were compared between birth cohorts with the Kruskal-Wallis test (non-parametric one-way ANOVA). KELA scores at age 4 years were compared among birth cohorts with the Wilcoxon rank-sum test (because only the two earliest birth cohorts had data for 4-year-old animals). Our general hypothesis was that scores would decrease among more-recent birth cohorts if the control program was working. Fecal-culture scores were compared among birth cohorts in a similar fashion. Cull risks were calculated using

frequency distributions and cross-tabulation. Kaplan-Meier survival analysis was used to graph time from birth until culling and the hazard of culling among all animals tested as heifers for KELA and fecal-culture; differences in survival by test score were evaluated with the multi-sample log-rank test ($P<0.05$ was considered significant). The software packages Statistix 7.0 and SPSS 10.1 were used.

Results

The distribution of different KELA and fecal-culture categories through time: The single animal that had a KELA score >164 was a heifer born in 1996 and tested in the first test year 1998 (Table 1). However, there were heifers in the second-highest category in each birth cohort.

For the fecal-culture scores (Table 2), there were animals shedding in the categories 'moderate' or 'many' in every test year in all birth cohorts. The Kruskal-Wallis test showed no differences for either KELA or fecal-culture categories among the test years within birth cohorts (all $P\geq0.8$).

Comparisons of birth cohorts at different ages of testing - Kruskal-Wallis tests and the Wilcoxon rank-sum test for KELA categories, showed no differences between the birth cohorts in any of the test years (all $P\geq0.05$). For the fecal-culture categories, a difference between birth cohorts was found ($P=0.008$) for animals tested as 3-yr-olds (all other P values were ≥0.38). The 1997 birth cohort had significantly fewer infected animals compared to 3-yr-olds in either 1996 or 1998 but that was not the consistent decrease that we had hoped to see through time. Hazard analysis indicated that the hazard of becoming a moderate or heavy fecal shedder was variable among the birth cohorts - but was not reduced to zero in any of the three cohorts (Fig. 1). The 1997 cohort had a consistently lower hazard of becoming a fecal shedder compared to the 1996 and 1998 cohorts ($P<0.05$).

Cull risks - Out of 455 cows in the study, 272 were culled, died or were sold in our time window of observation. Out of those animals, 43 were culled because of Johne's-disease clinical signs or high fecal-culture results. The cull risk in the birth cohort of 1996 was 20% (21/105 animals; 95% CI 12,28), 12% in 1997 cohort (12/100 animals; 95% CI 5,19) and 10% in 1998 cohort (10/100 animals; 95% CI 4,16). (For the non-culled animals, their observation time was censored at August 1, 2001; hence, a lower cull risk is expected for the more -recent birth cohorts.)

Table 1. KELA-test results (category of *Mycobacterium avium* subspecies *paratuberculosis*) for cows born in 1996, 1997 and 1998 in New York

		Year of test							
		1998		1999		2000		2001	
Year of birth	Test result	No.	%	No.	%	No.	%	No.	%
1996	0-39	18	75	50	76	39	81	17	81
	40-54	2	8	6	9	1	2	2	10
	55-164	3	13	10	15	8	17	2	9
	>164	1	4	0	0	0	0	0	0
1997	0-39			7	58	67	91	22	71
	40-54			2	17	5	7	4	13
	55-164			3	25	2	2	5	16
	>164			0	0	0	0	0	0
1998	0-39					16	76	42	82
	40-54					1	5	3	6
	55-164					4	19	6	12
	>164					0	0	0	0

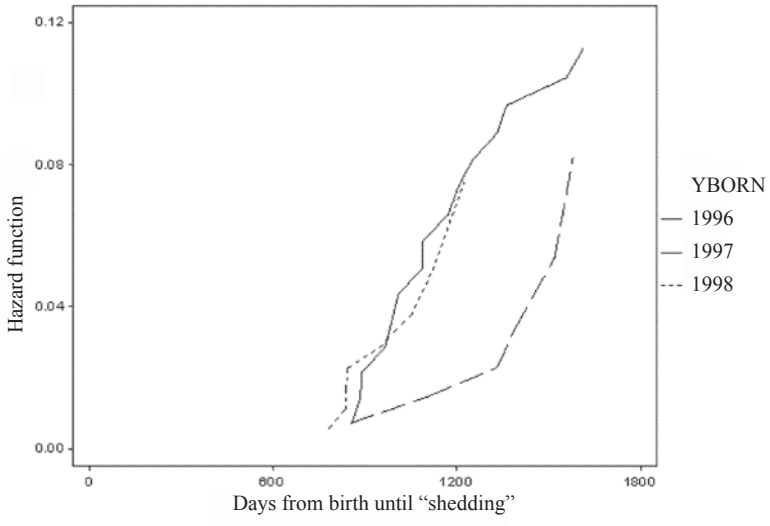

Fig. 1. Hazard of becoming a fecal-culture moderate or high among the 3 birth cohorts

Table 2. Fecal-test results for cows born in 1996, 1997 and 1998 in New York (CFUs/0.1g, *Mycobacterium avium* subspecies *paratuberculosis)*

		Year of test							
		1998		1999		2000		2001	
Year of birth	Test result	No.	%	No.	%	No.	%	No.	%
1996	neg	3	50	30	60	42	65	17	59
	few	2	33	10	20	8	12	7	24
	mod	0	0	5	10	7	11	2	7
	many	1	17	5	10	8	12	3	10
1997	neg			3	75	49	80	12	50
	few			0	0	10	17	7	29
	mod			1	25	2	3	2	8
	many			0	0	0	0	3	13
1998	neg					5	56	19	56
	few					0	0	8	23
	mod					0	0	2	6
	many					4	44	5	15

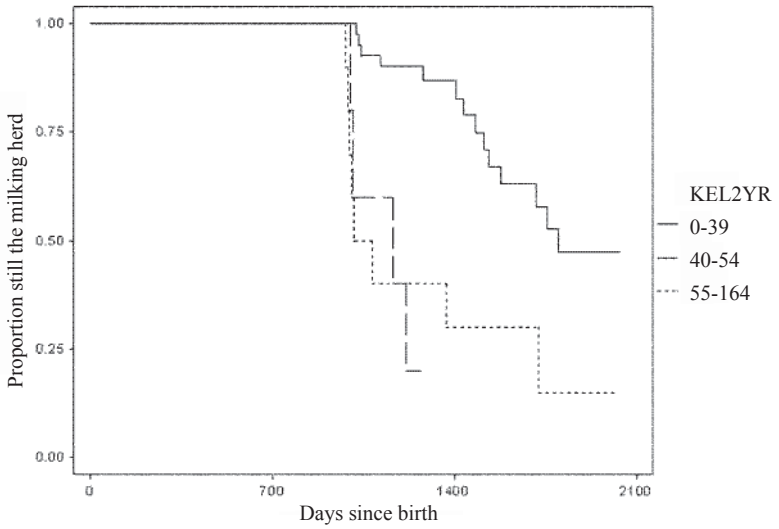

Fig. 2. Kaplan Meier survival time categorized by heifer KELA results

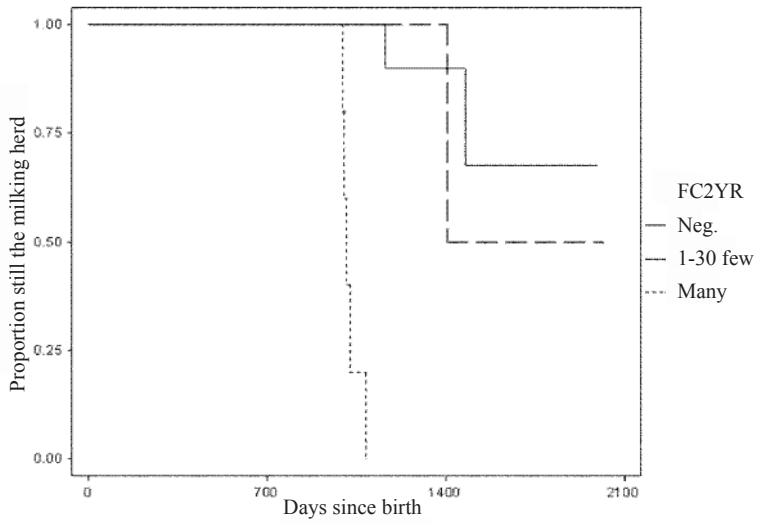

Fig. 3. Kaplan Meier survival time categorized by heifer fecal-culture results

Kaplan-Meier Survival Analysis and Log-rank test - Survival analysis for animals by KELA results for all animals tested as heifers (Fig. 2) shows that heifers in the 55-164 KELA category had the shortest survival time (median 1015 days on the farm, log rank p=0.003). Heifers in the lowest KELA category had the longest survival time (median 1777 days on the farm). The same analysis for fecal-culture scores (Fig. 3) shows that animals with the highest fecal-culture results survived the shortest amount of time (median 1007 days on the farm, log rank P=0.009).

Discussion

The KELA is useful for screening a whole herd for Johne's disease as described by DARGATZ et al. (2001), and the fecal-culture is specifically used to detect the heavy shedders. The sensitivity of KELA increases with repeated testing over time as recorded by WHITLOCK et al. (2000). But even after several years of testing, peaks in KELA reactor risks would not be surprising because the incubation period for reactors and clinical cases are 5.6 and 5.8 years, respectively. Reactor risks are expected to decline substantially after implementation of a successful Johne's eradication program (JUBB and GALVIN, 2000).

Each dairy herd needs an efficient control program for Johne's to help them control the disease. The results of our study indicate that Johne's disease could not reliably be eradicated in a short period of time using the methods used by this herd.

Blood test (KELA) and fecal culture of every cow at 120 days of gestation were performed for the last four years. The specificity of the KELA is considered high when fecal-culture is the comparison standard. However, KELA is not ideal for detecting individual cow infection due to relative low sensitivity.

Comparing test results at identical ages among birth cohorts should be a reliable method of evaluating control programs implemented in a given calendar year. In this herd, no difference was observed among age-specific comparison of birth cohorts for the KELA results. However, the fecal results showed a lower rate of infection among animals born in 1997 when evaluated at 3 years of age. Hence management practices in 1997 resulted in a lower (or delayed) rate of infection. Unfortunately, the 1998 birth-cohort showed a higher rate of infection and was very similar to the 1996 birth cohort (Fig. 1).

Heifers in the high KELA category and heifers within the group of highest fecal-culture scores had the shortest survival time in the herd. This is probably a direct consequence of culling all heavy shedders at the end of the lactation.

In this herd, refusal feed was fed to young heifers until recently and we observed a continuous spreading of Johne's disease. As of 2001, refusal feed has been fed only to dry cows. Future analysis of the 2001 birth cohort will show whether this change was valuable in reducing incidence of infection.

A Johne's-control program should be in place for every dairy herd. Evaluating birth cohorts as shown in this paper might provide a tool to monitor the efficacy of such a program.

Acknowledgements

The authors would like to thank the farm owners for allowing them to use the necessary data. Our gratitude also to the Diagnostic Laboratory and Quality Milk Production Services personnel of the College of Veterinary Medicine at Cornell University for all their help.

References

DARGATZ, D. A., B. A. BYRUM, L. K. BARBER (2001): Evaluation of commercial ELISA for diagnosis of paratuberculosis in cattle. J. Am. Vet. Med. Assoc. 218, 1163-1166.

JACOBSON, R. H., C. A. ROSSITER, Y. F. CHANG (1994): A new paradigm for interpretation of paratuberculosis serology: Profiling of herds based on multiple thresholds in ELISA. Proceedings of the 14th International Colloquium on Paratuberculosis, pp. 77-82.

JOHNSON, Y. J., J. B. KANEENE, J. C. GARDINER, J. W. LLOYD, D. J. SPRECHER, P. H. COE (2001): The effect of subclinical *Mycobacterium paratuberculosis* infection on milk production in Michigan dairy cows. J. Dairy Sci. 84, 2188-2194.

JUBB, T., J. GALVIN (2000): Herd testing to control bovine Johne's disease. Vet. Microbiol. 77, 423-428.

NORDLUND, K. V., W. J. GOODGER, J. PELLETIER, M. T. COLLINS (1996): Association between subclinical paratuberculosis and milk production, milk components and somatic cell counts in dairy herds. J. Am. Vet. Med. Assoc. 208, 1872-1876.

ROSSITER, C. A., W. S. BURHANS (1996): Farm-specific approach to paratuberculosis (Johne's disease) control. Vet. Clin. North Am. Food Anim. Pract. 12, 383-415.

SHIN, S. J. (1989): New methods for reduction in bacterial and fungal contamination from fecal culture for *Mycobacterium paratuberculosis*. Proceedings 93rd Annual Meeting of the USA Animal Health Association, 381.

STABEL, J. R. (1998): Johne's disease: a hidden threat. J. Dairy Sci. 81, 283-288.

STREETER, R. N., G. F. HOFFSIS, S. BECH-NIELSEN, W. P. SHULAW, D. M. RINGS (1995): Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am. J. Vet. Res. 56, 1322-1324.

SWEENEY, R. W. (1996): Transmission of paratuberculosis. Vet. Clin. North Am. Food Anim. Pract. Vol 12, 305-312.

SWEENEY, R. W., R. H. WHITLOCK, A. E. ROSENBERGER (1992): *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. Am. J. Vet. Res. 53, 477-480.

VAN SCHAIK, G., R. H. JACOBSON, Y. H. SCHUKKEN, S. M. STEHMAN, S. J. SHIN (2002): Predictive value of a kinetics ELISA to detect fecal shedding of *M. paratuberculosis* in New York State dairy herds. 7th International Colloquium on Paratuberculosis. p. 95.

WHITLOCK, R. H., S. J. WELLS, R. W. SWEENEY, (2000): ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet. Microbiol. 77, 387-398.

Received: 8 May 2008
Accepted: 4 June 2009

BAČIĆ, G., K. STIPETIĆ, Y. H. SCHUKKEN, C. GUARD, G. VAN SCHAIK, H. N. ERB: Povijesno kohortno istraživanje prevalencije paratuberkuloze u stadu mliječnih krava. Vet. arhiv 79, 323-332, 2009.

SAŽETAK

Svrha ovoga povijesnoga kohortnoga istraživanja bila je procijeniti djelotvornost predloženih kontrolnih mjera za paratuberkulozu (Johneovu bolest) na farmi mliječnih krava. Rabljene su arhivske datoteke iz računalnog programa Dairy Comp 305® za 455 krava holštajnsko-frizijske pasmine, oteljenih 1996., 1997. i 1998. godine. Pretražene junice bile su svrstane u skupine prema godini kada su bile oteljene. Opisani su rezultati kinetičkoga imunoenzimnoga testa (KELA) i rezultati izdvajanja uzročnika iz izmeta na čvrstoj (HEYM) i tekućoj hranjivoj podlozi (ESP Culture System II) te uspoređeni između promatranih skupina. Samo jedna životinja bila je pozitivna KELA testom. Usprkos tome rezultati izdvajanja uzročnika pokazali su da su postojale životinje s pozitivnim nalazom u promatranoj 2001. godini u svim istraživanim skupinama. Skupina životinja oteljenih 1997. godine pokazala je manju stopu bakteriološki pozitivnih uzoraka fecesa u usporedbi sa skupinama iz 1996. i 1998. godine. Od 455 krava promatranih u istraživanju, 43 su bile izlučene zbog kliničkih

znakova paratuberkuloze ili zbog pozitivnih rezultata pretrage fecesa. Uzročno specifični rizik za izlučivanje bio je 20% za životinje oteljene 1996. godine. Za životinje oteljene 1997. godine uzročno specifični rizik za izlučivanje bio je 12%, a 1998. godine spustio se na 10%. Analiza preživljavanja u kojoj smo životinje svrstali prema rezultatima KELA i izdvajanja bakterije iz svih životinja koje su bile pretražene kao junice, pokazala je da junice s najvišim pozitivnim nalazom KELA i bakterija imaju najkraću stopu preživljavanja u stadu. Analiza skupina prema godini otelenja omogućila je bolje razumijevanje učinkovitosti programa mjera za kontrolu paratuberkuloze u stadu. Rana pojava i pravovremena dijagnostika paratuberkuloze dovodi do skraćenja životnoga vijeka životinja u stadu.

Ključne riječi: paratuberkuloza, kontrolne mjere, mliječne krave, feces