Acta vet. scand. 1982, *23*, 53—64.

From the Departments of Clinical Pathology and Epidemiology & Preventive Medicine, University of California, Davis, USA, and the Department of Forensic and State Veterinary Medicine, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

CLINICAL BOVINE MYCOPLASMAL MASTITIS

AN EPIDEMIOLOGIC STUDY OF FACTORS ASSOCIATED WITH PROBLEM HERDS*

By

C. B. Thomas, D. E. Jasper and *P. Willeberg*

THOMAS, C. B., D. E. JASPER and P. WILLEBERG: *Clinical bovine mycoplasmal mastitis. An epidemiologic study of factors associated with problem herds.* Acta vet. scand. 1982, *23*, 53—64. — The California Dairy Herd Improvement Association records of 29 California dairies which experienced clinical mycoplasmal mastitis between January 1975 and December 1977 were examined and compared to the records of selected control herds. A 15-fold greater risk of clinical mycoplasmal mastitis was found among large herds as compared to small herds. On average, herds with clinical mycoplasmal mastitis culled 5 % more cows than did control herds (33 % vs 28 %). No difference was found in average milk production. These findings compare closely with the findings of a previous report where infected herds were identified by the presence of pathogenic mycoplasma in bulk tank milk. The similarity of results support the use of frequent bacteriologic culture of bulk tank milk as a routine surveillance strategy for mycoplasmal mastitis in endemic areas. The similarity of results also supports the use of routine clinical diagnostic data in the study of the epidemiology of diseases of veterinary importance.

mycoplasma; bovine mammary gland; mastitis; epidemiology; case-control study.

Bovine mycoplasmal mastitis has been diagnosed in California with increasing frequency since 1964 (*Jasper* 1981), and simultaneously the disease has appeared in other geographical areas with intensive milk production (*Boughton* 1979, *Jasper*).

* Supported in part by funds from the California Milk Advisory Board, the U.S. Department of Agriculture, Agricultural Research Service General Cooperative Agreement and a National Institutes of Health, Public Health Service Biomedical Research Support Grant.

In an attempt to identify some of the key herd factors which may act as determinants of the infection, a previous report (*Thomas et al.* 1981) dealt with the comparison of bulk tank positive and negative herds from a survey in California (the BTS study).

In the present report, a sample of herds in which laboratory confirmed clinical outbreaks of mycoplasmal mastitis had occurred is compared to a selected control group to evaluate the same herd factors as in the previous study, i.e., herd size, culling rate, and production parameters.

Thus the present study was performed to investigate whether herds with a clinical outbreak of mycoplasmal mastitis as reported to the diagnostic laboratory (problem herds) differ from herds classified in the bulk tank survey (BTS herds) with respect to certain key herd factors.

MATERIALS AND METHODS

Selection of case and control herds

Data were obtained from 2 sources. Data on herd factors were obtained from the California Dairy Herd Improvement Association (CDHIA), Cooperative Dairy Extension, University of California at Davis; the data supplied were the 1975, 1976 and 1977 year-end herd summaries for CDHIA official herds.

The identity of herds, which had experienced clinical mycoplasmal mastitis during the same years, was obtained as a subset from the laboratory records of the Mastitis Research Laboratory, Department of Clinical Pathology, School of Veterinary Medicine, University of California, Davis. A description of the full set of problem herds with confirmed mycoplasmal mastitis diagnoses spanning the years 1964 to 1978 has been presented (*Jasper et al.* 1979).

The major criterion for selection as a case herd for the present study was official record status for the year-end CDHIA herd summary during the year clinical mycoplasmal mastitis was identified. Of the 69 herds identified positive for clinical mycoplasmal mastitis from January 1975 to December 1977, 29 herds met this criterion. Table 1 presents the species distribution of all problem herds identified between January 1975 and December 1977. The species distribution of the subset of problem herds selected as case herds is also presented. Of the 3 case herds with mixed infection one was M. bovis and an unknown species, another was M. arginini and M. bovirhinis and the third was

Table 1. Frequencies and proportional distribution by mycoplasma species of all problem herds (1975—1977) and of selected case herds.

Species	All problem herds		Selected case herds	
	number	% of total	number	% of total
M. bovis	30	43.5	13	44.8
M. californicum	13	18.8	4	13.8
M. alkalescens	9	13.0	4	13.8
M. canadense	3	4.3	3	10.3
M. bovigenitalium	4	5.8	2	6.9
M. bovirhinis	1	1.4	0	0
M. arginini	1	1.4	0	0
Unknown species	3	4.3	0	0
Mixed species infections	5	7.2	3	10.3
Total	69	100.0	29	100.0

M. alkalescens, M. bovigenitalium and M. bovis. The percentage representation by species among the case herds closely approximates that of all problem herds for the period. There were too few case herds to allow stratification by mycoplasmal species in the subsequent analysis.

Table 2 presents the yearly distribution of all problem herds and those selected as case herds. Proportionally fewer problem herds identified positive in 1977 are represented among the latter.

Table 2. Frequencies and proportional distribution by year of all problem herds (1975—1977) and of selected case herds.

Year	All problem herds		Selected case herds	
	number	% of total	number	% of total
1975	7	10.1	5	17.2
1976	14	20.3	8	27.6
1977	48	69.6	16	55.2
Total	69	100.0	29	100.0

The laboratory records contained inadequate information for staging by severity of each epidemic in terms of prevalence of mycoplasmal mastitis within these herds. Therefore clinical mycoplasmal mastitis was defined as positive culture for mycoplasma from individual quarter or composite quarter milk samples from clinically mastitic cows and accompanied by a history of a herd mastitis problem. All case herds were Holstein-Friesian.

All control herds were Holstein-Friesian and had official record status for the year-end CDHIA herd summary for the appropriate year. Matching was employed in the selection of control herds from among all bulk tank negative CDHIA herds. In order to avoid yearly variation in production and culling variables due to extrinsic factors such as variation in feed cost, the CDHIA records from the year of identification of mycoplasmal mastitis were used both for the case herds and the matched control herd.

Two control herds were selected for each case herd from the same county, matching (within ± 10 %) by cow-years-in-herd. This is the herd size control group. All calculations, averages and changes reported by the CDHIA are based on the number of cow days converted to cow months and cow years (*Etchegaray et al.* 1976). Therefore, cow-years-in-the-herd is the most accurate and comparable measure of herd size reported by the CDHIA and was used throughout this study. In 1 instance an appropriate matching on herd size was impossible within the same county. There were 57 herd size control herds.

Two control herds were also selected for each case herd from the same county by matching (within ± 10 %) on average kg milk per cow per year. This is the production control group. There were 58 production control herds.

Methods of analysis

The means and standard deviations were computed for variables in the CDHIA data, and t-statistics were computed comparing the mean of the appropriate control group with the mean of the case group.

Variables examined were: cow-years-in-herd (Herd size); average percentage of cows dry (AveDry); percentage of cows leaving the herd during the year (% culled); average milk in kg/cow/year (AveMilk); average fat in kg/cow/year (AveFat); and average fat % $\times$ 100 for the herd (% Fat).

A computer program (*Willeberg* 1977) was used for the classification of herd summary records as well as for computation of relative risk (R) values and chi square statistics for chosen levels of each variable. The program used the Mantel-Haenszel method (*Mantel & Haenszel* 1959) for adjusting these R values and chi square statistics for the possible confounding influence of the other variable(s). Values of R greater than 1.5 were considered biologically important.

The population attributable risk percentage for the herd size variable was computed according to the method of *Cole & MacMahon* (1971). This involved the assumption that the distribution of herd size among control herds was representative of the CDHIA official herd population at large. Table 3 presents the frequencies and proportional distribution by herd size of 1010 CDHIA herds with official herd averages for the year 1977. Differences in the proportional representation of herd sizes between selected control herds and the CDHIA official herd population at large are considered minor.

Table 3. Frequencies and proportional distribution by 3 levels of herd size for production-matched control herds and for all negative CDHIA official herds for the year 1977.

	Production controls		CDHIA herds*	
Herd size	number	% of total	number	% of total
1—349	37	63.8	682	67.5
350—699	15	25.9	256	25.3
≧ 700	6	10.3	72	7.1
Total	58	100.0	1010	99.9

* 43 herds with CDHIA official records are not included because pathogenic mycoplasma were identified in their bulk tank milk.

Initially, the population relative risk was derived as the sum of the products of the herd size specific crude relative risk values and the proportion of control herds in the same range of herd size. Thus:

$$R_{pop}=(P_{1-349}\times 1)+(P_{350-699}\times R_{350-699})+(P_{700+}\times R_{700+})$$

where the P_i's are the proportions of control herds in the respective herd size ranges. Having computed the R_{pop}, the population attributable risk percentage is:

$$A_{pop}\,\% = [(R_{pop}-1)/R_{pop}]\times 100.$$

It expresses the proportion of clinical mycoplasmal mastitis appearing in the population of CDHIA herds which can be attributed to the effect associated with herd size above 350 cows.

RESULTS

Table 4 presents the means and standard deviations for 6 CDHIA production variables. Highly significant differences

Table 4. Means and (s) of 6 selected CDHIA production variables for case-herd and control-herd groups.

Group	Number of herds	Variables					
		Herd size (cow years)	AveDry (%)	% culled (%)	AveMilk (kg/year)	AveFat (kg/year)	% Fat (%×100)
Problem herd cases	29	671 (457)	14.7 (1.9)	33.5 (11.9)	7708 (781)	278 (26)	361 (17)
Production-matched control herds	58	389** (390)	14.7 (2.8)	27.2** (9.6)	7733 (743)	277 (27)	358 (18)
Herdsize-matched control herds	57	614 (386)	15.5 (3.5)	28.3* (8.6)	7577 (1025)	278 (35)	368 (25)

* $P < 0.05$
** $P < 0.01$

($P < 0.01$) were noted between the production-matched control herd group and the case herd group for 2 variables — herd size and % culled. Covariance analysis of a similar data set revealed a strong covariation between the herd size and % culled variables (data not shown). The smaller mean herd size of the production-matched control herd group is considered to account for the lower % culled value. A significant difference ($P < 0.05$) was noted between the herd size-matched control group and the case group for the % culled variable. Matching by herd size eliminates the potential biasing effect of herd size in the comparison of the mean % culled values between the case group and the control group.

In order to study the cause-effect relationship between clinical mycoplasmal mastitis and the higher percentage of culling, it was necessary to examine the culling percentages of the case herd group in the years preceding and/or subsequent to the year of the occurrence of clinical mycoplasmal mastitis. Twenty-four of the 29 selected case herds had CDHIA official records for all 3 years. Table 5 presents the mean value of the % culled variable for each year for the 24 herds grouped by the year clinical mycoplasmal mastitis was first identified. In each case the highest mean for the % culled variable occurred during the year the mycoplasmal mastitis epidemic was identified. To assess the statistical significance of this pattern repeated measures analysis of variance was used with the expectation that the interaction

Table 5. Average culling percentages for 1975, 1976, 1977 for case herds grouped by year of first mycoplasmal mastitis problem.

Year first positive	Number of herds	% culled			Mean
		1975	1976	1977	
1975	5	30.2	25.8	27.8	27.9
1976	8	30.4	37.1	31.3	32.9
1977	11	29.8	29.2	33.5	30.8
Overall	24	30.1	31.1	31.5	30.9

term would show significance. The interaction term attained a $P = 0.06$, while no significant differences existed among either of the 2 sets of marginal mean values.

The 2 variables — herd size and % culled — were the only variables having R values greater than 1.5. Table 6 presents the frequencies in the case and control groups stratified by the chosen levels of each of these variables. The adjusted and the crude approximate relative risk values are presented, and the level of significance of the chi square statistic is indicated. To remove the confounding effect of herd size on the % culled risk

Table 6. Frequencies and relative risk of mycoplasmal mastitis by 3 levels of herd size and 2 levels of culling for problem herds, as well as relative risk for the 50+ colony count group from the BTS study.

Factor/level	Number of herds		Adjusted R values[1]	
	case herds	production controls	case herds	BTS study 50+ colonies[2]
Herd size				
1—349	3	37	1	1
350—699	19	15	14.33* (15.62)	11.19
≧ 700	7	6	15.09* (14.39)	22.89
		herd size controls		
% culled				
<29 %	10	28	1	1
≧29 %	19	29	1.70 (1.83)	2.85

* $P < 0.005$.

[1] R values in parentheses are crude R values.

[2] BTS results from *Thomas et al.* (1981).

estimate, the analysis of the latter factor was done using the herd size-matched control herd group. *Jasper et al.* (1979) who initially reported on these data, concluded empirically that large numbers of pathogenic mycoplasma (more than 50 colonies from a 0.03 ml inoculum) in bulk tank milk were indicative of an active herd infection. Thus herds identified positive with 50 or more colonies of pathogenic mycoplasma in the BTS study were most comparable to the case herds in this study and the risk estimate for that group for the herd size and % culled variables are included in Table 6.

Table 7 presents the estimated population attributable risk percentage and its 95 % confidence interval for herd sizes of 350 cows or more. Similarly the A_{pop} % for the 50+ colony count group from the BTS study is presented for comparison.

Table 7. Population attributable risk percentage and 95 % confidence interval for the effects associated with herd size above 350 cows and the population attributable risk percentage for 50+ colony count herds of 350 cows or greater from the BTS study.

Problem herd study		BTS study[1]
A_{pop} %	95 % confidence interval	A_{pop} %
83.8 %	(68 %, 100 %)	83.6 %

[1] BTS results from *Thomas et al.* (1981).

DISCUSSION

The case-control research method has been both lauded and maligned. Proponents cite feasibility both in economic and ethical terms as the major advantages of the method compared to prospective studies or direct experimentation. The disadvantages cited are the difficulty in distinguishing true causality from spurious association and the vulnerability of the method to bias of selection or of chronology. General acceptance of an association between a proposed determinant and increased risk of the occurrence of a disease, discovered by the case-control method, comes when several studies, each scrutinized for the possibility of bias, produce consistent evidence which supports a biologically reasonable hypothesis (*Willeberg* 1977).

This report and the BTS study used the same source of production data for the study of candidate herd factors as deter-

minants of the occurrence of mycoplasmal mastitis and consequently, although this report spans a 3-year period of case herd accession, both reports make inference to approximately the same target population. The major difference between these 2 reports is in the method of case identification. Case herds in the BTS study were identified from a cross-sectional survey and derived some protection from bias by the high sampling fraction of the survey. No such systematic sampling frame existed in the accession of the problem herd series from which the subset of CDHIA herds were selected as the case group in this study. Therefore, the potential for a lack of representativeness in this case group exists.

Jasper et al. (1979) in commenting on the representativeness of the problem herd series, noted that the diagnostic laboratory records were believed to reflect most of the confirmed diagnoses of mycoplasmal mastitis made in California between May 1964 and July 1978. This observation along with the matching by county in selection of control herds protects against regional bias. However, selection bias involving the 6 CDHIA variables studied cannot be ruled out. As an example, suppose that larger herds were more likely to have submitted mastitic milk samples to the diagnostic laboratory for mycoplasmal culture. Such an occurrence would have led to a differential probability favoring detection of mycoplasma in larger herds and hence the statistical association between larger herd size and risk of mycoplasmal mastitis.

There is no direct way of assessing the magnitude or direction of such a bias in the assembled case herd group for the 6 CDHIA variables examined. *Lilienfeld & Lilienfeld* (1980) have pointed out, that potential selection bias' do not necessarily invalidate study findings. Several indirect means exist to determine that observed associations are real. Within a given study the strength of an association measured by the relative risk can be evaluated. Relative risk values greater than 2 or 3 are unlikely to arise from selection bias. The risk estimate for the herd size variable in this study is well above this threshold.

Consistency of results between studies is a major criterion for acceptance of case-control study results. The results of this report and the BTS study (*Thomas et al.* 1981) are remarkably similar. Not only were the same herd characteristics identified in both studies, but the relative magnitude of risk associated

with these herd factors measured either by relative risk or attributable risk were similar. Where different methods of case identification and independent assembly of control groups were employed in producing these results, confidence is enhanced that the results are real and not the artifact of the referral pattern to the diagnostic laboratory.

In Table 4 a difference of 5 percentage units in the mean % culled is noted between the case herd group and the herd size-matched control herd group (33 % vs. 28 %). In Table 5 approximately the same difference is noted within each herd group when comparing the mean % culled in the year of the outbreak to the other 2 years. Though the value of approximately 5 percentage units is consistent the authors feel it underestimates the level of culling associated with most mycoplasmal mastitis outbreaks and would be a conservative value to use in estimating the economic effects of culling due to mycoplasmal mastitis. Dairy farmers who have had to cull heavily due to an outbreak of mycoplasmal mastitis are likely to have compensated by retaining cows which in the absence of the outbreak would have been culled for various other reasons. Thus the proportion of the % culled attributable to a mycoplasmal mastitis outbreak may be greater than the mean difference of approximately 5 percentage units. *Jasper* (1981) in discussing the economic aspects of M. bovis mastitis noted that usually 10 % of a herd is infected by the time the diagnosis is made and usually not more than 25—30 % of the cows are infected before the epidemic is controlled.

The biological interpretation of the association between mycoplasmal mastitis and large herd size and increased percentage culling have been discussed in the BTS report. We have presented new information of a longitudinal nature in Table 5 which suggests that the risk of increased culling is the result of the presence of mycoplasma and not vice versa. Due to the temporal cross sectional nature of the BTS study this was not discernable.

Briefly, the interpretation of the herd size association which the authors favor is that herd size indirectly measures several factors of management, animal density and environment which are commonly found in herds of large size in California and which independently or in combination put these larger herds at higher risk of contracting mycoplasmal mastitis.

CONCLUSIONS

Specific

1) There is a 15-fold increase in risk of clinical mycoplasmal mastitis for CDHIA dairy herds which exceed 350 cows, and 85 % of the clinical mycoplasmal mastitis outbreaks in CDHIA dairy herds which occurred between January 1975 and December 1977 could be attributed to factors associated with herd sizes of 350 cows or greater.

2) A minimum estimate of the effect of a mycoplasmal outbreak on the mean annual culling percentage is an increase of 5 percentage units.

3) The similarity of findings between 2 studies, whether mycoplasmal positive herds were identified from bulk tank samples or by clinical mastitis samples, indirectly supports the routine use of bacteriologic culture of bulk tank milk samples as a surveillance strategy for early detection of herd outbreaks of mycoplasmal mastitis.

General

The similarity of results from this study, derived from the accession records of a clinical diagnostic laboratory, with those of the BTS study, derived from a population based survey, should encourage investigators responsible for veterinary clinical pathological and microbiological diagnostic services to make greater epidemiologic and analytic use of their laboratory data.

ACKNOWLEDGEMENT

Mr. F. Murrill of Cooperative Dairy Extension, University of California, Davis is cordially thanked for providing the CDHIA data.

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SAMMENDRAG

Klinisk mycoplasma mastitis hos kvæg. En epidemiologisk undersøgelse af determinanter for problem-besætninger.

Kontrolforeningsdata for 29 californiske malkekvægsbesætninger, hvori kliniske udbrud af mycoplasma mastitis forekom i perioden januar 1975 — december 1977, blev analyseret ved sammenligning med data fra „matchede" kontrol-besætninger. En 15-gange forøget risiko for sygdommen fandtes blandt store besætninger (over 350 års-køer) sammenlignet med mindre besætninger (under 350 års-køer). Sygdomsramte besætninger udsatte i gennemsnit 5 %-enheder flere køer end kontrol-besætningerne (33 % vs. 28 %). Der var ingen forskel i de gennemsnitlige ydelsestal. Disse resultater stemmer helt overens med resultaterne af en tidligere publiceret undersøgelse, hvor sygdomsramte besætninger blev identificeret ved hjælp af indsamlede tankmælksprøver. Dette understøtter anvendelsen af sådanne prøver i overvågningsprogrammer for mycoplasma-mastitis, ligesom det understøtter anvendelse af data fra indsendte diagnostiske prøver i forbindelse med undersøgelser af epidemiologiske forhold vedrørende sygdomsproblemer i veterinær praksis.

(Received January 8, 1982).

Reprints may be requested from: P. Willeberg, the Department of Forensic and State Veterinary Medicine, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.