Subclinical Ketosis: Prevalence and Associations with Production and Disease

I.R. Dohoo and S.W. Martin*

ABSTRACT

Cows in 32 southern Ontario Holstein herds were monitored for subclinical ketosis for a period of two and one half years. Milk samples were routinely collected and the level of milk ketone bodies determined by the use of a commercial nitroprusside based test powder (reactions scored as negative, +1 or +2). Approximately 92% of positive reactions were observed in the first 65 days of lactation and for that time period the prevalence of ketosis was 12.1%. Based on this prevalence, the minimum possible duration of subclinical ketosis would be 7.9 days and the minimum possible lactational incidence rate would be 12.1%. The prevalence during the first 65 days of lactation in individual herds ranged from 0 to 33.9%.

Subclinical ketosis was more likely to be found in cows experiencing metritis than in unaffected cows. Detection of elevated levels of milk ketones also indicated that the cow had a significantly higher risk of having clinical ketosis, metritis or cystic ovaries diagnosed within the following four days. Milk ketone scores of +1 and +2 were found to be associated with a reduction in daily milk production of 1.0 and 1.4 kg of milk respectively.

Key words: Ketosis, subclinical, prevalence, effect, production, associations.

RÉSUMÉ

Cette étude s'échelonnait sur une période de deux ans et demi et elle consistait à tenter de déceler l'acétonémie subclinique, chez les vaches de 32 troupeaux Holstein du sud de l'Ontario. On préleva régulièrement, à cette fin, des échantillons de lait dans lesquels on rechercha la présence de corps cétoniques, à l'aide d'une poudre commerciale qui contenait un sel de l'acide cyanhydrique; on enregistra les résultats de la façon suivante: négatifs, +1 ou +2. Environ 92% des réactions positives survinrent pendant les premiers 65 jours de la lactation, période au cours de laquelle la fréquence de l'acétonémie atteignit 12,1%. D'après ces résultats, la durée la plus brève possible de l'acétonémie subclinique serait de 7,9 jours, tendis que le taux le plus bas possible de sa fréquence, au cours de la lactation, se situerait à 12,1%. La fréquence de l'acétonémie durant les premiers 65 jours de la lactation varia de 0 à 33,9%, selon les troupeaux.

L'acétonémie subclinique s'avéra plus susceptible d'affecter les vaches atteintes de mammite que les autres. La détection d'une haute teneur du lait d'une vache en corps cétoniques indiquait aussi qu'elle courait un plus grand risque de manifester de l'acétonémie, de la métrite ou des kystes ovariens, dans les quatre jours suivants. Des réactions lactées de +1 et +2 se révélèrent respectivement associées à une baisse de 1 et 1,4 kg de la production de lait quotidienne.

Mots clés: acétonémie, subclinique, fréquence, effet, production, associations.

INTRODUCTION

Ketosis can exist in both clinical and subclinical forms with clinically ketotic cows representing one end of a continuous spectrum of the condition (1). Detection of the subclinical form of the disease by the use of a nitroprusside based test powder has been shown to be a relatively simple and reliable procedure (2). However, very little is known about the incidence or prevalence of the subclinical condition.

In general, diseases which are subclinical in nature are becoming the ones of greatest economic importance in the dairy industry (3). Although it is reasonable to assume that subclinical ketosis has a detrimental effect on milk production very little is known about the magnitude of that effect. If a program to monitor and perhaps treat the disease is to be considered, it is essential to have a thorough understanding of the associations between this disease, other diseases and milk production.

The purpose of this study was to determine the overall prevalence and distribution of subclinical ketosis in 32 southern Ontario dairy herds. In addition, relationships between subclinical ketosis and several other diseases were evaluated as was the association between subclinical ketosis and milk production.

MATERIALS AND METHODS

A research project designed to evaluate relationships amongst diseases, production and survivorship was carried out in 32 southern Ontario Holstein herds between February 1979 and August 1981. Details of the project have been reported elsewhere (4). All herds were enrolled on a production testing program (Record of Performance or Dairy Herd Improvement Association) and a veterinary herd

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health program, with the latter either being provided by private practitioners (24 herds) or the Ontario Veterinary College (OVC) farm service (eight herds). On average the herds were slightly higher producing than other herds on production testing programs in Ontario (averages of 140 and 130 breed class average units respectively). Health and fertility records were maintained on all farms by the dairymen and their veterinarians and were routinely collected by the senior author. Production data were obtained directly from the production testing organizations. During the project, health, fertility and production data were recorded for 2876 lactations in 2009 cows.

In addition to this data, milk samples were collected for testing for subclinical ketosis and subclinical mastitis. For the eight herds closest to the OVC, composite (cow) milk samples were collected at each visit by a milk production testing fieldman (approximately 10-12 times per year). For the remaining 24 herds, samples were collected four times per year. All samples were refrigerated and shipped fresh to the OVC. Samples were tested, within three days of collection, for ketone bodies using a qualitative nitroprusside based test (Ketotest, Denver Laboratories, Montreal, Canada). Approximately 0.5 mL of milk was added to approximately 10 g of test powder and the colour reaction was recorded after three minutes. Results were scored as negative, +1 or +2.

Test results were merged with the health, fertility and production data by a series of interactive computer programs. The prevalence of positive test reactions in the whole lactation and in the first 65 days of the lactation were determined. This latter value was used to estimate a range of possible lactational incidence rates based on the range of possible durations of subclinical ketosis. The distributions of positive test results by farm and by number of days postpartum were determined using the Statistical Package for the Social Sciences (5).

Associations between test results and other diseases were evaluated using contingency table analysis (6). All diseases which were diagnosed in at least ten cows during the four days immediately prior to the collection of a milk sample and also in at least ten cows during the four days following a milk sampling were identified. The presence or absence of the disease during each four day time period was crosstabulated with the presence or absence of a positive test result and the strength of the association was measured by the odds ratio (OR) (7). The significance of the odds ratio was tested using a chi-square statistic incorporating Yate's correction for continuity.

Least squares multiple linear regression (5) was used to evaluate the association between subclinical ketosis and test day milk production according to the model:

$$Y_{ijk} = u + k_i + h_j + \beta_1(a_{ijk}) + \beta_2(d_{ijk}) + \beta_3(d_{ijk2}) + \beta_4(p_{ijk}) + \beta_5(s_{ijk}) + E_{ijk}$$

where Y_{ijk} = milk production in kg for the k^{th} observation in the j^{th} herd in the i^{th} ketone score.
u = population mean
k_i = fixed effect of the i^{th} ketone score
h_j = fixed effect on the j^{th} herd
β_1 to β_5 = regression coefficients for the age at calving (a_{ijk}), days in milk (d_{ijk} and d_{ijk}^2), previous lactation milk production (p_{ijk}) and the $\log_e$ somatic cell count (s_{ijk}).
E_{ijk} = random error term.

The effects of subclinical ketosis were also expressed in terms of percentage of mean production.

PREVALENCE OF POSITIVE KETONE TESTS BY DAYS POSTPARTUM

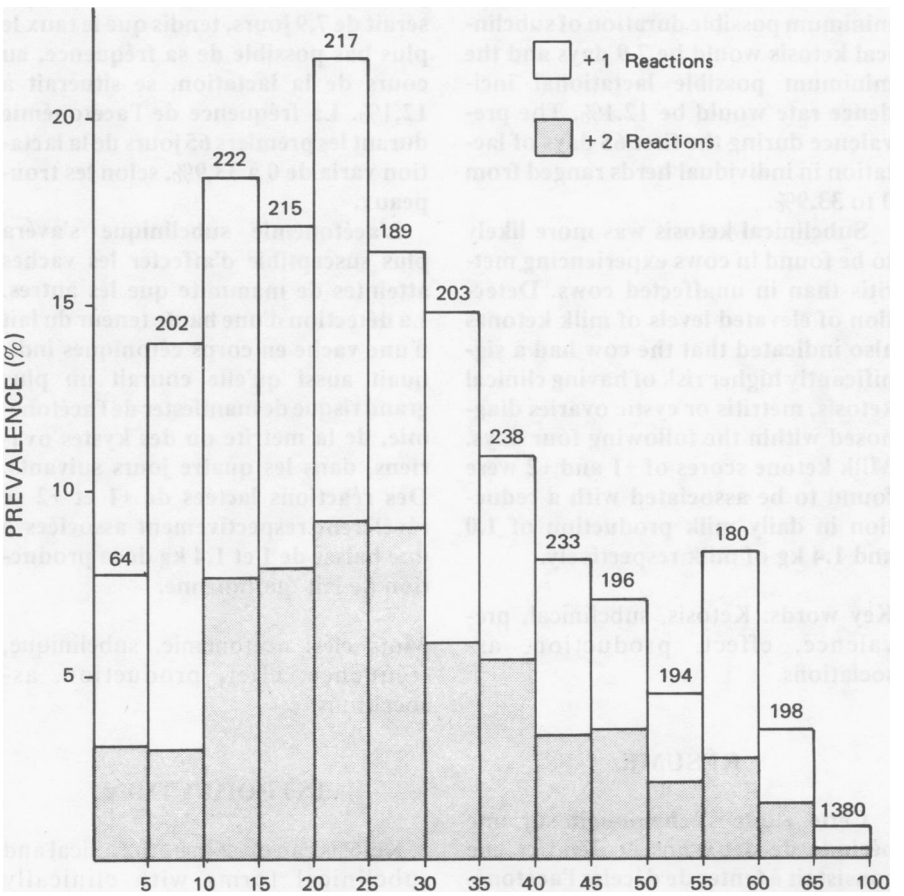

Fig. 1. Prevalence of positive ketone tests by time postpartum. Number of tests performed is given at the top of each column.

RESULTS

During the study 11,453 milk samples were collected and ketosis test scores were recorded for 11,424 of them. Of these, 335 (2.9%) had a positive reaction (+ 1 or + 2). Most positive reactions (91.6%) occurred within 65 days of calving and a total of 2551 tests were carried out in that time period. Of these, 307 (12.1%) had a positive reaction (+ 1 or + 2) and 131 (5.1%) were scored as + 2.

The distribution of reactions by five day intervals postpartum is given in Figure 1. The prevalence of positive test results peaked in the 21-25 day period postpartum and 56% of the positive results were found between days 11 and 35. In each time period the proportion of the positive results which were classified as + 2 remained relatively constant at 40-45% of the total number of reactions.

For individual farms the overall prevalence of positive tests (based on the whole lactation) ranged from 0 to 8.5%, while the prevalence based on tests performed in the first 65 days of the lactation ranged from 0 to 33.9%. The distribution of herds by prevalence for the 65 day postpartum period is shown in Figure 2. The prevalence of positive reactions within a herd on any one test day ranged from 0 to 26.5% of the milking cows.

Based on the value of 12.1% for the prevalence of positive tests in the first 65 days of lactation, a range of possible lactational incidence rates was calculated. The lactational incidence rate is the proportion of cows developing subclinical ketosis during their lactation (4). Figure 3 shows the relationship which would have to exist between the incidence rate and the average duration of positive test results in order for a prevalence of 12.1% to be realized. The minimum average duration would be 7.9 days if all cows were affected (lactational incidence rate = 100%) and conversely the minimum incidence rate would be 12.1%, if those cows had a positive result for the entire 65 day period.

Four disease conditions were diagnosed in at least ten cows within four days before a milk sampling and also in at least ten cows within four days following a sampling. These conditions were metritis (included all bacterial infections in the reproductive tract), cystic ovaries, ketosis (clinically apparent) and mastitis. The odds ratios measuring the association between these conditions and the ketone test results are given in Table I. None of the odds ratios for diseases occurring prior to the test were significant, and for three of the four conditions there were no positive ketone test results within the four days after the condition was diagnosed. On the other hand a positive test result indicated a significantly higher risk of metritis, cystic ovaries or ketosis being diagnosed in the subsequent four day periods.

A total of 6443 test day results were used to evaluate the effect of subclinical ketosis on the test day milk production. Of the 5010 observations which were not used, most were eliminated because they did not have values for milk production in the previous lactation. All results based on milk samples from heifers would fall into this group. The regression function had a coefficient of determination (r^2) of 0.83 and the fixed effects for ketone scores of + 1 and + 2 were -1.03 and -1.40 kg respectively (effects significantly dif-

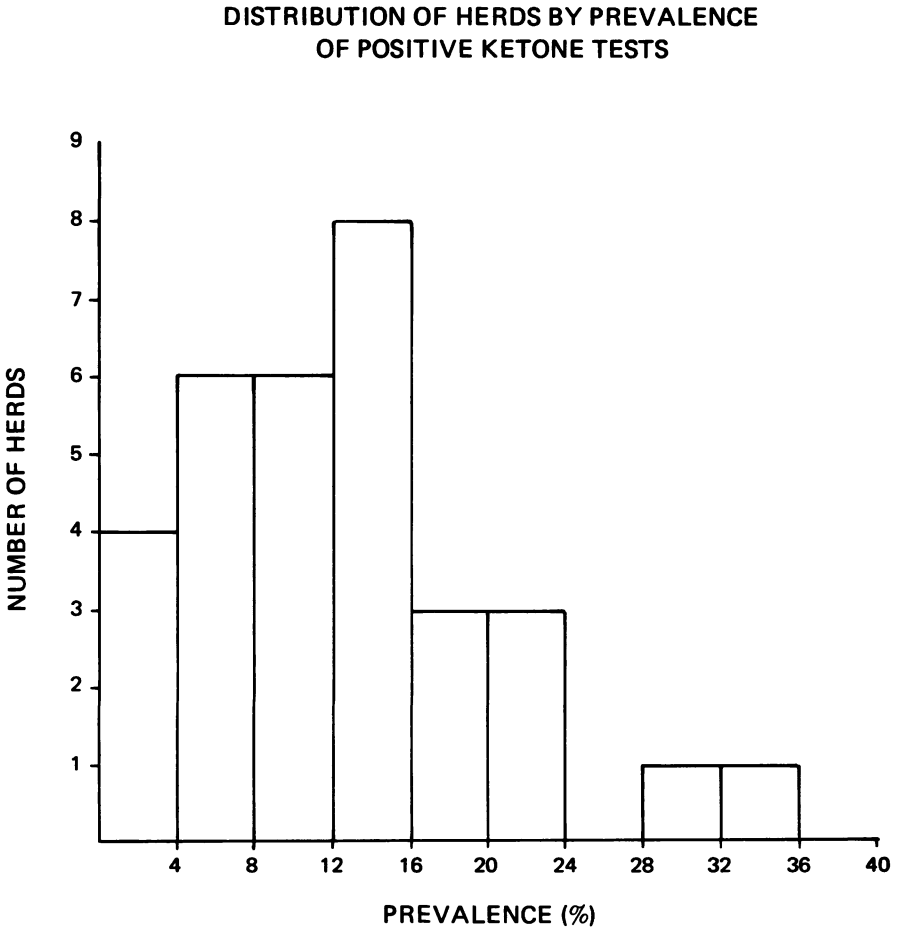

Fig. 2. Distribution of number of herds by prevalence of positive ketone tests in the first 65 days postpartum.

TABLE I. Number of Diagnoses (n) of a Disease within Four Days of a Milk Sampling and the Odds Ratio (OR) of its Association with a Positive Ketone Test

	Disease in the four days prior to a milk sampling		Disease in the four days following a milk sampling	
Disease	n	OR	n	OR
Metritis	36	1.95	48	10.1[a]
Cystic ovaries	26	nc	21	5.6[a]
Ketosis	13	nc	12	33.7[a]
Mastitis	31	nc	42	0.8

[a]Significant at $p<0.01$

nc = Not calculated because no positive ketone tests occurred within four days of the diagnosis of the disease

ferent from zero at $p < 0.05$). The mean production was 23.5 kg so the losses associated with ketone scores of + 1 and + 2 were approximately 4.4 and 6.0% of the mean production respectively.

DISCUSSION

Subclinical ketosis can be detected by measuring ketone levels in the blood, milk or urine. Urine ketone levels have been shown to be unreliable for the detection of hyperketonemia because of frequent false positive results (i.e. lack of specificity). On the other hand milk ketone levels are approximately 35-50% of the blood levels (2,8,9) and a commercially available test powder will detect milk ketone (acetone plus acetoacetate) levels in excess of approximately 4.0 mg% (2,9). Consequently, the milk test will usually detect blood ketone levels over 10 mg%, which is generally accepted as the lower limit for a cow to be considered subclinically ketotic (2,10).

The temporal distribution of positive test results shown in Figure 1 is in close agreement to previously published results based on 31 cows from a University dairy herd (11). In that study, peak ketone levels were observed about day 28 but levels were substantially elevated from day 10 to day 35. The relatively tight clustering of positive results suggests that if a program is set up to monitor milk ketone levels, cows would only have to be tested for the first two months of the lactation.

The large farm to farm variation in the rate of subclinical ketosis is shown in Figure 3. Although most farms had a prevalence of positive test results of between four and 16% during the first 65 days of lactation, two farms had levels over 28% and one farm had none at all. The etiology of ketosis is complex and factors leading to its development have been reviewed elsewhere (1,2,12,13,14,15). It is likely that different sets of conditions on the farms in this study resulted in the wide range in the prevalence of the disease, however evaluation of the role of specific causal factors was beyond the scope of this project.

The prevalence of a disease is the number of animals affected with the disease at a point in time. (In this paper the prevalence is expressed as a proportion of the population at risk and strictly speaking should be referred to as a prevalence proportion. However, in general usage the term prevalence is used for both measures.) The prevalence of subclinical ketosis in cows in the first 65 days of their lactation was 12.1%. In contrast to prevalence, the incidence of a disease is the number of new cases in a given period of time. For a stable disease, the prevalence is related to the product of the incidence of the condition and its average duration (16). Since the average duration of the subclinical ketotic state in a cow is not known, the incidence rate was estimated based on a range of possible durations (Figure 3). The duration is taken to be the total number of days that the cow has a positive milk ketone test during the first 65 days of her lactation. It has been estimated that approximately 50% of cows go through a state of subclinical ketosis (8), and if that value is correct it would suggest an average duration of about 16 days.

It has been reported that detection of elevated ketone levels in milk frequently indicates the impending onset of either clinical ketosis or some other disease condition (8,17). Results of this project generally support this contention. Both odds ratios shown in Table I for the association between metritis and a positive milk ketone test are greater than one (indicating a positive association), although only one of the two is significant ($p<0.01$). Since metritis is a condition which generally commences at calving, the results suggest that cows with metritis are more likely to develop subclinical ketosis. For both cystic ovaries and ketosis, the probability of diagnosing those diseases was significantly higher after a

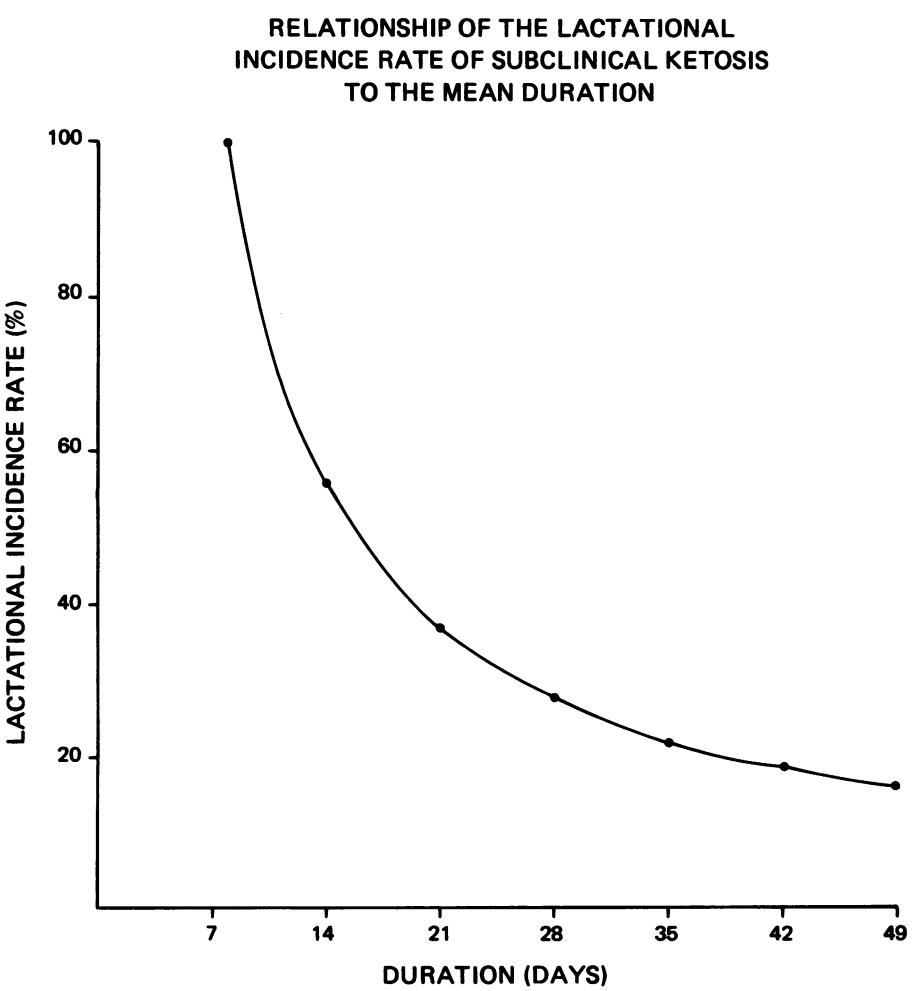

Fig. 3. Relationship between the average duration of positive test results and the lactational incidence rate based on a prevalence of positive tests in the first 65 days postpartum of 12.1%.

positive milk ketone test than after a negative one (odds ratios of 5.6 and 33.7 respectively). This suggests that elevated milk ketone levels may be an early sign in the course of both of those diseases. The fact that no elevated ketone levels were observed in the four days following the diagnosis of cystic ovaries or ketosis suggests that the therapy that these cows received was in fact very effective. There did not appear to be any significant association between subclinical ketosis and mastitis. This is in contrast to the positive association between clinical ketosis and cases of acute mastitis which was found in this study (18).

Losses of 1.0 and 1.4 kg of milk per day were found to be associated with test results of + 1 and + 2 respectively. These values represent approximately 4.4 and 6.0% of the mean daily production. Reduced milk production associated with subclinical ketosis has also been reported from a study involving 31 dairy cows, but the magnitude of the loss was not stated (11). Treatment of cases of subclinical ketosis with propylene glycol has been reported to increase milk production by 4.3 lb/day more than in control animals (8) or by 350 kg over the whole lactation (19). The first value (4.3 lb = 1.95 kg) is similar to the losses which were found in this study. The gain of 350 kg over a whole lactation as a result of five days of propylene glycol feeding suggests that the beneficial effects on milk production persist long after the therapy is stopped. A gain in milk production of 0.448 kg/day of life was found to be associated with the occurrence of clinical ketosis in cows in this study (20). Since the average calving interval for these cows was 401 days, that value would correspond to 180 kg over the whole lactation. This gain was attributed to the beneficial effects of the therapy (which usually incorporated a period of supportive therapy such as propylene glycol feeding) and although less than the previously reported value of 350 kg still suggests that the beneficial effects of the therapy persist after the termination of the therapy.

The results of this study show that subclinical ketosis is associated with reduced milk production, however whether or not it is worthwhile to establish a program to monitor cows for subclinical ketosis depends on several factors. Firstly the sensitivity and specificity of the milk test for subclinical ketosis must be evaluated and that will require an independent method of classifying cows as ketotic or normal. This classification could be based on other metabolic parameters or on functional parameters (such as reduction in milk production). Secondly, the potential for the prevention of the condition through nutritional management must be investigated. Thirdly the value of therapy for the condition must be further evaluated. If, as is suggested by some studies (19,20), the benefit of therapy persists long after the ketotic state has been eliminated then it may be beneficial to routinely monitor cows for subclinical ketosis during the first two months of lactation.

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