Survey of ketolactia, determining the main predisposing management factors and consequences in Hungarian dairy herds by using a cow-side milk test

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ABSTRACT
The aims of the survey were to determine the prevalence of ketosis in dairy herds by measuring the concentration of beta-hydroxybutyrate (BHBA) in milk by Keto-Test (Sanwa Kagaku Kenkyusho, Nagoya, Japan); risk factors and the relationship with postpartum diseases were investigated. 1667 early lactating (days in milk 0–75) cows were tested in 52 dairy herds in 2013 and 2014 years. In total, 29.3 per cent of samples were positive (BHBA ≥100 µmol/l), including 3.7 per cent high positives (BHBA ≥500 µmol/l). The prevalence was similar in herds with less than or more than 9000 kg milk yield (0.34 and 0.38, respectively, P=0.4); however, it was higher in the herds with more than 1000 cows than in smaller herds (<500 and 500–1000 cows) (0.46, P=0.03). The BHBA level in milk was in a non-linear positive relationship with parity (P=0.01), associated with retained placenta (P=0.0006), mastitis (P=0.02) and clinical ketosis (P<0.001). The results confirm the high prevalence of ketolactia in Hungarian dairy herds and its links to herd-related and cow-related risk factors and diseases occurring commonly in fresh cows.

INTRODUCTION
Ketosis, a common metabolic disorder of the peripartal period, is associated with several consequential diseases and economic losses. Clinical (CK) and subclinical (SCK) forms have been described.1 With a lack of clinical signs, the presence of SCK often remains undiscovered, and the true prevalence (TP) is underestimated in dairy herds. Ketosis has detrimental effects on cow health, productivity and reproduction.23 Ketosis predisposes cows to other diseases and reproductive disorders3–12 and is associated with depressed milk production.8

Despite energy-rich feed rations, negative energy balance frequently develops in the peripartal period in dairy cows.13

CK and SCK are different in the presence or absence of symptoms.1 Due to a lack of symptoms, SCK is often not recognised.6 Prevalence of SCK has previously been reported between 7 and 73 per cent in dairy herds.3 12 14–21

Diagnosis is based on determination of the level of ketone bodies in blood (ketonaemia), urine (ketonuria) or milk (ketolactia).15 22

The ‘gold standard’ for diagnosing ketosis is the determination of blood beta-hydroxybutyrate (BHBA) concentration because of its high sensitivity (SE) and specificity (SP).23 As laboratory results are not immediately available, cow-side tests may be preferred.24 The cost of cow-side test (US$1–2) also is an advantage, as usually it is lower than the cost of laboratory testing (US$15/hour).25 26 The high SE (73–95 per cent) and SP (68–96 per cent) of a colorimetric semi-quantitative BHBA cow-side test (Keto-Test, Sanwa Kagaku Kenkyusho, Nagoya, Japan) has been previously reported.27–31

The aim of the study was to determine the prevalence of ketolactia by Keto-Test milk tests in Hungarian dairy herds. Risk factors causing elevated BHBA levels and links between ketosis and other diseases were analysed.

MATERIALS AND METHODS
Data gathering
A cross-sectional observation study was enrolled from July 30, 2013 to August 27, 2014, involving 52 large-scale dairy herds. The herds were selected randomly from a database, and if the owner or operator of the farm accepted the survey, the sampling was executed (Fig 1).

A total of 1669 Holstein-Friesian dairy cows were sampled between 0 and 75 days in milk (DIM). The majority of animals (97 per cent, n=1620) were sampled between 1 and 27 completed DIM and only 3 per cent (n=47) on day 0 (day of the calving) or ≥28 DIM. Two cows were excluded from further analysis.

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Three herd categories were created from the database for analysis. Small (187–500 cows, 37 per cent of all herds), medium (501–1000 cows, 40.7 per cent of all herds) and large (1001–1815 cows, 22.2 per cent of all herds) categories were constructed according to numbers of cows. The distribution of sampled cows was 724 (43.4 per cent), 505 (30.3 per cent) and 438 (26.2 per cent) in small, medium and large herd categories, respectively. We further differentiated lower (<9000 kg, 22 herds, 42.3 per cent) and high (≥9000 kg, 30 herds, 57.7 per cent) producing categories of these herds based on the average 305 days’ milk yield. In total, 665 samples (39.9 per cent) were taken in lower producing and 1002 samples (60.1 per cent) in high producing herds.

Disease data were recorded on the days of milk sampling. Cases of retained placenta (RP), metritis, mastitis, displaced abomasum (DA), dystocia, milk fever, gastrointestinal disorders, lameness and CK that occurred until the day of the sampling were reported by a local veterinarian. The definition of diseases was based on the same standard in all examined herds.32 Premature parturitions (calving maximum three weeks earlier than expected) and twins also were recorded.

Milk samples were taken at the morning milking session in the milking parlour. Following Oetzel’s study and based on the recommendation of the manufacturer of Keto-Test, a minimum of 12 cows were tested once, except for that five instances where only between 9 and 11 cows were available at a single day of visit. The cows that had ketosis treatment before the test day were excluded. All milk samples were collected in 10 ml sterile plastic tubes from one quarter of the udder, after preparation for milking but before attaching the milking machine. Keto-Test and semiquantitative diagnostic strips (Sanwa Kagaku Kenkyusho) were used for determining BHBA levels in raw milk via a colorimetric reaction.

The milk samples were measured at a temperature of 20°C. Before dipping the strips in milk, the tubes were gently shaken to homogenise butterfat in the sample. After 60 seconds, the colour of the strip was evaluated by comparing with the colour code scale supplied by Keto-Test (Sanwa Kagaku Kenkyusho). Results from the Keto-Test were denoted as 0, 50, 100, 200, 500 and 1000 µmol/l BHBA concentration in milk, respectively. The manufacturer defines the level of ≥100 µmol/l BHBA in milk as a cut point for SCK.

Results were recorded on a paper-form datasheet immediately after reading and were entered a digital database for further analysis.

**Statistical analysis**

The adjusted prevalence was calculated by the Rogan-Gladen estimator33 using test SE and SP. A binomial generalised linear mixed model was fit to study the occurrence of ketolactia (dichotomised BHBA variables as dependent variables) in association with the independent variables as fixed effects and herd as a random effect adding a random term to the intercept.34 All data processing and analysis was performed in R environment (R Core Team 2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (http://www.R-project.org).

As these diagnostic tools and methods are used in veterinary practice, false-positive or false-negative results are possible, thus there are two types of prevalence, true and apparent defined in literature. Knowing reliable SE (73 per cent) and SP (96 per cent) characteristics of a familiar

![FIG 1](locations-of-the-herds-tested-in-hungary.hr-croatia-ro-romania-sk-slovakia-slo-slovenia-srb-serbia-ua-ukraine.png)
test, TP was calculated by the formula: TP=(AP+(SP−1))/(SE+(SP−1)) and shown in Table 1.

### RESULTS

**Distribution of BHBA\text{MILK} categories and prevalence of ketosis**

The overall distribution of samples among BHBA\text{MILK} categories (n=1667) is presented in Table 2.

Prevalence data of ketosis are presented in Table 1.

**Relationships between prevalence of ketosis and risk factors**

#### Herd size

The logistic regression (binomial generalised linear mixed model) analysis—including the herd as a random effect—showed that risk of developing severe ketosis (BHBA\text{1000}) was higher in larger herds. Highly positive cases were identified at a more than three times higher frequency in the largest dairies (>1000 cows) than in the two other smaller herd size categories (OR: 3.74, 95% CI 1.1 to 13.2, P=0.02). Furthermore, the odds of BHBA\text{1000} cases were more than four times higher in largest (>1000 cows) herds compared with smallest (<500 cows) herds (OR: 4.57, 95% CI 1.1 to 22.3, P=0.03).

#### Herd productivity

There was no significant association between the prevalence of ketosis and average herd milk production (< or >9000 kg) level (BHBA\text{100} P=0.6; BHBA\text{500} P=0.8; BHBA\text{50} P=0.7; BHBA\text{100} P=0.4; BHBA\text{ALL POSITIVE} P=0.4).

#### Parity

The distribution of cows according to parity is presented in Table 3. The relationship between parity and odds of positive results is presented in Fig 2.

There was a significant (P=0.01), non-linear positive relationship detected between the parity and the probability of ketosis. The probability of ketosis with respect for BHBA\text{100} and all positive cases peaked at the third and fourth lactations (OR: 2.02, 95% CI 1.32 to 3.10, P=0.0008; OR: 1.99, 95% CI 1.35 to 2.92, P=0.0003, respectively).

**Days in milk**

The highest number of positive cases was detected around the 10th day of lactation. The probability of development of a positive BHBA\text{MILK} was significantly higher in the first 10 days of lactation (OR: 1.6, 95% CI 1.1 to 2.31, P=0.009) than afterwards.

**Twins**

A higher probability of ketosis was found among twin-calving cows (n=42) in case of higher positive (BHBA\text{500} and BHBA\text{1000}) categories (OR: 4.17, 95% CI 1.03 to 12.42, P=0.02; OR: 4.73, 95% CI 0.51 to 21.02, P=0.08, respectively). In the case of lower BHBA(100, 200) this relationship was not significant.

### Premature calving (calving maximum three weeks earlier than expected)

Premature delivery of calves (n=21) resulted in a significantly higher risk of SCK in the BHBA\text{100} category (OR: 2.68, 95% CI 0.95 to 7.03, P=0.04). There were no significant relationships observed in other BHBA categories or in primiparous cows.

**RP and metritis**

RP (n=155) was associated with significantly increased odds of a positive ketosis test result (OR: 1.85, 95% CI 1.30 to 2.63, P=0.0006). On dividing the positive cases into BHBA categories, a relationship was found to be significant for BHBA\text{100} only (OR: 1.94, 95% CI 1.31 to 2.84, P=0.0008), but not in any other BHBA categories.

The odds of elevated BHBA\text{MILK} was not significantly associated with metritis cases (n=140).

### Table 1: Prevalence of ketosis

| Groups                  | Apparent prevalence | 95% CI       | True prevalence | 95% CI       |
|-------------------------|---------------------|--------------|-----------------|--------------|
| Production <9000 kg     | 0.34                | 0.28 to 0.40 | 0.44            | 0.35 to 0.52 |
| Production >9000 kg     | 0.38                | 0.33 to 0.43 | 0.49            | 0.41 to 0.57 |
| Herd size <500 cows     | 0.34                | 0.28 to 0.41 | 0.44            | 0.35 to 0.53 |
| Herd size 500–1000 cows | 0.32                | 0.26 to 0.39 | 0.41            | 0.33 to 0.50 |
| Herd size >1000 cows    | 0.46                | 0.38 to 0.55 | 0.61            | 0.49 to 0.74 |

BHBA, beta-hydroxybutyrate.
Mastitis
The odds of a positive Keto-Test and mastitis were significantly increased when data were analysed from all BHBA categories together (n=114) (OR: 1.63, 95% CI 1.07 to 2.45, P=0.02). When analysing individual positive categories, BHBA\textsubscript{100}, BHBA\textsubscript{200} and BHBA\textsubscript{1000} found positive associations (OR: 1.66, 95% CI 1.04 to 2.60, P=0.03; OR: 1.08, 95% CI 0.44 to 2.28, P=0.8; OR: 2.60, 95% CI 0.48 to 9.27, P=0.1, respectively). However, in the BHBA\textsubscript{500} category, this association was not found (OR: 0.998, 95% CI 0.20 to 3.21, P=1).

Milk fever, lameness
There were no significant relationships found between reported milk fever (n=16) and lameness (n=41) cases and a positive milk BHBA result.

Clinical ketosis
As it was evidently expected, a much greater chance of highly elevated BHBA\textsubscript{MILK} results in cases of CK (n=27) was detected (BHBA\textsubscript{100}: OR: 4.87, 95% CI 0.90 to 17.04, P=0.03; BHBA\textsubscript{1000}: OR: 26.17, 95% CI 6.79 to 85.71, P<0.0001).

DISCUSSION
The overall summary of our findings about relationships between each investigated management factor and health issues and ketolactia is presented in Table 4.

One of the most important economic problems on dairies is the association between hyperketonaemia and depressed milk production in early lactation.\textsuperscript{8} The monetary value of economic losses caused by hyperketonaemia has been calculated to €257/cow/lactation.\textsuperscript{35} This is mainly composed of 350–500 kg of milk production losses plus the costs of treatment of related diseases experienced during the 305-day lactation period.\textsuperscript{36,10,12,15}

In cases of SCK, milk composition may also be affected, which could be used as an indicator of the problem on a herd level.\textsuperscript{31}

In this study, there was a remarkably high prevalence of hyperketonaemia detected via BHBA concentration from raw milk samples. In total, 29 per cent of examined milk samples which were diagnosed to be BHBA positive (≥100 µmol/l) during the period was observed. In considering the limitations of the present study, it is reasonable to interpret the prevalence of ketosis in higher lactations (fifth to eighth) very carefully due to limited number of animals in those classes.

There have been various data reported in literature (7–73 per cent) regarding the prevalence of SCK in dairy herds.\textsuperscript{3,12,14–21} In another survey from the UK where approximately 43,000 dairy cows were examined on 1200 dairy farms, 1.4 per cent of the cows (10–20 DIM) were affected by CK and 27 per cent of cows were SCK positive (1.0–2.9 mmol/l BHBA\textsubscript{BLOOD}).\textsuperscript{3} In a Hungarian study, 12.9 per cent of examined cows (n=294) had blood-BHBA values higher than 1.4 mmol/l and 55.2 per cent of the cows had blood-BHBA values higher than 0.8 mmol/l.\textsuperscript{36}

In a UK audit, 765 cows from 15 dairy herds were tested using a milk BHBA test. The prevalence of SCK averaged...
### TABLE 4: Effects of investigated factors on OR of each ketolactia categories

|                    | BHBA 0 OR | 95% CI | P values | BHBA 50 OR | 95% CI | P values | BHBA 100 OR | 95% CI | P values | BHBA 200 OR | 95% CI | P values | BHBA 500 OR | 95% CI | P values | BHBA 1000 OR | 95% CI | P values |
|--------------------|-----------|--------|----------|------------|--------|----------|------------|--------|----------|------------|--------|----------|-----------|--------|----------|-------------|--------|----------|
| DIM                | 1.03      | 1.01 to 1.04 | 0.0038* | 0.98      | 0.96 to 1.00 | 0.033* | 0.99      | 0.97 to 1.01 | 0.045* | 0.98      | 0.94 to 1.03 | 0.038 | 0.99      | 0.99 to 1.13 | 0.11 |
| Parity             | 0.89      | 0.83 to 0.95 | 0.0007* | 0.98      | 0.90 to 1.07 | 0.0003* | 0.99      | 0.97 to 1.01 | 0.0001* | 0.96      | 0.92 to 1.03 | 0.044 | 1.16      | 0.86 to 1.56 | 0.334 |
| Twins (n=42)       | 0.83      | 0.43 to 1.61 | 0.94    | 0.50      | 0.15 to 1.30 | 0.51    | 0.10      | 0.40 to 2.25 | 0.032 | 1.51      | 1.03 to 2.24 | 0.02268* | 4.73      | 0.51 to 21.02 | 0.0807 |
| Dystocia (n=37)    | 1.20      | 0.59 to 2.45 | 0.62    | 1.04      | 0.41 to 2.37 | 0.84    | 0.66      | 0.2 to 1.74 | 0.0527 | 0.81      | 0.09 to 3.21 | 1.00  | 0.00      | 0.00 to 3.94 | 0.6229 |
| Dystocia in first lactation | 0.69 | 0.24 to 2.00 | 1.51 | 0.41 to 4.67 | 0.3884 | 0.90 | 0.10 to 4.01 | 1.00 | 0.21 to 9.29 | 0.2988 | 0.00 | 0.00 to 15.37 | 1.00 | 0.00 to 61.88 |
| Dystocia in 2+ lactation | 1.62 | 0.59 to 4.68 | 0.3563 | 0.70 | 0.13 to 2.46 | 0.7786 | 0.68 | 0.13 to 2.39 | 0.7791 | 0.00 | 0.00 to 2.94 | 0.6345 | 0.00 | 0.00 to 7.16 | 1.00 | 0.00 to 52.52 |
| Premature calving (n=23) | 0.92 | 0.35 to 2.40 | 1.00 | 0.40 | 0.04 to 1.65 | 0.2815 | 2.68 | 0.95 to 7.03 | 0.04295* | 0.71 | 0.02 to 4.50 | 1.00 | 0.00 | 0.00 to 7.28 | 1.00 | 0.00 to 17.99 |
| RP (n=155)         | 0.77      | 0.55 to 1.09 | 0.1515  | 0.60      | 0.36 to 0.97 | 0.0293* | 1.94      | 1.31 to 2.84 | 0.0008* | 0.75      | 0.31 to 1.58 | 0.6089 | 1.89 | 0.70 to 4.39 | 0.1796 | 2.65 | 0.63 to 8.44 | 0.09219 |
| Milk fever (n=16)  | 0.98      | 0.69 to 1.41 | 0.9299  | 1.26      | 0.30 to 4.20 | 0.7564  | 1.43      | 0.33 to 4.77 | 0.5225 | 0.94      | 0.02 to 6.25 | 1.00  | 0.00 | 0.00 to 9.84 | 1.00  | 0.00 | 0.00 to 24.28 |
| Metritis (n=140)   | 0.98      | 0.69 to 1.41 | 0.9299  | 0.77      | 0.46 to 1.22 | 0.2786  | 1.13      | 0.71 to 1.75 | 0.5736 | 1.10      | 0.50 to 2.18 | 0.7231 | 1.42 | 0.43 to 3.68 | 0.4105 | 2.07 | 0.38 to 7.36 | 0.2094 |
| Metritis (n=114)   | 0.62      | 0.41 to 0.93 | 0.01522* | 1.07      | 0.65 to 1.71 | 0.8144  | 1.66      | 1.04 to 2.60 | 0.02527* | 1.08 | 0.44 to 2.28 | 0.8443 | 0.998 | 0.20 to 3.21 | 1.56 | 0.48 | 0.92 to 9.27 | 0.1354 |
| Clinical ketosis (n=27) | 0.59 | 0.24 to 1.38 | 0.2442 | 1.33 | 0.47 to 3.31 | 0.4802  | 0.16 | 0.004-0.995 | 0.04484* | 0.54 | 0.013 to 3.36 | 1.00 | 4.87 | 0.90 to 17.04 | 0.03217* | 26.17 | 6.79 to 85.71 | <0.0001* |
| Lameness (n=41)    | 1.99      | 0.997 to 4.13 | 0.0399* | 0.77 | 0.29 to 1.80 | 0.6976  | 0.46 | 0.12 to 1.28 | 0.1582 | 0.35 | 0.01 to 2.10 | 0.5171 | 1.94 | 0.22 to 7.92 | 0.2946 | 0.00 | 0.00 to 8.76 |
| Digestive disorders (n=12) | 3.06 | 0.76 to 17.64 | 0.08918 | 0.00 | 0.00 to 1.36 | 0.00822 | 0.86 | 0.09 to 4.04 | 1.00 | 0.00 | 0.00 to 5.14 | 1.00 | 3.41 | 0.08 to 24.43 | 0.2751 | 0.00 | 0.00 to 33.61 |

*P value (bold) shows significance. BHBA, beta-hydroxybutyrate; DIM, days in milk; RP, retained placenta.
30 per cent, with levels in individual herds varying from 10 to 60 per cent.\textsuperscript{11}

The Pearson correlation coefficients between blood BHBA and milk BHBA are strong (0.89), justifying the use of milk BHBA tests for determination of the prevalence of hyperketonaemia on an herd-level basis.\textsuperscript{37} BHBA levels in the blood are estimated at six\textsuperscript{37} or eight times higher than in the milk.\textsuperscript{38} There are wide variations in BHBA threshold levels used to describe SCK in the literature from 1.0\textsuperscript{19} 39 40 to 1.2 µmol/l\textsuperscript{15} 41 to 1.4 µmol/l BHBA in the blood. However, a lower threshold level of BHBA\textsubscript{BLOOD} (0.8 µmol/l) is also used in routine herd monitoring programmes in Hungary.\textsuperscript{36} 42 The applied threshold depends on the applied determination method and it has limited influence on the interpretation of the results.\textsuperscript{6}

In our study, the highest proportion of positive cases was found around day 10 post partum. It is quite similar to previously published results which found peak occurrence in the first or second week of lactation.\textsuperscript{8} 22 24 43 Results from a large 1010-cow, 25-herd study in Ontario demonstrate that the ORs of SCK prevalence (serum BHBA >1200 µmol/l) were 12.17 and 12.20 on the first and second weeks of lactation, respectively. A peak (OR: 24.37) was found in the second week of lactation and the cumulative value was OR: 39.8 until the ninth week of lactation.\textsuperscript{22}

There was a significant (P=0.01), non-linear positive relationship detected between parity and the probability of ketosis. The probability of ketosis with respect to all positive cases peaked at the third and fourth lactations. However, as we supposed above, this is a limitation of our study, that there were a limited number of samples that were examined from older cows. It is recommended to take into account when investigating this result.

Parity is an important determinant in the development of ketosis.\textsuperscript{44} In a study which analysed 3586 lactations in a 17-year period (January 1980 to December 1996), it was shown that parity is significantly related to the presence of ketosis, and the highest incidence was reported in third and fourth lactations.\textsuperscript{45} A significant, non-linear correlation was reported between positive milk BHBA and parity, which was partly in harmony with previously reported results.\textsuperscript{45}

We found that the probability for development of ketosis was increased in twin-calving cows, which correlates with the results of others.\textsuperscript{46} 47

Environmental and management factors such as herd size, season of calving, feeding frequency or ration composition, poor dry cow management and rumen adaptation may play a role in the manifestation of this metabolic disorder, and have a serious effect on the variation in prevalence and the incidence of ketosis in dairy herds.\textsuperscript{41} 42 44 The effects of herd size and production level were investigated, inspired by an earlier work.\textsuperscript{41} A positive correlation between herd size and the risk of severe ketosis was found in our cross-sectional observation study. The fact that approximately 40 per cent of the samples originated from 6 out of 52 farms made us worry that this major result might be confounded by herd bias. The potential effect of herd bias was examined by a logistic regression (binomial generalised linear mixed model) analysis—including the herd as a random effect—to confirm that results were not confounded. Our findings were supported by other publications as well.\textsuperscript{48} Producers in smaller herds tend to overestimate the incidence of CK and producers in larger herds tend to underestimate the incidence of CK.\textsuperscript{43} We assume that in larger herds (>1000 cows), each farm operator is responsible for larger numbers of cows and may pay less attention to individuals, increasing the risk of unrecorded problems compared with smaller herds. In large-scale farms, it is more difficult to manage a production system which meets the requirements of all cows in the system.

Our results do not show any influence of level of herd milk production on the prevalence of ketosis. In this section, it is necessary to highlight that in this study, not individual but herd production level was investigated. The finding is similar to earlier studies.\textsuperscript{48} Other studies also show that there is no direct link between ketosis and milk production level within herds.\textsuperscript{3} However, it is conceivable that an existing higher presence of SCK within an herd may depress the productivity in the lactation resulting reduced milk production in the affected herd.\textsuperscript{3} 6 10 12 15

Similar to previous results,\textsuperscript{8} 11 49 50 our study showed that the elevated level of BHBA in milk was associated with a greater risk of premature parturition, dystocia, RP, as well as with the clinical mastitis cases were diagnosed before the milk ketone tests were done. It is well documented that negative energy and protein balance contribute to the development of a depression in immune function\textsuperscript{4} 5 and a subsequent increased incidence of related diseases in early lactation.\textsuperscript{7} 9 As ketosis is induced by a deficiency of glucose, it is frequently associated with a decrease in immune function. Insufficient immune function leads to the clinical manifestation of several infectious diseases.\textsuperscript{51}

Controversially with other publications\textsuperscript{7} 12 20 92 we did not find association between metritis and elevated level of BHBA in milk. The explanation would be that the association with metritis may be influenced by the sampling time, as metritis may be diagnosed in the first three weeks of lactation thus possibly after milk sample collection was performed in our study. In other words, it is a limitation of our results that we missed all the metritis cases that occurred after milk ketone test.

Summarising our results, we can conclude that the prevalence of SCK by measurement of ketolactia is high in large-scale dairy herds. Prevalence peaks within the third to fourth lactation, and twinning is associated with a higher risk of ketosis. Larger herd sizes were identified as a management-related risk factor for ketosis. Links between ketosis and other fresh cow diseases such as dystocia, RP and mastitis were revealed.

Monitoring of ketosis by using a semiquantitative, colorimetric milk BHBA test may assist in the early detection
of ketosis and in the management and prevention of consequent fresh cow diseases in dairy herds.

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Contributors PH has been involved in substantial contributions to the conception and design of the work, analysis, and interpretation of data for the work. GZ has been involved in substantial contributions to the conception and design of the work. CC has been involved in substantial contributions to the conception and design of the work. CC has been involved in substantial contributions to the conception and design of the work. LC has been involved in substantial contributions to the conception and design of the work. All authors have been involved in drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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Competing interests GZ is employed by ELANCO Animal Health, Eli Lilly Regional Operations, the provider of Keto-Test milk BHBA test strips, used in this study. PH has been involved in substantial contributions to the conception and design of the work; and the acquisition, analysis and interpretation of data for the work. All authors have been involved in drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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