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Risk factors and prevalence of subclinical ketosis in dairy cows in Croatia

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ABSTRACT

The purpose of this study was to assess the risks or associations between the prevalence of subclinical ketosis (SCK) and some periparturient diseases in Holstein cows, by determining beta-hydroxybutyrate (BHBA) serum levels using a Precision Xceed® BHBA meter (Abbott, USA) or spectrophotometer, and to evaluate the reproductive performance of cows positive for SCK. The study was conducted on 841 cows, from 107 randomly selected farms, aged 2-8 years, divided into two groups: positive (POS = BHBA level≥1200 µmol/L), with clinical or SCK, and a negative (control) group (NEG = BHBA level<1200 µmol/L). Blood samples were collected from lactating cows in the period between 1-15 days after parturition. There was a positive correlation ($r = 0.99$; $P<0.0001$) between BHBA determined spectrophotometrically and by using Precision meter. The correlation between BHBA concentration and milk yield (total lactation) was not significant ($r = 0.04$; $P = 0.26$). The POS group of cows had a significantly higher mean lactation yield ($P<0.001$) than the NEG group of cows (7076 kg *vs*. 6409 kg). The cows in the NEG group had a significantly shorter period of median days open to pregnancy (MDOP) compared to the POS group (124 *vs*. 138; $P<0.0001$). The hazard ratio (HR) for days open to pregnancy (DOP) in the POS group of cows was 0.66 ($P<0.0001$). The results showed that multiparous cows had 1.41 times more chance of being affected with SCK than primiparous cows. The prevalence of SCK was

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15.8% in Holstein cows from central, northern, northwestern and eastern parts of Croatia. About 55.6% of the cows with SCK were affected by some of the observed periparturient diseases (relative risk was 1.91; P = 0.001).

Key words: beta-hydroxybutyrate (BHBA), subclinical ketosis, risk factor, cow

Introduction

Ketosis is a common metabolic disorder in dairy cattle, which occurs 2-4 weeks after parturition, characterized by increased concentrations of ketones, such as beta-hydroxybutyrate (BHBA), acetoacetate (AcAc), and acetone, in milk, urine and blood (DUFFIELD et al., 1997; ZHANG et al., 2012). The predominant circulating ketone body in ruminants is BHBA. There is a strong correlation between the whole blood concentrations of BHBA and AcAc (KAUPPINEN, 1983). Cows affected by clinical ketosis have clinical symptoms such as: anorexia, abnormal licking and chewing, rapid loss of body condition, and decreased milk production (YOUSSEF et al., 2010). Reducing the severity and duration of negative energy balance (NEB) is crucial in the prevention of ketosis (OVERTON and WALDRON, 2004; KOČILA et al., 2013).

Subclinical ketosis (SCK) is characterized by increased concentrations of circulating ketone bodies i.e. serum BHBA level more than 1.2 mmol/L, without the clinical signs of ketosis (ANDERSSON, 1988). It is associated with a loss in milk production and increased risk of periparturient diseases. Subclinical ketosis in the first or second week after calving is associated with increased risk of displacement of abomasum (DA), clinical metritis, clinical ketosis, clinical endometritis, a prolonged postpartum anovulation period, increased severity of mastitis, and lower milk production in early lactation. There are several validated and practical tools for cow-side measurement of ketone bodies (Ketostix, Ketur-Test, Ketodiabur, Medi-Test-Keton, etc.) (LeBLANC, 2010). In a study by CARRIER et al. (2004), SCK was more prevalent in the multiparous cows than in primiparous cows and was more prevalent in the first two weeks of lactation, based on a BHBA concentration in serum of ≥1400 μmol/L (14.4 mg/dL). Cows with a serum BHBA level of ≥ 1400 μmol/L are three times more at risk for DA and clinical ketosis (OETZEL, 2004). This increase in ketone bodies concentration in the serum of cows immediately after parturition has a negative impact on the health of the cows, and it is associated with reduced milk production (DUFFIELD et al., 2000; FOLNOŽIĆ et al., 2015). The overall prevalence of subclinical ketosis in dairy cows in early lactation is estimated at 7.5%-14% (GEISHAUSER et al., 2000; ENJALBERT et al., 2001). The cows with a body condition score (BCS) ≥3.75 out of 5.0 at calving have increased risk of ketosis. Early detection of ketosis, including SCK, may enable earlier intervention and minimize the loss of milk production (DUFFIELD et al., 2009). Prevention of ketosis depends on several factors, including proper transition-cow nutrition, management of body condition, and the use of certain feed additives, such as niacin, propylene glycol and ionophores (GORDON et al., 2013). The first report on comparative use of an electronic human BHBA meter (MediSense Precision, Abbott, Abingdon, UK) and spectrophotometer for BHBA concentration measurement in dairy cows resulted in the suggestion that the electronic BHBA meter is suitable for detecting SCK in dairy cows (IWERSEN et al., 2009). The aims of this study were to determine the risk factors and reproductive performance of cows diagnosed with SCK, the association between SCK and some periparturient diseases, such as milk fever, mastitis, retained placenta, displacement of abomasum, claw diseases, metritis etc., and the prevalence of SCK in dairy cows from Croatia using an electronic BHBA meter (Precision Xceed BHBA) compared with serum concentrations of BHBA as determined by spectrophotometer.

Materials and methods

The study was conducted on 841 Holstein cows, aged 2-8 years (Mean ± SD of parity was 2.6 ± 1.6), from 107 randomly selected farms (by simple randomization method) located in the central, northern, northwestern and eastern parts of Croatia. The majority of the cows, depending on the farm, were housed in free-stall barns, with straw bedding. On the participating farms there were between 26 and 395 Holstein cows. The cows were fed with corn and grass silage, and concentrate (depending on the milk production on each farm) and milked twice daily. On this basis, the animals on all the farms had the appropriate conditions and similar herd management. The herds' average milk yield was 7350 ± 976.13 kg per lactation on all farms. The cows were divided into two groups: (1) the positive group (POS; n = 133) with serum BHBA levels of ≥1200 µmol/L (affected by clinical ketosis or SCK), and (2) the negative (NEG; n = 708) or control group, with serum BHBA levels of <1200 µmol/L. Blood samples were collected from lactating cows in the period from parturition, on the 1st to 15th day after parturition (Mean ± SD; 7.9 ± 4.3). The samples for analysis were taken from the caudal vein using a vacutainer set. Then one droplet of blood was applied to the test strips of a Precision Xceed® BHBA device (Abbott, USA). Each animal was tested only once. For laboratory spectrophotometry, the blood samples were stored for about 30 minutes at room temperature and centrifuged for 15 minutes at 700 g. Following 15 minutes of centrifugation, the serum was separated and the samples were immediately stored at -20 °C until analyzed. In the obtained blood serum, the concentration of BHBA was determined (up to a maximum of 3 months after freezing according to laboratory protocols) by absorptive spectrophotometry using an Olympus AU 600 analyzer (Olympus Diagnostica GMBH, Hamburg, Germany). Cows with infectious diseases, as well as animals treated with any metabolic drugs, vitamin and mineral preparations, within 2 weeks before the blood collection, were excluded from the study. Animals on all the farms had similar herd management. All cows were examined, including vaginal and transrectal uterine palpation after calving, and thereafter every 3 weeks, and 45 days after insemination with transrectal ultrasonography using a 5 MHz linear-array transducer (SonoVet 2000, Medison, Seoul, South Korea) until confirmation of pregnancy. Following examination, the cows with diseases, such as cystic ovarian follicles, ovarian atrophy, etc. were managed according to the usual herd management procedures (including hormonal therapy or another therapy) and the values were censored when observations were terminated for reasons beyond the control of the investigator. Total lactation was calculated by employees of the Croatian Agricultural Agency, according to the AT4 methods (milk control every 4 weeks) and projection of the quantity and quality of milk yield up to 305 days of lactation, according to International Committee for Animal Recording (ICAR) protocols. Date of parturition, parity, pregnancy, culling, date of artificial inseminations, milk yield, postpartal diseases (recumbence, ketosis, cystic ovarian follicles, clinical endometritis, mastitis, etc.) were monitored and documented. Cows were artificially inseminated with frozen-thawed semen by experienced inseminators upon observed estrus. Reproductive performance and culling data of individual animals were collected up to the end of lactation (305 days) for each cow in the study.

Statistical analysis and modeling. Statistical analyses were performed using SAS 9.3 software (2002-2010 SAS Institute Inc., Cary, NC, USA). To assess the reproductive performance of cows, the interval from calving to first insemination (days to first service, DFS), the interval from calving to pregnancy (days open to pregnancy, DOP) were measured. The non-parametric, log-rank test and Wilcoxon test of equality across strata (PROC LIFETEST) were used for categorical variables. Kaplan-Meier curves were plotted and survival estimates were used to calculate the median days to first service (MDFS) and median days open to pregnancy (MDOP). Plots were made with significant covariates included, to generate predicted survival curves of the two group using the GPLOT procedure. Values were censored when observations were terminated for reasons beyond the control of the investigator, such as when the cows were culled from the herd during the study period before becoming pregnant, or were not pregnant at the end of the study period. The percentage of cows censored in the NEG and POS groups was 3.38% and 5.26%, respectively. For continuous variables, a semi-parametric model, Cox proportional hazard regression (PROC PHREG) was used. To test the influence on the group in the model, the following parameters were included: peripartal diseases, parity and lactation. The final model was built using stepwise regression analysis (PROC PHREG) with a P value <0.05 to remain and <0.15 to enter the model. The peripartal diseases (milk fever, mastitis, retained placenta, displacement of abomasum, claw diseases, metritis and clinical ketosis) were pooled into a single category, because the incidence was low. To verify the assumption of proportionality using Cox proportional hazards regression, timedependent covariates were included in the model, and interactions with log (time) were used. The exponential function of the estimated parameters was used to calculate the hazard ratio (HR) of differences between the covariates, using the PHREG procedure in SAS. A HR was interpreted as the relative daily probability of conception or relative pregnancy rate (daily hazard of conception or daily hazard of pregnancy). Power analysis was done using the PROC POWER procedure in SAS (Log-Rank test for two survival curves). The power of the test was calculated using piecewise linear survival curves with a two sided test (α = 0.05), different group weights and the total number of cows. The computed power of the test (1-β) was 0.852. Average lactation between POS and NEG groups was estimated using the GLM method with included the effect of parity and herd. To obtain the relative risk (RR) or prevalence ratio of POS cows, the GENMODE procedure, with log link function and binomial distribution, was used. The Pearson coefficient of correlation was calculated between the two methods for determining BHBA, and between the level of BHBA and milk yield using the CORR procedure.

Results

A positive and significant correlation (r = 0.99; P<0.0001) was found between BHBA as determined spectrophotometrically or electronically by a BHBA meter (Precision Xceed, Abbott, USA), while the correlation between BHBA concentration and milk yield was not significant (r = 0.04). The average lactation was higher in the POS group of cows by 667 kg (P<0.001) (Table 1). The MDFS was slightly shorter in the NEG group of cows but this was not statistically significant. The cows in the NEG group had a significantly shorter period of MDOP compared to the POS group (Table 1, Figs 1 and 2).

The prevalence of SCK was 15.8% in Holstein cows from central, northern, northwestern and eastern parts of Croatia. The percentage of cows diagnosed with clinical ketosis was 1.07%. Multiparous cows had 1.41 times more chance of being affected with SCK than primiparous cows (Table 1). About 55.6% of the cows with SCK were affected by some of the observed diseases. The RR or prevalence ratio determined for the POS group of cows was 1.91, (P = 0.001), which means that POS group of cows had a 1.91 times greater chance of being affected with at least one of the diseases diagnosed in this research (clinical metritis, retention of placenta, claw disease, left displacement of abomasum, milk fever etc.) (Tables 1 and 2).

Table 1. Differences in some parameters between POS cows (BHBA serum level ≥1.20 mmol/L) and NEG cows (BHBA <1.20 mmol/L).

		Group		P value
		POS	NEG	
N (%)		133 (15.8)	708 (84.2)	
Mean lactation in kg (95%, CI)		7076 (6732-7420)	6409 (6271-6547)	<0.001
Mean lactation in kg by parity (95%, CI)	1	6761 (6311-7210)	6400 (6191-6609)	n.s.
	2	7062 (6521-7603)	6519 (6273-6765)	n.s.
	3	7220 (6419-8021)	6256 (5988-6525)	n.s.
	4	6614 (5348-7880)	6626 (6358-6895)	n.s.
	5	7371 (6527-8216)	6155 (5685-6625)	n.s.
	6	7428 (6533-8323)	6496 (6034-6959)	n.s.
MDFS (95%, CI)		111 (106-112)	107 (106-109)	0.08
MDOP (95%, CI)		138 (130-142)	124 (122-126)	<0.0001
PPD N (%)		66 (55.6)	183 (25.8)	<0.0001
RR (95%, CI)		1.91 (1.55-2.37)		
PARITY	Primiparous N (%)	81 (14.0)	497 (86.0)	<0.05
	Multiparous N (%)	52 (19.7)	211 (80.3)	
RR (95%, CI)		1.41 (1.03-1.93)		

n.s. - non significant; MDFS - Median days to first service; MDOP - Median days open to pregnancy; CI - Confidence interval; PPD - Percentage of cows with at least one diagnosed peripartal disease; RR - Relative risk

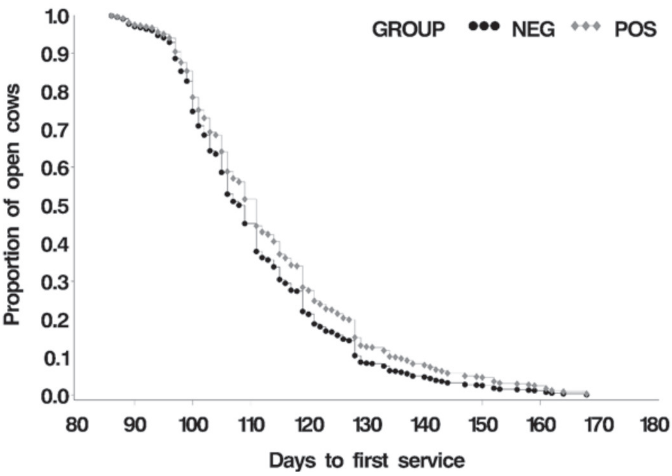

Fig. 1. Survival curve: the relationship of the proportion of open cows and DFS for the NEG (BHBA <1.20 mmol/L) and the POS (BHBA serum level ≥1.20 mmol/L) cows.

Table 2. Incidence of some diseases between POS cows (BHBA serum level ≥1.20 mmol/L) and NEG cows (BHBA <1.20 mmol/L)

	Group		RR (95% CI)	P value
	POS (n = 133)	NEG (n = 708)		
Metritis n (%)	14 (10.53)	31 (4.38)	2.40 (1.31-4.39)	>0.01
Milk fever n (%)	7 (5.26)	9 (1.27)	4.14 (1.56-10.92)	>0.01
Retention of placenta n (%)	11 (8.27)	44 (6.21)	1.33 (0.70-2.50)	n.s.
Left displacement of abomasum n (%)	2 (1.50)	1 (0.14)	10.6 (0.97-116.57)	n.s.
Mastitis n (%)	9 (6.77)	41 (5.79)	1.16 (0-58-2.34)	n.s.
Claw disease n (%)	28 (21.05)	75 (10.59)	1.98 (1.34-2.94)	>0.001

n.s. - non significant; RR - Relative risk; CI - Confidence interval

Table 3. The HR of the POS group of cows as compared to the NEG group of cows

	Positive cows comparing to negative cows		P
	Estimate	95% CI	
DOP	HR = 0.66	0.55, 0.80	<0.0001
Covariates			
Lactation	HR = 1.00	1.00, 1.00	<0.01
DFS	HR = 0.86	0.71, 1.04	0.08
Covariates			
Parity	HR = 0.95	0.91, 0.99	<0.05

DOP - Days open to pregnancy; DFS - Days to first service; HR - Hazard ratio; CI - Confidence interval

Table 3 shows the HR of the POS group of cows. The HR for the DOP was 0.66 (0.55-0.80; P<0.0001), implying that cows in the POS group were open longer until pregnancy, that is, the relative rate of pregnancy decreased by 34% in the POS group, while all other variables remained constant. The only other variable that had a significant association with DOP was lactation (P<0.01). The HR for the DFS was 0.86 (0.71-1.04; P = 0.08). The only other variable that had a significant association with DFS was parity (Table 3).

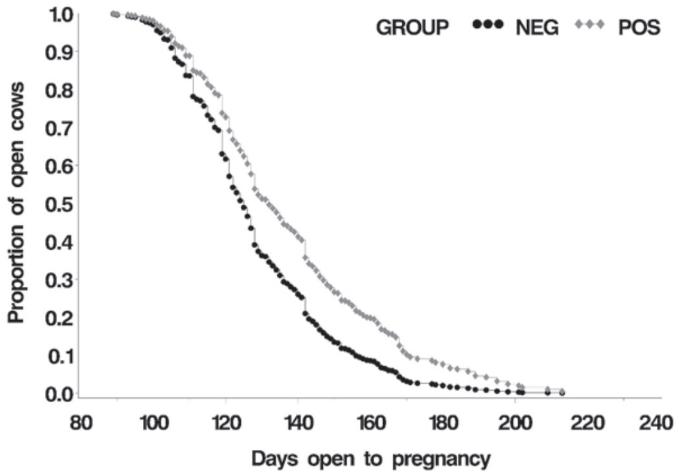

Fig. 2. Survival curve: the relationship of the proportion of open cows and DOP for the NEG (BHBA <1.20 mmol/L) and the POS (BHBA serum level ≥1.20 mmol/L) cows.

Discussion

According to COMPTON et al. (2014) SCK was manifested when blood BHBA concentration was found to be ≥1.4 mmol/L, while in our study SCK was diagnosed similarly as in the investigation by McART et al. (2012), who reported that blood BHBA concentration was between 1.2 to 2.9 mmol/L. The first study on using an electronic human BHBA meter (MediSense Precision, Abbott, Abingdon, UK) for testing dairy cows reported a high correlation (r = 0.99) with BHBA concentration when determined spectrophotometrically (gold standard) (JEPPESEN et al., 2006), as in our study. The SCK in cows has a prevalence ranging from 7% to 41% (GEISHAUSER et al., 2000; ENJALBERT et al., 2001). The peak prevalence of SCK in cows occurred between 4 and 19 days after parturition (GARRO et al., 2014), or on average at 5 days in milk (DIM) (McART et al., 2012), when 28.9% of the cows had a SCK-positive test (McART et al., 2012). In mostly pasture-grazed dairy cows in New Zealand the prevalence of SCK was on average either 14.3% (0%-60.0%) or 2.6% (0%-24.4%) between 7-12 days and 35-40 days post-calving, respectively. It was greater in Southland (13.3%) than Waikato and Canterbury herds (6.9% and 4.7%, respectively) (COMPTON et al., 2014). In our study the prevalence of SCK in dairy cows was higher than that (10.3%) found by GARRO et al. (2014) and lower from that reported by McART et al. (2012), but similar to the results of COMPTON et al. (2014) in Southland herds. When ketosis incidence (blood BHBA tests at 1, 2, 3, and 6 weeks after calving) and average early lactation ketosis prevalence were measured in a large field study (DUFFIELD et al., 1997), the cumulative incidence of ketosis (45%) was about 2.2 times higher than the average prevalence of ketosis (20%). In the same study, the reported incidence of clinical ketosis was only 1.5%. In our study, only 1.07% cows were affected by clinical ketosis.

TONI et al. (2011) found the incidences of retained placenta, left displaced abomasum, clinical metritis and clinical endometritis increased with milk fat to protein ratio ≥2.00. Milk fat to protein ratio can be a useful indicator for SCK (DUFFIELD et al., 1997; KROGH et al., 2011).

Cows who were first positively diagnosed with SCK from 3 to 7 DIM were 0.7 times more likely to conceive on first service, and produced 2.2 kg less milk per day for the first 30 DIM in comparison with the cows who were first tested positive at 8 DIM or later (McART et al., 2012). In our study, the cows in the POS group had longer MDOP of 14 days and MDFS of 4 days than the cows from the NEG group. The cows with SCK had 4.9 (95% CI; 1.17-20.98) times more risk of developing clinical metritis than cows without SCK (GARRO et al., 2014). In our study, 6.15% more cows with clinical metritis were found in the POS group. There was no significant association between mastitis incidence in the cows with SCK compared to the control group (4.7% *vs*. 5.2%) in our previous study (ĐURIČIĆ et al., 2015). In this study, this frequency was slightly, but not significantly higher in the POS group than in the NEG group (6.77% *vs.* 5.79%).

Cows who were first positively tested for SCK from 3 to 5 DIM were 6.1 times more likely to develop displacement of the abomasum than the cows who were first positively tested for SCK at 6 DIM or later (McART et al., 2012). SCK was associated with a three times greater risk of displacement of the abomasum (ĐURIČIĆ et al., 2015). Cows in the POS group in the current study had 1.98 times greater risk of developing claw disease than the cows from the NEG group. Retained placenta was diagnosed in 2.06% more cows in the POS than in the NEG group, but the difference was not statistically significant.

Cows with BHBA concentrations equal to or higher than 1.2 mmol/L had a 1.91 times greater chance to be affected by some of the following diseases: clinical metritis, retention of placenta, claw disease, left displacement of abomasum, milk fever etc.. Also, cows with SCK had days open longer until pregnancy in comparison to cows without SCK.

Our assumption is that average lactation and milk yield per cow are much lower than in the herds in the investigations by other authors mentioned above, and thus the prevalence of SCK in our study is slightly lower, as well as the number of cows in the herds affected by clinical ketosis. The prevalence of SCK in dairy cows from Croatia was 15.8%.

In conclusion, it was confirmed that this simple and cheap method is a very precise and useful tool for diagnosing SCK. Evaluation of the prevalence of SCK is very useful in dairy herds because cows with SCK, in early lactation, tend to have increased incidence of some periparturient diseases, and increased risk factors. This study brings new findings about risk factors and the prevalence of SCK in Croatia.

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SAŽETAK

Svrha ovog istraživanja bila je procijeniti rizike ili povezanost između prevalencije supkliničke ketoze (SKK) i nekih peripartalnih bolesti u krava holštajnske pasmine, određivanjem serumske razine beta-hidroksibutirata (BHBA) s pomoću elektroničkog uređaja Precision Xceed® BHBA device (Abbott, SAD) ili spektrofotometrijski te vrednovati reprodukcijska obilježja u krava pozitivnih na SKK. Istraživanje je provedeno na 841 kravi s nasumično odabranih 107 farmi, u dobi od 2 do 8 godina, podijeljenih u dvije skupine: pozitivne (POZ = BHBA razina ≥1200 µmol/L), s kliničkom ili SKK te negativnu (kontrolnu) skupinu (NEG = BHBA razina <1200 µmol/L). Uzorci krvi su prikupljani od krava u laktaciji tijekom razdoblja od 1. do 15. dana nakon teljenja. Ustanovljena je pozitivna korelacija (r = 0,99; P<0,0001) razine BHBA određene spektrofotometrijski ili pomoću elektroničkog uređaja Precision Xceed (Abbot, SAD). Korelacija između koncentracije BHBA i proizvodnje mlijeka (ukupne laktacije) nije bila značajna (r = 0,04; P = 0,26). POZ skupina krava imala je značajno višu prosječnu proizvodnju mlijeka (P<0,001) od NEG skupine krava (7076 kg *vs.* 6409 kg). Krave iz NEG skupine imale su značajno kraće razdoblje medijan dana do koncepije u usporedbi s POZ skupinom krava (124 *vs.* 138; P<0,0001). Omjer rizika za dane do koncepcije u krava iz POZ iznosio je 0,66 (P<0,0001). Rezultati ovog istraživanja su pokazali da su multiparne krave imale 1,41 puta veću vjerojatnost oboljeti od SKK nego primiparne krave. Prevalencija SKK u krava holštajnske pasmine u središnjim, sjevernim, sjeverozapadnim i istočnim dijelovima Hrvatske iznosila je 15,8%. Oko 55,6% krava sa SKK oboljelo je od nekih praćenih peripartalnih bolesti (relativni rizik je iznosio 1,91; P = 0,001).

Ključne riječi: beta-hydroksibutirat (BHBA), supklinička ketoza, čimbenici rizika, krava