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Subclinical ketosis: Prevalence and risk factor analysis in early lactation dairy cattle of Thrissur district#

Anjaly Francis[1], V. R. Ambily[2*], Usha Narayana Pillai[3], Deepa Chirayath[4], N. Madhavan Unny[5] and M. Shynu[6]
Department of Veterinary Clinical Medicine, Ethics and Jurisprudence
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651,
Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Present study was conducted in Kerala Veterinary and Animal Sciences University farms, various organized and unorganised farms and individual households in Thrissur district during the period of May 2021 to April 2022 with the objectives of estimation of prevalence of subclinical ketosis (SCK) by screening dairy cows on 14th and 28th day post-partum. Multiparous cows with a blood beta-hydroxy butyrate (BHB) value ≥ 1.2 mmol/L without any overt clinical signs were considered as positive for SCK. One hundred and fourteen animals from second to seventh lactation were screened for SCK. Prevalence of SCK was 23.7 per cent on 14th day and 14.04 per cent on 28th day with a total prevalence of 27.2 per cent. Occurrence of SCK was highest in animals in second lactation (54.8 per cent) with more occurrence in crossbred Holstein Friesian cows. Maximum number of positive cases were observed in dairy cows of age between four to six years (54.8 per cent). Body condition score of diseased animals were higher than normal animals.

Prevalence was highest in animals calved between months of July to September and 58.1 per cent of affected animals had male calf. Concurrent abnormalities during transition period were also assessed based on clinical examination and farm records.

Keywords: *Subclinical ketosis, beta-hydroxy butyrate, multiparous cows, transition period*

 # Forms part of the M.V.Sc thesis submitted by the first author to the Kerala Veterinary and Animal Sciences University, Poookode, Wayanad, Kerala.

1. *M.V.Sc student*
2. *Assistant Professor*
3. *Professor and Head*
4. *Veterinary Surgeon*
5. *Professor*
6. *Professor, Department of Veterinary Biochemistry*

**Corresponding author: ambily.vr@kvasu.ac.in, Ph: 9400447332*

The transition stage of a dairy cow, spanning from three weeks before to three weeks after calving, is critical as it is associated with a higher incidence of metabolic, nutritional, and infectious diseases. The increased energy requirement associated with the commencement of lactation is not balanced by an equivalent increase in energy intake, resulting in a worsening of existing negative energy balance during periparturient period. Frequent monitoring of metabolic disorders during early lactation is very essential for the evaluation of successful management strategies during transition period. Unlike clinical illnesses, subclinical metabolic disorders are considerably difficult to identify; they demand extra diagnostic procedures, and gaining a comprehensive picture is not always feasible as there will be no overt clinical presentations. This leads to severe and unexpected economic impact to farms. Subclinical ketosis is an important metabolic disorder in early lactation due to failure of adaptation to existing negative energy balance. In the present study, post-partum cows with blood BHB value of ≥ 1.2 mmol/L were considered as positive for SCK (Oetzel, 2004).

Subclinical ketosis causes severe economic impacts on production and profit in farms. Epidemiological risk factors like age and parity, quarter of calving, body condition of animal, management practices, herd size and days in milk and are considerably affect the incidence of SCK. Published data regarding scientific researches on incidence and epidemiological aspects of SCK among cattle population in Kerala are meagre. With these considerations, present study was conducted with objective of screening of postpartum dairy cows in Thrissur district for subclinical ketosis (SCK) and investigation of epidemiological aspects of SCK.

Materials and methods

One hundred and fourteen multiparous animals from various farms and individual households at 14th and 28th day post-partum were screened for SCK. Post-partum cows with blood BHB value of ≥ 1.2 mmol/L were considered as positive animals.

Estimation of blood BHB

For sampling procedures, the cows were restrained properly for collecting a single drop of capillary blood form ear tip. An electronic handheld device, FreeStyle optimum H (Abbott Diabetic care Ltd. Range Road, Witney, Oxon, UK) was used to analyse the BHB concentration in capillary blood. After inserting the test strip (FreeStyle optimum-H β ketone test strips) into the handheld device, the front edge of the strip was dipped directly on to the drop of blood. There was a ten second count-down before the blood BHB result display.

Epidemiological investigation and analysis of risk factors

Details on parity, breed, age, body condition score of animal and sex of the calf were collected from all the 114 animals. Calendar year was divided into four quarters-first (January-March), second (April-June), third (July-September) and fourth (October-December) and data on quarter of the year in which the animals calved was also collected. Data regarding concurrent transition period abnormalities were collected from farm records.

The association of risk factors on the incidence of SCK was tested using chi-square tests. The breeds of animals were categorised into crossbred Holstein Friesian and crossbred Jersey. Parity was categorised into second parity (C_2) and above C_2, age into up to 6 years and above 6 years, season of calving as the first and second half of the year and body condition score as ≤ 3 and above 3. Transition period abnormalities in diseased and normal animals were compared using Z-test for independent proportions. Blood BHB values were compared using independent t-test and body condition score of diseased and normal animals were compared using Mann-Whitney U test. All the statistical analysis was done using SPSS version 24.0.

Results and discussion

Screening of animals

During the study period from May

2021 to April 2022, 114 early post-partum multiparous animals were screened for SCK on day 14 and 28 post-partum. Subclinical ketosis was confirmed in 27 (23.7 *per cent*) animals on 14th day and 16 (14.04 *per cent*) animals on 28th day post-partum. Incidence of disease was highest in 14th day post-partum when compared with 28th day. Similar observations were previously reported by Dar *et al*. (2018). Mohammed *et al*. (2019) recorded 23.5 *per cent* incidence of SCK during second week of lactation in multiparous cows in Bikaner, Rajasthan. The mean concentration of blood BHB value of diseased and normal animals on day 14 and 28 post-partum are presented in Table 1. The blood BHB concentration of diseased animals on 14th and 28th day were 1.71 ± 0.13 and 1.37 ± 0.17 mmol/L. Similar results were previously observed by Mezzetti *et al*. (2019). Corresponding values of normal animals were 0.74 ± 0.04 and 0.77 ± 0.03 mmol/L. This was in accordance with observations by Anisha (2017). Blood BHB concentration of diseased animals on both test days were significantly higher ($p \leq 0.01$) than normal animals.

Risk factors associated with occurrence of SCK

Multiparous animals in second to seventh lactation were screened in the present study (Table 2). Incidence of SCK was highest in post-partum animals of parity 2 (C_2) (54.8 *per cent*), followed by parity 3 (C_3) (32.3 *per cent*) and parity (C_4) (12.9 *per cent*). Present study found no association between parity and incidence of SCK on risk analysis; similar to Garro *et al.* (2014). Higher incidence of SCK was noticed in crossbred Holstein Friesian cows (90.3 *per cent*), followed by crossbred Jersey (9.7 *per cent*) (Table 3). The incidence was more in Holstein Friesian crossbred herds in the studies of Mohammed *et al.* (2019) in Rajasthan, India and Hossain and Samad (2019) in Bangladesh. Higher incidence of SCK was observed in animals of age 4 - 6 years (54.8 *per cent*), followed by 6 - 8 years (35.5 *per cent*). Lower incidence (9.7 *per cent*) was reported in animals of 2 - 4 years in the present study (Table 4). Asl *et al*. (2011) observed that incidence of SCK increased with age of the cow. This is due to more active metabolic and physiologic adaptations at younger age of animals. As age advances, physiologic processes become slower. A non-significantly higher BCS (3.5) was recorded in diseased animals on day 14 (Table 5). This was in agreement with Gillund *et al*. (2001) and Bewley and Schutz (2008). Samiei *et al*. (2015) observed a reduction of more than 0.5 units on BCS of animals during the first month of lactation and reported that BCS of animals on 3rd, 14th and 28th day post-partum exhibited a strong correlation with body condition score at calving.

A non-significant reduction of 0.25 units was observed in the diseased animals on 28th day. No significant change was observed in median BCS of normal animals on 14th and 28th day. Samiei *et al*. (2015) observed no association between BCS on test days and occurrence of SCK. Similar results were obtained in the present study. The highest incidence of SCK was occurred in animals that calved in the months of July to September, followed by January to March. Low incidence was noticed in animals that calved in months of October to December (Table 6). This was similar to the observations of Vanholder *et al*. (2015). In SCK affected group, 58.1 *per cent* of animals had male calf and 41.9 *per cent* had female calf. But no significant association was noticed between occurrence of SCK and sex of the calf in the present study. Results were similar to Garro *et al*. (2014), who observed no association between sex of the calf and occurrence of hyperketonemia in

Table 1. Comparison of blood BHB of diseased and normal animals on 14th and 28th day post-partum

Variable	Period	Diseased (Mean ± SD)	Normal (Mean ± SD)	t-value	p-value
Blood BHB (mmol/L)	Day 14	1.71 ± 0.13	0.74 ± 0.04	7.319**	< 0.001
	Day 28	1.37 ± 0.17	0.77 ± 0.03	3.487**	0.002
	t-value (p-value)	2.536* (0.018)	0.751ns (0.455)		

Table 2. Parity wise distribution of screened animals

Parity	SCK positive (n=31)		Normal (n=83)	
	Number	*Per cent*	Number	*Per cent*
C2	17	54.8	38	45.8
C3	10	32.3	25	30.1
C4	4	12.9	15	18.1
C5	0	0.0	3	3.6
C6	0	0.0	1	1.2
C7	0	0.0	1	1.2

Table 3. Breed wise distribution of screened animals

Breed	SCK positive (n=31)		Normal (n=83)	
	Number	*Per cent*	Number	*Per cent*
CBHF	28	90.3	71	85.6
CBJ	3	9.7	9	10.8
Vechur	0	0.0	3	3.6

Table 4. Age wise distribution of screened animals

Age	SCK positive (n=31)		Normal (n=83)	
	Number	*Per cent*	Number	*Per cent*
2 - 4	3	9.7	12	14.5
4 - 6	17	54.8	29	34.9
6 - 8	11	35.5	26	31.3
Above 8	0	0	16	19.3

Table 5. Comparison of BCS between diseased and normal animals on day 14 and 28 post-partum

Period	Median (Inter quartile range)		Z-value
	Diseased	Normal	
Day 14	3.50 (0.75)	3.25 (0.25)	1.051^{ns} (0.293)
Day 28	3.25 (0.31)	3.25 (0.50)	1.713^{ns} (0.087)
Z-value (P-value)	1.807^{ns} (0.071)	1.117^{ns} (0.201)	

ns non-significant

cattle maintained in grazing production system. Contradictory to this, Nydam *et al.* (2013) and Alberghina *et al.* (2015) stated that sex of calf was an important predictor for the occurrence of SCK in early lactation multiparous animals. According to these reports, male calves required more energy in late gestation due to their larger size, and this higher energy requirement led to a larger negative energy balance in early lactation, putting them at risk for hyperketonemia.

Assessment of risk factors revealed no significant association between all the above-listed factors and the incidence of SCK on analysis of risk.

Concurrent transition period abnormalities

The occurrence of other transition period abnormalities which were diagnosed on or before the test days in SCK positive and normal animals were evaluated in the present study. No significant difference was noticed in incidence of these abnormalities in SCK positive and normal healthy animals in the present study (Table 8). Mastitis was the most common abnormality noticed in diseased group (32.0 per cent). Higher incidence of clinical mastitis was previously reported among early lactation dairy cattle in Thrissur district (Easaw *et al*., 2022). Dohoo and Martin (1984) and Suthar *et al*. (2013) could not find any significant

Table 6. Classification of screened animals based on quarter of calving

Quarter	Diseased (n=31)		Normal (n=83)	
	Number of animals	*Per cent*	Number of animals	*Per cent*
1(January-March)	9	29.0	22	26.5
2(April-June)	3	9.7	8	9.6
3(July-September)	11	35.5	23	27.7
4(October-December)	8	25.8	30	36.1
Total	**31**	**100.0**	**83**	**100.0**

Table 7. Association of risk factors on incidence of SCK

Factors	Category	Diseased		Normal		χ^2 Value	P-value
		Number	*Per cent*	Number	*Per cent*		
Breed#	CBHF	28	90.3	71	88.8	0^{ns}	1.0
	CBJ	3	9.7	9	11.3		
Parity	C_2	17	54.8	38	45.8	0.423^{ns}	0.515
	Above C_2	14	45.2	45	54.2		
Age	Upto 6	20	64.5	41	49.4	2.074^{ns}	0.150
	Above 6	11	35.5	42	50.6		
Period of calving	First half	12	38.7	30	36.1	0.064^{ns}	0.801
	Second half	19	61.3	53	63.9		
Sex	Male	18	58.1	51	61.4	0.108^{ns}	0.742
	Female	13	41.9	32	38.6		
BCS	≤ 3	8	26.7	21	27.3	0.004^{ns}	0.949
	Above 3	22	73.3	56	72.7		

ns non-significant

 # Yates correction was applied

Table 8. Transition period abnormalities in diseased and normal groups

Abnormalities	Diseased		Normal		Z-value	p-value
	No of cases	*Per cent*	No of cases	*Per cent*		
Mastitis	8	32.0	18	20.0	1.172^{ns}	0.241
metritis	3	12.0	0	0.0	1.846^{ns}	0.065
RFM	2	8.0	1	1.1	1.244^{ns}	0.213
Hypocalcemia	0	0.0	0	0.0	-	-
Lameness	0	0.0	2	2.2	1.430^{ns}	0.153
Dystocia	1	4.0	5	5.6	0.338^{ns}	0.735
Haemoparasites	1	4.0	10	11.1	1.386^{ns}	0.166
Abortion and still birth	1	4.0	2	2.2	0.422^{ns}	0.673
Anorexia and weakness	1	4.0	0	0.0	1.021^{ns}	0.154
Prolonged gestation	1	4.0	0	0.0	1.021^{ns}	0.154

ns non-significant

association between occurrence of subclinical ketosis and mastitis. Incidence of metritis (12.0 *per cent*) and retention of foetal membrane (8.0 per cent) were also recorded in diseased group. According to Garro *et al*. (2014), cows with metritis had 4.9 times higher risk of SCK than cows without metritis. Other recorded transition period abnormalities in SCK affected animals in current study were dystocia (4.0 per cent), haemoparasitic infection (4.0 per cent), abortion (4 per cent), anorexia and weakness (4.0 per cent) and prolonged gestation (4.0 per cent).

Conclusion

Present study evaluated the epidemiological aspects of subclinical ketosis in early lactation dairy cattle in various farms and individual households in Thrissur district. The overall prevalence of SCK was 27.2 per cent with highest incidence on 14th day (23.7 per cent). Prevalence of disease was more in animals of second parity within age group of 4-6 years. Crossbred Holstein Friesian cows were more affected and 58 per cent of affected animals had male calf. Occurrence was high in animals that calved in months of July to September. Mastitis and metritis were the most common concurrent abnormalities detected, but were not associated with occurrence of SCK. Present study revealed no association between risk factors like parity, breed, age, body condition score of animals, sex of the calf, season of calving and presence of concurrent transition period abnormalities and occurrence of SCK. Further studies are warranted with larger uniform herds for evaluation of risk factors to avoid confounding effect of management practices and nutrition on occurrence of subclinical ketosis.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Alberghina, D., Piccione, G., Giannetto, C., Morgante, M. and Gianesella, M. 2015. Sex of offspring influences metabolism during early transition period in dairy cows. *Arch. Anim. Breed*. **58**: 73-77.

Anisha, J.P. 2017. Metabolic profile of crossbred dairy cows during transition period. *M.V.Sc thesis,* Kerala Veterinary and Animal Sciences University, Pookode, 76p.

Asl, A.N., Nazifi, S., Ghasrodashti, A.R. and Olyaee, A. 2011. Prevalence of subclinical ketosis in dairy cattle in the Southwestern Iran and detection of cutoff point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. *Prev. Vet*. Med. **100**: 38-43.

Bewley, J.M. and Schutz, M.M. 2008. An interdisciplinary review of body condition scoring for dairy cattle. *Prof. Anim. Sci*. **24**: 507-529.

Dar, A.M., Malik, H.U., Beigh, S.A., Hussain, S.A., Nabi, S.U., Dar, A.A., Dar, P.A. and Bhat, A.M. 2018. Clinico-biochemical alternation in bovine ketosis. *J. Entomol. Zool. Stud*. **6**: 1146-1150.

Easaw, A.M., Vijayakumar, K., Davis, J., Shyma, V.H. and Surya, S. 2022. Molecular characterisation of virulence genes in Staphylococcus aureus associated with clinical bovine mastitis. *J. Vet. Anim. Sci*. **53**: 279-284.

Dohoo, I.R. and Martin, S.W. 1984. Subclinical ketosis: prevalence and associations with production and disease. *Can. J. Comp. Med.* **48**: 1-5.

Garro, C.J., Mian, L. and Cobos Roldan, M. 2014. Subclinical ketosis in dairy cows: prevalence and risk factors in grazing production system. *J. Anim. Physiol. Anim. nutr*. **98**: 838-844.

Gillund, P., Reksen, O., Grohn, Y.T. and Karlberg, K. 2001. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. *J. Dairy Sci*. **84**: 1390-1396.

Hossain, S.M.S. and Samad, M.A. 2019. Prevalence of sub-clinical ketosis and its associated cow level risk factors in lactating dairy cross-bred cows in Bangladesh. *J. Vet. Med. OH Res*. **1**: 29-38.

Kanz, P., Drillich, M., Klein-Jobstl, D., Mair, B., Borchardt, S., Meyer, L., Schwendenwein, I. and Iwersen, M. 2015.

Suitability of capillary blood obtained by a minimally invasive lancet technique to detect subclinical ketosis in dairy cows by using 3 different electronic hand-held devices. *J. Dairy Sci*. **98**: 6108-6118.

Khol, J.L., Freigassner, K., Stanitznig, A., Tichy, A. and Wittek, T. 2019. Evaluation of a handheld device for the measurement of beta-hydroxybutyrate in capillary blood obtained by the puncture of the vulva as well as in venous whole blood in cattle. *Pol. J. Vet. Sci.* **22**: 557-564.

Mezzetti, M., Minuti, A., Piccioli-Cappelli, F., Amadori, M., Bionaz, M. and Trevisi, E. 2019. The role of altered immune function during the dry period in promoting the development of subclinical ketosis in early lactation. *J. Dairy Sci*. **102**: 9241-9258.

Mohammed, N., Jaiswal, M. and Bihani, D.K. 2019. Prevalence of subclinical and clinical ketosis in cattle in and around Bikaner. *Biol. Rhythm Res*. **53**: 1-9.

Nydam, D.V., Ospina, P.A., McArt, J.A., Oetzel, G. and Overton, T.R. 2013, April. Monitoring negative energy balance in transition dairy cows for herd results. In *Proceedings of the 22nd Tri-State Dairy Nutrition Conference. Michigan State University, Fort Wayne, Indiana, USA. p9-22*.

Oetzel, G.R. (2004) Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. North Am. Food Anim. Pract*. **20**:651–674.

Samiei, A., Liang, J., Ghorbani, G., Hirooka, H., Ansari-Mahyari, S., Sadri, H. and Tufarelli, V. 2015. Relationship between dietary energy level, silage butyric acid and body condition score with subclinical ketosis incidence in dairy cows. *Adv. Anim. Vet. Sci*. **3**: 354-361.

Suss, D.A.V.I.D., Drillich, M., Klein-Jobstl, D., Wagener, K., Krieger, S., Thiel, A., Meyer, L., Schwendenwein, I. and Iwersen, M. 2016. Measurement of β-hydroxybutyrate in capillary blood obtained from an ear to detect hyperketonemia in dairy cows by using an electronic handheld device. *J. Dairy Sci*. **99**: 7362-7369.

Suthar, V.S., Canelas-Raposo, J., Deniz, A. and Heuwieser, W. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *J. Dairy Sci*. **96**: 2925-2938.

Vanholder, T., Papen, J., Bemers, R., Vertenten, G. and Berge, A.C.B. 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. *J. Dairy Sci.* **98**: 880-888. ■