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Subclinical ketosis: Prevalence and some risk factors in cross breed and imported breed dairy cows in Mosul, Iraq

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Abstract

Although ongoing control strategies are attempting to minimize certain metabolic disorders such as hyperketonemia, factors such as genetics, management, environment, and geography still present a potential challenge to the achievement of the desired results. In light of this, this study was conducted (i) to determine the prevalence of subclinical and clinical ketosis based on the concentration of ketone bodies (BHB) using ELISA Kit, and (ii) to examine a number of epidemiological risk factors related to the conditions. From October, 2020 to the January, 2021. A 80 blood samples were collected in a cross-sectional survey in different areas of Mosul. Data included age, management systems, parity, and origin. At the cut-off point ≥1.2-≤ 1.4 mmol / L, the prevalence of subclinical ketosis was 27.5% and for clinical ketosis it was 5%. Results revealed that the animals aged > 3 years were more at risk for subclinical ketosis compared to animals aged ≤ 3 years RR = 2.6471. Multiparous animals were more at risk compared to the primiparous (two or less births) RR = 2.0652. No significant difference between the animals' origins RR = 0.6863. Indoor and outdoor system animals were more at risk for subclinical ketosis compared to the indoor system only RR = 2.1389. In conclusion, hyperketonemia is prevalent in Mosul. Certain risk factors should be taking into account when planning control programs. Attention should be paid to diagnosis, management, and control of this disease during the risk stage to avert economic loss to the dairy farmers.

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Introduction

Ketosis (Hyperketonemia) in dairy cows is a predominant problem faced by the animal husbandry industry worldwide, it is a frequently recurring and costly disorder in ruminants with significant economic implications to farm profitability in terms of substantially decreased milk production, minimizing the insemination rate and incurring additional treatment costs. An increase of ketone bodies in the numerous body fluids accompanied by clinical signs such as loss of appetite, unrepresentative licking, and chewing, rapid body weight loss, and decreased milk yield are referred to as clinical ketosis (CK), while subclinical ketosis (SCK) is evident in an increase of ketone bodies in the blood, plasma, serum, urine and milk beyond the standard range in a cow without detectable signs of disease (1). Most of the dairy cows at the transition period (three weeks before and after calving) are affected by diverse physiological, hormonal, and metabolic changes, besides change in feeding and housing, which in turn significantly increases the incidence of the diseases in this timeframe. During the onset of lactation, most dairy cows have a substantially high requirement for glucose and inadequate adaption in this period can result in a negative energy balance and increased risk for occurrence of ketosis and affect animal health and production. Globally, the literature on the prevalence of SCK ranges between 10% and 40% or from 8.5% to 58.8%, depending on the various diagnostic techniques used and thresholds, with the most prevalence during the first 21 days of calving (2). The

common authoritative risk factors (including both the herd and Cow-level risk factors) for ketosis are management, the season, herd size, breed, dystocia, and parity (3). Economically subclinical ketosis has a significant impact on dairy farms, and losses from undiagnosed SCK may exceed losses caused by CK. With subclinical ketosis, performance and production efficiency of animals is affected obviously by increasing the culling rates, reproductive decline, decreased milk yield, metritis, withdrawal insemination index, mastitis, abomasal displacement, and risk of developing CK (4). Several biochemical tests to diagnose ketosis by measuring the ketone bodies, predominantly β-hydroxybutyrate (BHBA) in biological fluids are available. Normal cows have plasma BHBA concentrations less of than 1000μmol/L and cows with clinical ketosis have concentrations often in excess of 2500μmol/L. Measurement of blood B-hydroxybutyrate BHBA levels in serum or plasma with a cut-off point of 1.2 to 1.4 mM is the gold standard test for diagnosis of subclinical ketosis and commonly used to differentiate cows with and without SCK (1,5). Several inexpensive cowside tests are also commercially available, but with lower sensitivity and specificity in comparison with the gold standard test (6,7).

In Mosul, Iraq, the occurrence and risk factors for subclinical ketosis are not known, so, the objectives of this study are formulated to address this issue, and bridge this gap to determine the prevalence of subclinical ketosis in cows in Mosul city, Iraq, by measuring blood BHBA levels in serum using ELISA test, in addition to; to examine a number of epidemiological risk factors related to the prevalence of subclinical ketosis.

Materials and methods

Animals and study location

In total, 80 blood samples were collected from the newly-calved cows in a cross-sectional survey in different areas of Mosul city, Iraq, which included Gogjalee, Shalalat, Bazawayah, Al-Intisar, Julechwan, and Tehrawa, from the beginning of October, 2020 to the end of January, 2021. The ages of the animals ranged from two to 10 years, from different management systems (indoor, indoor and outdoor), parity (primiparous and multiparous) and of different origins: local crossbreed and imported breed. Epidemiological information, clinical signs, and clinical vital parameters (pulse, respiratory rate, rumen movements, and heart rate) were recorded in a preformed clinical card.

Samples

A total of 7 ml of the blood was collected from the middle coccygeal vein using sterile syringes after cleaning the area and sterilizing it with 70% ethyl alcohol in the morning (Between 8-9 A.M.). Blood samples were placed in sterile 10 ml glass tubes without anticoagulant to obtain blood serum for estimating the concentration of BHB in serum. Animals with BHB concentration in serum <1mmol/L were considered as control group.

Laboratory analysis

The concentration of ketone bodies (Beta-hydroxy butyrate BHB) in serum samples was measured with an ELISA kit (from Bioassay Technology Laboratory, Shanghai, China) following the manufacturer's instructions. The plate was read by an Elisa Reader at 450 nm wavelength. The test was performed at room temperature.

Statistical analysis

The prevalence was calculated using descriptive statistics on Excel program 2010, and the Relative Risk was analyzed for epidemiological factors using the statistical program Epi-Info V.7.2, and the Significant $P<0.05$ differences between affected groups and control group, (8).

Results

The results of the present study indicated that the prevalence of subclinical ketosis in cows in Mosul was 22/80 (27.5%) depending on the concentration of BHB in the serum by ELISA test at the cut-off point ≥1.2-≤ 1.4 mmol/L, and the prevalence of clinical ketosis was 5% (4/80) at a threshold 2.9 mmol/L (Table 1). The study findings also indicated that 36% and 2% of subclinical and clinical ketosis cases respectively, were diagnosed in the first three weeks of birth, and 5% of cases of clinical ketosis at six weeks after birth.

Table 1: Concentration of BHB in the serum of the cows by ELISA test

BHB mmol/L	Total No. tested	No. of Animals	Percentage (%)
≥1.2-≤ 1.4	80	22 (Sub Clinical Ketosis)	27.5
≥2.9		4 (Clinical Ketosis)	5
<1		54 (-ve control group)	67.5

This study showed significant ($p <0.03$) difference between the affected animals depending on the age. Animals aged > 3 years were more at risk for subclinical ketosis compared to animals aged ≤ 3 years, and with a relative risk (RR=2.6471, CI= 1.34 - 5.21) (Table 2). Findings also indicated a significant ($p <0.05$) difference between the affected animals depending on the number of births, where the multiparous animals (three and more births) were more at risk for subclinical ketosis compared to the primiparous (two or less births) with a RR=2.0652, CI= 1.04 - 4.04 (Table 2).

The findings indicated that no significant ($p <0.4$) difference between affected animals, depending on the animals' origin, as the relative risk between local

crossbreed and imported infected animals (RR=0.6863, CI=0.28 - 1.64). The results of this study also indicated a significant (p <0.04) difference between the affected animals depending on the management systems, where the animals with indoor and outdoor systems were more at risk for subclinical ketosis compared to the animals with the indoor system with a relative risk (RR=2.1389, CI= 1.01 - 4.52) (Table 2).

Table 2: Risk factors for subclinical ketosis based on BHB concentration

Factors	No. case tested	No. of +ve (%)	RR	CI	P
Age					
<4years	9	5 (6.25%) [a]	1		
≥4 years	71	17 (21.25) [b]	2.6471	1.34 - 5.21	0.03
Parity					
≤ 2	23	10 (12.5%) [a]	1		
≥ 3	57	12 (15%) [b]	2.0652	1.04 - 4.04	0.05
Origin					
Local	24	5 (6.25%) [a]	1		
Imported	56	17 (21.25) [a]	0.6863	0.28 - 1.64	0.4
Management					
Indoor	36	14 (17.5%) [a]	1		
Indoor and outdoor	44	8 (10%) [b]	2.1389	1.01 - 4.52	0.04

Values significantly (P < 0.05) different labeled with different letters (a, b or c). RR (Relative Risk), CI (Confidence interval), P (probability).

Discussion

The current study showed a high prevalence of subclinical ketosis in cows in Mosul, Iraq, the occurrences of subclinical and clinical ketosis were 27.5% and 5%, respectively, depending on the BHBA concentration in serum by ELISA test. The prevalence rate of the current study may agree with or differ from other studies conducted in other countries. In Iran, the prevalence of subclinical ketoacidosis was 14.4% and 18.42% (9,10). In Turkey, it was 33.3% (11). Suthar *et al.* (4) indicated in a study that included several European countries include Italy, Hungary, Poland, Serbia, Slovenia, Spain, Germany, Portugal and Turkey that the overall occurrence of subclinical ketosis was 21.8%, ranging from 11.2 to 36.6%. Several reasons, such as geographical area, season, number of animals examined, techniques used for diagnosis, management system, breed, the threshold used, and type of sample, could explain the difference in prevalence in this study and other studies in other regions or countries. These findings are in accordance with those recorded by (11-14). In general, numerous studies indicated that the global prevalence rates for subclinical ketosis range from 8-40% (15), and clinical ketosis 2-15% (16).

This study showed that the animals aged > 3 years were significantly more at risk for subclinical ketosis compared to animals ≤ 3 years of age, relative risk (RR = 2.6471). The reason could the multiparity and increased milk production. Or in other hand, it may be due to the fact that young cows may increase metabolic activities during early lactation, against stressor conditions (i.e., NEB). This adaptation and activity moderately decrease with the progress of age. This finding agrees with results of other studies (3,17).

Results also showed that the multiparous animals (three and more births) were significantly more at risk for subclinical ketosis compared to the primiparous (two or less births) with RR = 2.0652. It is possible that the reason is the high milk production, which may be predisposed to negative energy, as the cows in the first or second calving may not reach the peak of milk production in their early stages of completion compared to multiparous cows. This result is in agreement with the outcomes of other studies by (18,19).

The finding of this study indicates that there is no significant difference between the animal's origin (RR = 0.6863). This result is in agreement with (20). This can be attributed to the absence of a difference in the quantities of milk production between imported and local crossbreed cows in addition to the difference in management and feeding systems and environmental conditions, which are among the critical factors that affect the ability of cows to adapt to the different conditions that exist between different countries. This result is supported by previous studies (14,21). Contrary to the results of this study (7,20) indicated a significant difference in the occurrence of subclinical ketosis between the animal breeds, and the prevalence rate may be high in animals with high genetic potential milk production, as these animals are unable to withstand the pressures of high nutritional requirements for milk production and experience a negative energy phase.

The results of this study revealed significant difference between the affected animals depending on the management systems, whereby the animals exposed to

indoor and outdoor systems are more at risk for subclinical ketosis compared to the animals exposed to the indoor system only (RR = 2.1389). The results of the present study are in agreement with (22). This could be attributed to the availability of concentrated nutrition for animals raised by the indoor feeding system and its reduction in cows raised in the mixed system (indoor and outdoor feeding), as reducing the concentrated diet may decrease the ability of cows to obtain sufficient energy to meet the requirements for milk production and subsequently risk the development of negative energy balance. Yang *et al.* (23) confirmed that decreasing the feed intake coinciding with the requirement to maintain a high milk production is the cause of ketosis in milk-producing cows.

Conclusions

In conclusion, findings of the current study showed that SCK is prevalent in dairy cows in Mosul, Iraq, and can be observed at various rates in many countries worldwide. The major risk factors for SCK are age, parity, and management system. More epidemiological studies are recommended to evaluate the magnitude of the disease and its economic impact.

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Conflict of interest

Authors declare no conflict of interests of the manuscript.

References

1. Benedet A, Manuelian CL, Zidi A, Penasa M, De Marchi M. Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance. Animal. 2019;13(8):1676-1689. DOI: 10.1017/S175173111900034X
2. Duffield TF, Lissemore K D, McBride BW, Leslie KE. Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci. 2009;92:571-580. DOI: 10.3168/jds.2008-1507
3. Vanholder T, Papen J, Bemers R, Vertenten G, Berge AC. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J Dairy Sci. 2015;98(2):880-888. DOI: 10.3168/jds.2014-8362
4. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci. 2013;96(5):2925-2938. DOI: 10.3168/jds.2012-6035
5. Rahawy MA. Study on the post-partum disorders and their relationship with the reproductive performance in Iraqi cow-buffaloes. Iraqi J Vet Sci. 2021;35(2):313-317. DOI: 10.33899/ijvs.2020.126771.1387
6. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. J Biomed Informat. 2014;48:193-204. DOI: 10.1016/j.jbi.2014.02.013
7. Ospina PA, Nydam DV, Stokol T, Overton TR. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J Dairy Sci. 2010;93(2):546-554. DOI: 10.3168/jds.2009-2277
8. Nooruldeen MY, Jaafar SE, Salih AI. Seroprevalence of Neospora caninum infections in cattle in Kirkuk province. Iraqi J Vet Sci. 2021;35(2):331-334. DOI: 10.33899/ijvs.2020.126832.1394
9. Sakha M, Ameri M, Sharifi H, Taheri I. Bovine subclinical ketosis in dairy herds in Iran. Vet Res Comm. 2007;31(6):673-679. DOI: 10.1007/s11259-007-0053
10. Sharif T M, Hadadi M, Noughabi HH, Mohammadi A, Rostami F, Sharifi H. Bovine subclinical ketosis in dairy herds in Nishaboor, Iran. Comp Clin Pathol. 2012;21(6):1637-1641. DOI: 10.1007/s00580-011-1340-2
11. Voyvoda H, Erdogan H. Use of a hand-held meter for detecting subclinical ketosis in dairy cows. Res Vet Sci. 2010;89(3):344-351. DOI: 10.1016/j.rvsc.2010.04.007
12. Anna C B, and Vertenten G. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. J Dairy Sci. 2014;97 :2145-2154. DOI: 10.3168/jds.2013-7163
13. Biswal S, Nayak DC, Sardar KK. Prevalence of ketosis in dairy cows in milk shed areas of Odisha state, India. Vet World. 2016;9(11):1242. DOI: 10.14202%2Fvetworld.2016.1242-1247
14. Brunner N, Groeger S, Canelas Raposo J, Bruckmaier RM, Gross JJ. Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe. Trans Anim Sci. 2019;3(1):84-92. DOI: 10.1093/tas/txy102
15. Al-Obaidii1WA, Al-Obaidi QT, Hasan SD. Detection of Trichomoniasis in cattle in Nineveh province. Iraqi J Vet Sci. 2021;35(2):287-290. DOI: 10.33899/ijvs.2020.126790.1380
16. McArt JA, Nydam DV, Oetzel GR. Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci. 2012;95(9):5056-66. DOI: 10.3168/jds.2012-5443
17. Garro CJ, Mian L, Cobos Roldán M. Subclinical ketosis in dairy cows: Prevalence and risk factors in grazing production system. J Anim Physiol Anim Nutrient. 2014;98(5):838-44. DOI: 10.1111/jpn.12141
18. Elise H, Tatone T F, Duffield S J, LeBlanc JD, Jessica LG. Investigating the within-herd prevalence and risk factors for ketosis in dairy cattle in Ontario as diagnosed by the test-day concentration of β-hydroxybutyrate in milk. J Dairy Sci. 2016;100:1308-1318. DOI: 10.3168/jds.2016-11453
19. Mohammed ZA. Association between clinical and subclinical mastitis and reproductive performance of cows at Nottingham dairy centre. Iraqi J Vet Sci. 2021;35(2):343-350. DOI: 10.33899/ijvs.2020.126843.1398
20. Berge AC, Vertenten G. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. J Dairy Sci. 2014;97(4):2145-54. DOI: 10.3168/jds.2013-7163
21. Madreseh-Ghahfarokhi S, Dehghani-Samani A, Dehghani-Samani A. Ketosis (acetonaemia) in dairy cattle farms: Practical guide based on importance, diagnosis, prevention and treatments. J Dairy Vet Anim Res. 2018;7(6):299-302. DOI: 10.15406/jdvar.2018.07.00230
22. Compton CW, McDougall S, Young L, Bryan MA. Prevalence of subclinical ketosis in mainly pasture-grazed dairy cows in New Zealand in early lactation. New Zealand Vet J. 2014;62(1):30-7. DOI: 10.1080/00480169.2013.823829
23. Yang W, Zhang B, Xu C, Zhang H, Xia C. Effects of ketosis in dairy cows on blood biochemical parameters, milk yield and composition, and digestive capacity. J Vet Res. 2019;63(4):555. DOI: 10.24782Fjvetres-2019-0059

الخلونمية تحت السريرية: الانتشار وبعض عوامل الخطورة في أبقار الحليب الهجينة والمستوردة في الموصل، العراق

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الخلاصة

على الرغم من أن استراتيجيات السيطرة مستمرة للتقليل من الاضطرابات الأيضية مثل فرط كيتون الدم، إلا أن بعض العوامل مثل الوراثة والإدارة والبيئة والمنطقة الجغرافية لا تزال تمثل تحديًا محتملاً لتحقيق النتائج المرجوة. في ضوء ذلك، أجريت هذه الدراسة لتحديد مدى انتشار الخلونمية تحت السريري والسريري اعتمادا على تركيز أجسام الكيتون نوع بيتا هيدروكسي بيوتيريت باستخدام تقنية الإنزيم المناعي الممتز، وللتحقق من دور بعض عوامل الخطورة الوبائية على الانتشار. منذ بداية تشرين الأول ٢٠٢٠ وحتى نهاية كانون الثاني ٢٠٢١، تم جمع ٨٠ عينة دم من أبقار حديثة الولادة في مسح مقطعي لمناطق مختلفة من الموصل. تضمنت البيانات الوبائية العمر وأنظمة الإدارة وعدد الولادات والمنشأ. اعتمادًا على نقطة القطع ≤١,٢-≤ ١,٤ مللي مول / لتر، أظهرت النتائج أن انتشار الخلونمية تحت السريرية كان ٢٧,٥% وبالنسبة الحالة الخلونمية السريرية، كانت ٥%. الحيوانات التي يزيد عمرها عن ثلاثة سنوات كانت أكثر عرضة للإصابة بالخلونمية تحت السريرية مقارنة بالحيوانات التي يبلغ عمرها ≤ ٣ سنوات بخطورة نسبية ٢,٦٤٧١ كانت الحيوانات متعددة الولادة أكثر عرضة للإصابة مقارنة بالحيوانات (ولادتان أو أقل بخطورة نسبية ٢,٠٦٥٢). لم يكن هناك فرق معنوي بين منشأ الحيوانات بخطورة نسبية ٠,٦٨٦٣. الحيوانات ذات نظام التربية الداخلي والخارجي كانت أكثر عرضة للإصابة مقارنة بنظام التربية الداخلي فقط بخطورة نسبية ٢,١٣٨٩ فإن الخلونمية منتشر في الموصل. يجب أن تؤخذ عوامل الخطورة في الاعتبار عند التخطيط لوضع برامج السيطرة. يجب الاهتمام بتشخيص هذا المرض وإدارته ومكافحته خلال مرحلة الخطر لتجنب الخسارة الاقتصادية لمزارعي الألبان.