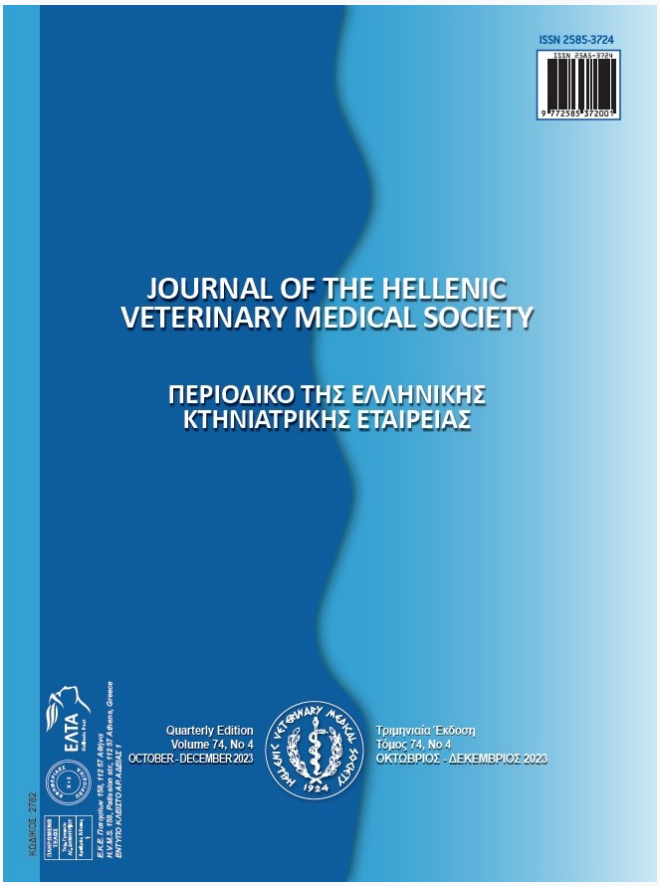
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Research article
Ερευνητικό άρθρο

Prevalence of subclinical ketosis in small backyard Holstein dairy farms in western cities of Turkey

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ABSTRACT: The prevalence of subclinical ketosis (SCK) was investigated in small, family-run backyard dairy farms with 5-20 Holsteins in the villages of western cities in Turkey. A total of 168 multiparous and 60 primiparous cows in130 farms were examined with a cow-side beta-hydroxybutyric acid (BHBA) analyser. The BHBA cut-off point for SCK diagnosis was set at ≥ 1.2 mmol/L. Cows in the study were grouped according to days 0-7 (PP0-7), 8-15 (PP8-15), 16-28 (PP16-28), 0-15 (PP0-15), and 0-28 (PP0-28) postpartum. Overall SCK prevalence in PP0-28 was 26.8%. It was 16.4, 26.4 and 35.8% in PP0-7, PP8-15, and PP16-28 ($p<0.05$), respectively. It was 23.7 and 35.6% in multiparous and primiparous, respectively. For multiparous it was 19.4 and 35.6% and for primiparous 35.1 and 36.4 % in PP0-15 and PP16-28, respectively. The odds ratio was 2.26 and 1.04 for primiparous compared to multiparous in PP0-15 and PP16-28, respectively. Odds were 1.55 and 2.85 for PP16-28 compared to PP8-15 and PP0-7, respectively. It was 1.83 for PP8-15 compared with PP0-7. The prevalence was much higher in primiparous cows than in multiparous cows in seasons other than summer ($p<0.05$). In conclusion, the prevalence of SCK was much higher than in the large integrated farms previously reported in Turkey. The risk of SCK is higher in PP8-15 and PP16-28 than in PP0-7. Primiparous cows were more frequently affected by SCK than multiparous cows, especially in the first two weeks after calving and in the other seasons except for summer.

***Keywords*:** Backyard farm; Holstein; subclinical ketosis

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INTRODUCTION

Subclinical ketosis (SCK) is an early stage of clinical ketosis manifested by elevated ketone bodies in the blood and milk without clinical signs. SCK is one of the most important metabolic diseases in Holstein dairy farms worldwide (Brunner et al., 2019; Deniz et al., 2020; Suthar et al., 2013) and is also reported in Turkey (Aksoy et al., 2022; Sahal et al., 2017; Sentürk et al., 2016; Suthar et al., 2013). In many cases, SCK is defined as an increased concentration of beta-hydroxybutyric acid (BHBA) in the blood≥1.20 mmol/L (Aksoy et al., 2022; Brunner et al., 2019; Sentürk et al., 2016; Suthar et al., 2013) or in milk at cut-off points ≥100 or 200 µmol/L (Aksoy et al., 2022; Berge and Vertenten, 2013). It has a huge economic impact on the profitability of dairy farms due to milk yield losses averaging 300 kg per lactation (Deniz et al., 2020), or Euro 130 - 150 per single SCK case (Mostert et al., 2017; Steeneveld et al., 2020) and other associated metabolic diseases and early culling (Deniz et al., 2020; Raboisson et al., 2015). Based on meta-analyses (Loiklung et al., 2022) and prevalence studies of SCK (Brunner at al., 2019; Suthar et al., 2013), rates of 22.7 %, 24 % and 21.8 % globally and 12.0 % (Başbuğ et al., 2014), 11.2 % (Suthar et al., 2013), 19.4 % (Sentürk et al., 2016) and 8.3 % (Aksoy et al., 2022) in Turkey were reported in large integrated Holstein dairy farms. An important reason for the development of SCK is a negative energy balance (NEB), which develops shortly after calving due to the high energy demand for milk production. NEB led to fat mobilisation and consequently to higher fatty acid oxidation, resulting in increased BHBA levels in the blood or milk (Deniz et al., 2020; Kolk et al., 2017). Other reasons include overfeeding in the prepartum period causing fatty liver (Roche et al., 2018), a high body condition score at calving (Aksoy et al., 2022; Seifi et al., 2011; Vanholder et al., 2015) and seasonal effect (Ha et al., 2023). Various prevention and treatment methods against SCK have been reported, e.g. with propylene glycol (Gordon et al., 2017), monensin controlled released capsule (Melendez et al., 2006) and injectable butafosfan and cyanocobalamin combination (Deniz et al., 2022; Gordon et al., 2017; Sahal et al., 2017). SCK prevalence has mainly been studied in large integrated dairy farms where a professional ration system and farm management were applied. However, in countries like Turkey, there are also small, non-integrated backyard dairy farms with 5-20 animals in villages where the farm is run directly by the owner and where there is no regular veterinary control and no professional ration system. So far, the prevalence of SCK in this type of dairy farming has not been investigated in a multicentre study. The aim of the present study was to investigate the prevalence of SCK in small-sized backyard Holstein dairy farms in the villages of selected western cities in Turkey.

MATERIAL AND METHODS

A total of 168 multiparous and 60 primiparous Holstein dairy cows in 1st to 10th lactation were randomly sampled from 130 small, non-integrated backyard farms managed by family members in villages in İzmir, Aydın, Muğla and Denizli (Aegean region) in Turkey. The number of animals in these farms ranged from 5 to 20 and there was no daily veterinary control and no professional ration-based feeding system. The study animals were divided into the groups according to the postpartum days (PP) on which the blood was collected. This grouping was necessary to observe prevalence differences at different early stages of lactation (Aksoy et al., 2022; Carrier et al., 2004; Duffield, 2000). The number of study cows are n=55 (PP0-7, days 0-7 postpartum), n=106 (PP8-15, days 8-15 postpartum), n=67 (PP16-28, days 16-28 postpartum), n=161 (PP0-15, days 0-15 postpartum), n=228 (PP0-28, days 0-28 postpartum). Ninety-three of the cows (40.8%) were examined in the summer period (June, July and August) and the rest of the cows were examined in the other months of the year (n=135, 59.2%). The number of multiparous cows was n=70 in summer and n=99 in the other months. Whole blood was collected from the coccygeal vein and analysed for BHBA in one drop of whole blood using an easy-to-use cow-side analyser (Khol et al., 2019) (Medtrust Wellionvet Belua, Med Trust Handelsges. GmbH., Austria). The cut-off value for BHBA in blood for SCK diagnosis has been set at ≥ 1.2 mmol/L (Aksoy et al., 2022; Brunner et al., 2019; Sentürk et al., 2016; Suthar et al., 2013) for Holstein dairy cows that did not show signs of clinical ketosis such as constipation, anorexia, a sudden drop in milk yield, fatigue, depression and reduced rumen contraction (Sentürk et al., 2016).

Statistical analysis

Descriptive statistics were performed to determine the percentage of SCK-affected cows in the groups. A chi-square test was used to determine the significant difference between groups PP0-7, PP8-15, PP16-28 as well as between PP0-15 and PP16-28, and seasonal effects. A binary logistic regression test was per-

formed to determine the effect of parity and grouping and the effect of parity and season on SCK prevalence. The Odds ratios for the groups, parities and seasonal effect were calculated for the risk determination.

RESULTS

The prevalence of SCK in all cows, including multiparous and primiparous cows, was shown in Figures 1, 2 and 3. Furthermore, a comparative table (Table 1) was created to discuss the SCK prevalence with the results of others. The average prevalence of SCK was 26.8%. It was 23.7% in multiparous and 35.6% in primiparous Holstein cows (odds: 1.78, p=0.07). The overall prevalence of SCK was 29% in the summer season (June, July and August) and 25.2% in the other months of the year (odds: 1.21, p>0.05). A significant group effect between PP0-7, PP8-15 and PP16-28 (p=0.05) and between PP0-15 and PP16-28 (p<0.05) was found in the SCK prevalence. The odds for SCK were 1.55 and 2.85 for PP16-28 compared to PP8-15 and PP0-7, respectively. For PP8-15, the odds were 1.83 compared to PP0-7. The risk for SCK was high for PP8-15 and PP16-28 compared to PP0-7. Odds ratios were 2.26 and 1.04 for PRP versus MUL in PP0-15 and PP16-28, respectively. Primiparous cows were more likely to be affected by SCK than multiparous cows, especially in the first two weeks after calving, the SCK trend between multiparous and primiparous cows was controversial in the summer and the rest of the year, which was significant (p<0.05) (Figure 3).

DISCUSSION

Overall prevalence of SCK in all dairy Holstein in the present study was higher than the average prevalence reported by different researchers (Aksoy et al., 2022; Ayvazoğlu and Gökçe, 2020; Başbuğ et al.,

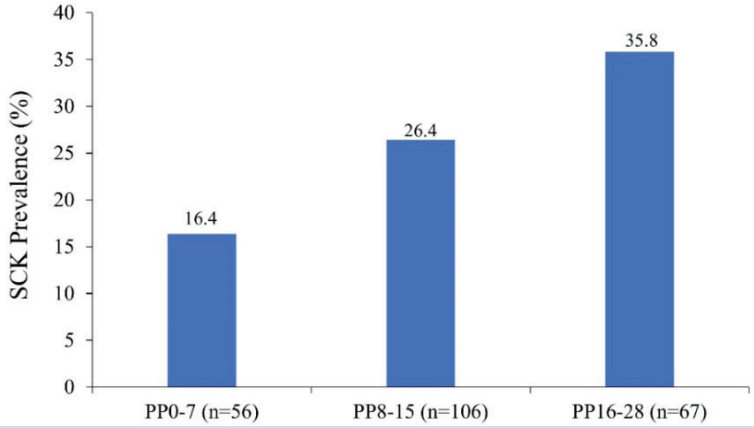


Figure 1. Prevalence of subclinical ketosis (SCK) at days 0-7, 8-15 and 16-28 postpartum (PP) in small and backyard dairy Holstein farms in villages of the Aegean region in Turkey.

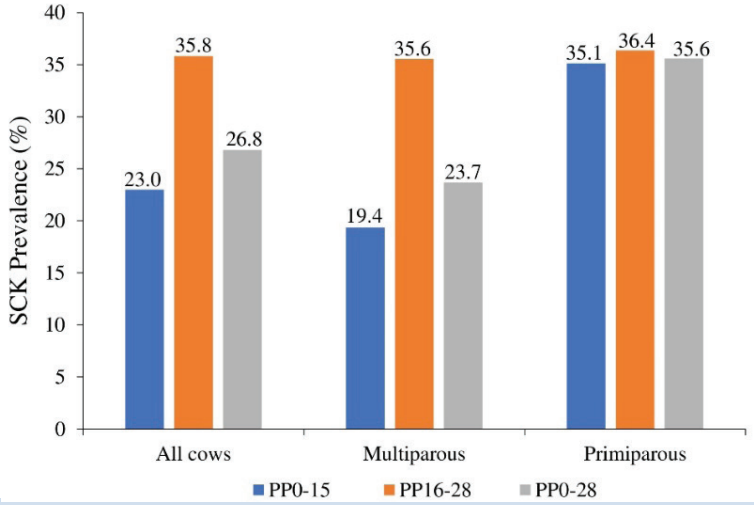


Figure 2. Prevalence of subclinical ketosis (SCK) in small and backyard dairy Holstein farms in villages of the Aegean region in Turkey at days 0-15, 16-28 and 0-28 postpartum (PP).

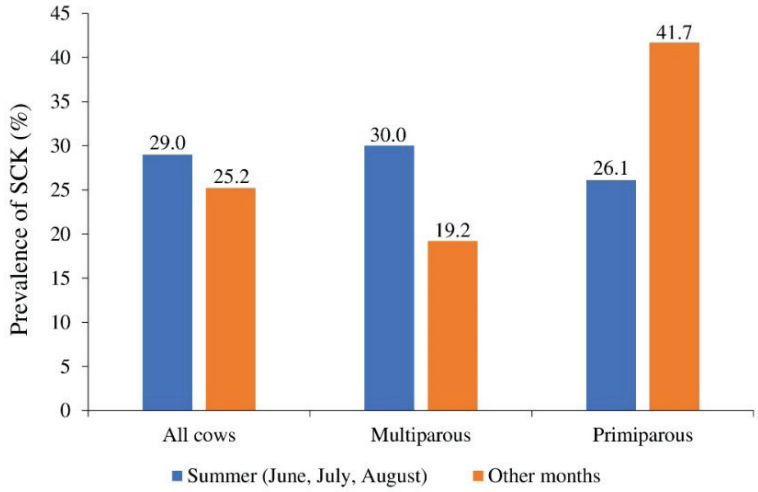


Figure 3. Prevalence of subclinical ketosis (SCK) in summer (June, July and August) and other months of the year in small and backyard dairy Holstein farms in villages of the Aegean region in Turkey.

Table 1. Prevalence of subclinical ketosis (SCK) detected in blood tests in Turkey in the last years.

Source	Region/City in Turkey	Number of animals	Number of farms	Breed of dairy cows	BHBA test day at PP	BHBA cut-off (mmol/L)	SCK prevalence (%)
Present study[1]	Aegean	228	130	Holstein	0-28	≥1.2	26.8
Aksoy et al. 2022[2]	Aegean	206	8	Holstein	14	≥1.2	8.3
Aksoy et al. 2022[2]	Aegean	203	8	Holstein	28	≥1.2	4.7
Ayvazoğlu and Gökçe 2020	Ardahan	200	97	Dairy	7	1.0-1.4	10.0
Ayvazoğlu and Gökçe 2020	Ardahan	200	97	Dairy	14	1.0-1.4	8.5
Şahal et al. 2017[3]	CA	544	13	Holstein	7-14	1.0-3.0	9.7
Şentürk et al. 2016[4]	Mediterranean	315	2	Dairy	14 - 21	≥1.2	14.8
Şentürk et al. 2016[4]	Aegean	325	2	Dairy	14 - 21	≥1.2	16.6
Şentürk et al. 2016[4]	Marmara	340	2	Dairy	14 -21	≥1.2	22.3
Başbuğ et al. 2014	Sivas	200	NEI	Holstein	7 - 56	>1.0	12.0
Suthar et al. 2013[5]	NEI	872	24	Holstein	1-15	≥1.2	11.2
Kennerman 1999[6]	Bursa	244	67	Dairy	Various	>1.0	16.4

BHBA: betahydroxy-butyric acid, NEI: no exact information available, PP: postpartum. CA: Central Anatolia. [1]: Randomised prevalence study in small, non-integrated Holstein farms in backyards. [2]: Randomised prevalence study in large integrated dairy farms. [3]: Randomised study not specific for prevalence in large integrated Holstein dairy farms, prevalence rate was calculated. [4]: Prevalence study in large integrated dairy farms without breed information. [5]: Prevalence study in large integrated Holstein dairy farms without information on region. [6]: Sixty-five of the total 67 farms were owned by farmers (individual peasant), the rest were semi-intensive farms. Thirty-nine cows were only within one month postpartum; 74 cows were in the last month of the prepartum period, 111 cows were between 2 - 3 months postpartum.

2014; Kennermann, 1999; Sahal et al., 2017; Sentürk et al., 2016; Suthar et al., 2013), in large integrated dairy Holstein farms in Turkey. However, it was similar to the average rate reported in other countries (Brunner et al., 2019; Loiklung et al., 2022; Suthar et al., 2013). Furthermore, a slight difference was found between Aegean and Mediterranean regions, and a little higher rate was found in the Marmara region in Turkey in terms of SCK prevalence (Sentürk et al., 2016). In this study, the breed of dairy cows was not reported. The rate of SCK prevalence in the Aegean region looked even slightly lower than the data reported in the present study. The present study was conducted on small backyard farms operated by the owner of the farm. The difference in prevalence could be due to unprofessional management, including feeding, especially in early lactation when cows in small backyard farms need much more energy for milk production shortly after calving. In contrast, a much higher incidence of SCK (36.5%) was found

in a large integrated dairy farm in central Anatolia in a controlled study (Uyarlar et al., 2018). However, the exact timing of blood sampling after calving was unclear, and it was not a prevalence study, which is not comparable to the present study. A similar study (Kennermann, 1999) to the present study was reported, but instead of reporting SCK prevalence in small family member-managed farms, a combined prevalence was reported in both semi-intensive Holstein farms and small family member-managed farms, and the timing of blood collection was also very different (prepartum and postpartum). Thus, the timing of the BHBA test after calving, the BHBA cut-off point, the dairy breed and type of farm are important factors for the SCK prevalence rate, as shown in the comparative table of the present study. In addition to a high body condition score (>4.0) at the time of calving (Aksoy et al., 2022; Ha et al., 2023; Roche et al., 2018), poor silage quality (Roche et al., 2018) and time of year (Ha et al., 2023), one of the most important reasons for the development of SCK is inappropriate feeding, including an unbalanced ration at the beginning of the lactation period shortly after calving, when lactating animals need immediate energy for milk production. Probably, the dairy farms run by family members in the present study did not comply to the need of cows for energy at the start of lactation and during the first month of lactation, especially in primiparous cows. In other European countries (Berge and Vertenten, 2013) and Turkey (Aksoy et al., 2022), the prevalence of ketosis in milk has been reported to be much higher. It is well known that SCK causes significantly reduced milk production (Deniz et al., 2020; Duffield et al., 2009; Sahal et al., 2017) and this was found roughly 6.7 kg/day in Holstein cows tested positive for SCH at the second week postpartum in Turkey (Aksoy et al., 2022). It also predisposes dairy cows to other metabolic diseases such as retained placental, displaced abomasum, metritis, mastitis, ovarian dysfunction, low conception rate, culling and multiple diseases (Aksoy et al., 2022; Brunner et al., 2019; McArt et al., 2012; Suthar et al., 2013; Uyarlar et al., 2018) and imposes huge costs on dairy farms (Deniz et al., 2020; Mostert et al., 2017; Steeneveld et al., 2020). Although the diseases incidences and milk production associated with SCK were not the objective of the present study, it can be speculated that SCK may cause more economic impact in small backyard, non-integrated dairy farms in Turkey, because there are still an enormous number of small-sized backyard dairy farms run by family members in which the number of culling rates increased (Aksoy et al., 2021).

Additionally, the present study showed the prevalence of SCK was increasing weekly up to day 28 postpartum in small backyard farms. The overall prevalence and likelihood of SCK were remarkably high in PP8-15 and PP16-28 groups compared to PP0-7 group. The higher prevalence of SCK in the second week postpartum has already been reported by others in dairy farms (Aksoy et al., 2022; Azizi et al., 2022; Carrier et al., 2004; Duffield, 2000; Suthar et al.,2013). The present study showed much higher prevalence rate in the PP16-28 group, even compared to the previously reported studies in Turkey. However, Carrier et al. (2004) reported a higher prevalence at postpartum week 1 and 2 than postpartum week 3 and thereafter. Similarly, others (Azizi et al., 2022) reported a higher prevalence rate of SCK in the first week postpartum. The present results showed that the prevalence pattern is slightly different between large-sized integrated and non-integrated small, backyard Holstein farming. The prevalence and odds ratio of SCK were much higher during the third and fourth weeks postpartum in the present study. That result was in line with the statement of Dohoo and Martin (1984), but they determined ketosis by milk testing. The reason for this was the higher prevalence of SCK in primiparous Holstein cows both in the first two weeks and last two weeks within a month postpartum in the present study, in contrast to multiparous Holstein showed lower prevalence rate in the first two weeks postpartum. That resulted in an average 12.1% higher prevalence rate at the first two weeks postpartum (PP0-15) in primiparous cows compared to multiparous Holstein cows. The present study has shown that the prevalence pattern of SCK differed between primiparous and multiparous cows in the first month after calving in small backyard dairy farms.

The higher prevalence rate in primiparous cows is generally contradictory in comparison to the previously published reports up to now for large-sized integrated dairy Holstein farms, because others reported SCK was much more prevalent in multiparous than primiparous cows (Aksoy et al., 2022; Azizi et al., 2022; Brunner et al., 2019; Ha et al., 2023; Vanholder et al., 2015). The most important reason for the higher prevalence of SCK in multiparous cows was the higher milk production in early lactation on the large integrated dairy farms compared to primiparous cows. However, there are also consistent reports with the present study in primiparous Holstein (Carrier et al.,

2004; Steen et al., 1996), and Jersey (Chandler et al., 2018). One could speculate that primiparous Holstein cows on the small Holstein backyard farms are more likely to suffer from SCK throughout the first month postpartum, especially during the first two weeks. The reason for this could be inappropriate feeding, management and adaptation to the milk production in early lactation. For example, SCK diagnosed in the second week after parturition caused a milk yield loss of 7 kg/day in primiparous Holstein cows in integrated large dairy farms (Aksoy et al., 2022) although large integrated farms have continuous veterinary control and appropriate ration system. Considering that the overall prevalence in the present study was higher than in large integrated farms in Turkey, each reason mentioned above applies to both primiparous and multiparous cows in small backyard farms. However, it seems that primiparous cows were more susceptible to inappropriate ration and management during the transition period to the start of first milk production in the first two weeks after calving. This could indicate that primiparous cows on small backyard farms are much more affected by SCK than on the integrated large dairy farms where the ration system during the transition period and the management are more professional for the start of milk production. The transition period refers to the 3 weeks before and after parturition, and the energy content, minerals, vitamins and amino acids of the ration are different and adapted according to the energy needs of dairy cows to prevent NEB in early lactation (Deniz et al., 2020; Deniz and Aksoy, 2022; Duffield, 2000; Mcart et al., 2012). Primiparous cows had more difficulty than multiparous cows in adapting to the onset of milk production in Holstein backyard farms in the present study. It could be suspected that the management and ration preparation in small backyard Holstein farms during the transition period were not sufficient for the start of milk production and that this led to NEB and higher SCK prevalence, especially in primiparous cows. The number of milking frequencies, body condition scores (Aksoy et al., 2022; Seifi et al., 2011; Vanholder et al., 2015) as well as silage quality and overfeeding before parturition (Roche et al., 2018) are other reasons for the development of SCK that need to be carefully evaluated in the small Holstein backyard farms, especially in relation to primiparous cows.

In the present study, a slight, non-significant effect was found in the prevalence rate of SCK in relation to the summer season. In contrast, a seasonal effect on SCK was already reported, especially for cows calving in the spring particularly in the summer were significantly at higher risk of severe ketosis (Ha et al., 2023). Interestingly, in the present study, multiparous and primiparous Holstein cows had similar prevalence rates of SCK in summer. Primiparous Holsteins, however, were more susceptible to SCK than multiparous during the rest of the year, the difference being 22.7%. This is something new for the small backyard Holstein dairy farms. This could relate to the calving season in backyard farming in villages. This economically important silent disease can severely affect the profitability of backyard Holstein family farms. However, some prevention and treatment methods have already been reported for SCK (Deniz et al., 2022; Gorden et al., 2017; Mendelez et al., 2005; Sahal et al., 2017). In addition to improving ration and management during the transition period, preventive measures can also be taken in the small backyard family farms.

CONCLUSION

The prevalence of SCK was much higher on small Holstein backyard dairy farms managed by family members than on the large integrated dairy farms previously reported in Turkey. The risk of SCK was much higher in the third and fourth weeks than in the first week after calving. Primiparous Holsteins were more frequently affected by SCK than multiparous cows, especially in the first two weeks after calving and during the rest of the year, except in summer. The results of the present study can be confirmed by using a much larger population of dairy farms in cross-regional and multicenter studies, and a meta-analysis can be performed. Since farming and animal husbandry on small backyard dairy farms are important economic activity for farmers and their families, educating farmers on the prevention and management of SCK through the veterinary chambers or state veterinary offices is crucial to prevent the economic impact on their profitability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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