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Original Article
Livestock Diseases

Determination of the causative agent of periparturient period interdigital dermatitis that adversely affects reproduction and milk production in cows by MALDI-TOF[1]

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ABSTRACT.- Takci A., Mogulkoc M.N., Sancak T. & Kivrak M.B. 2023. **Determination of the causative agent of periparturient period interdigital dermatitis that adversely affects reproduction and milk production in cows by MALDI-TOF.** *Pesquisa Veterinária Brasileira 43:e07341, 2023.* Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye. E-mail: mbkivrak@cumhuriyet.edu.tr

In recent years, problems associated with high milk yield in dairy cows have increased considerably. Today, interdigital dermatitis (ID) is one of the most significant problems across the dairy industry, threatening animal health and welfare and inducing serious productivity losses. The etiology and mechanism of damage caused by this disease, which disrupts quality of life, milk yield, and reproduction, have not yet been fully understood. In order to achieve the study objectives, 40 biparous cows (3 years old – second calving) that had lameness associated with ID from prepartum day 30 to postpartum day 60 (Study group) and 40 biparous cows (3 years old) without lameness symptoms during the specified period (Control group) constituted the material of the present study. There was no difference in the levels of non-esterified fatty acids in the blood samples collected from those animals during the dry period (45-60 days before parturition; $P=0.38$). There were differences between the groups in certain parameters, including beta-hydroxybutyric acid level in the postpartum period, weight loss in the first month, number of ovarian cysts in the first 60 days, milk production in the first month, and the first corpus luteum determination process. ($P<0.001$) There was no difference between the groups in terms of reproductive parameters (mode of delivery, litter viability, litter weight, litter weight, expulsion of membranes, etc.) and presence of infection (acute puerperal metritis) during birth ($P>0.05$ for all parameters). In addition to all of the above, *Wohlfahrtiimonas chitiniclastica* bacteria were isolated for the first time in microbiological samples collected from ID, which is a significant condition for dairy cows during the periparturient stage, contributing to the etiology of the disease, which has not been understood so far.

INDEX TERMS: Interdigital dermatitis, periparturient, postpartum, reproduction, milk yield.

INTRODUCTION

In recent years, modern dairies have resorted to selections to achieve high-yielding cows. Nevertheless, introducing high milk-yielding cows has brought further infertility problems (Lucy 2001, Dillon et al. 2006, Walsh et al. 2011). The periparturient period, which includes late pregnancy, parturition, and early lactation, is very important for milk production, health status, and reproductive performance of dairy cows (Hut et al. 2019). Higher dry matter consumption should occur during the said period, but this cannot be achieved due to pregnancy. Therefore, cows may not tolerate the increased metabolic load of pregnancy and lactation (Salin et al. 2017, Triwutanon & Rukkwamsuk 2021). Certain factors, such as lameness, are associated with further reduction in dry matter consumption (Stevenson et al. 2020, Becker et al. 2021). Concomitant foot diseases lead to excessive stress in dairy cows through many processes, including lactation

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and preparation for new pregnancy (Tsousis et al. 2022). Unfortunately, the incidence of foot diseases is quite high in the first 90 days postpartum, i.e., the preparation phase for a new pregnancy and any disruption may adversely affect fertility maintenance (Lucey et al. 1986, Bergsten 2001). The incidence of metabolic diseases, infertility, and mastitis increases with foot diseases in the periparturient stage, further complicating the process for dairy farms (Roche 2006).

Therefore, comorbid lameness with infertility is associated with increased labor costs, decreased reproduction, and decreased milk yield (Walsh et al. 2011). Lameness has an adverse effect on cows at all stages of reproduction. This begins with a delayed postpartum physiological process. After that, the same delays occur in the onset of cyclic activity upon disruption of follicle development and ovulation. This increases the number of inseminations per pregnancy and the time to first insemination (Garbarino et al. 2004, Hultgren et al. 2004). On the other hand, even in the cases where estrus does occur, the fact that it passes in the form of silent estrus exerts much difficulty on the facility regarding reproduction management (Walker et al. 2008, Bahonar et al. 2009).

Interdigital dermatitis (ID), i.e., the main cause of lameness in cows, is a wet, ulcerative, and erosive bacterial disease that manifests across the coronary band or interdigital space on the plantar surface of the feet (Refaai et al. 2013, Wilson-Welder et al. 2015, Palmer & O'Connell 2015). Bovine ID predisposes dairy cows to infectious and metabolic diseases due to their vulnerable immune system, decreased energy levels and dry matter consumption. As a result, dairy farms may turn into enterprises with decreased reproductive efficiency, higher prevalence of infectious and metabolic diseases, and significantly reduced milk yield (Corlevic & Beggs 2022). The exact etiology of ID is not fully understood despite the range of adverse effects associated with it. However, since treponemes have been isolated from cow hooves in many cases with similar lesions, it was reported that treponemes might also account for the occurrence of ID (Knappe-Poindecker et al. 2013).

Several previous studies have suggested that all foot diseases, including ID, caused a decrease in reproduction and milk yield. The present study aimed to investigate how the well-established adverse activity of ID as one of the most important problems for dairy cattle farms today on reproduction and milk yield occurs, which mechanisms are disrupted in association in addition to that, and what is involved in its etiology.

MATERIALS AND METHODS

The present study was approved by the Animal Research Ethics Committee, Cumhuriyet University, upon decision No. 717 on March 17, 2023.

The study was conducted in a private dairy farm with 300 Brown Swiss cows in Ortaklar Village, Yıldızeli Town, Sivas Province, Turkey, with coordinates 39.83371433796894, 36.34688098838113 and an altitude of 1290 meters. The bovines were placed in shelters designed based on a free-stall system with adequate walking space in the facility. The study was carried out from February to May. The temperature inside the shelter ranged between 6°C and 12°C.

The animals' body condition score (BCS) varied between 3.5 and 3.75 at the time of parturition, and all groups were fed with the same ration and under the same conditions.

Animals were metabolically monitored throughout the periparturient period. Accordingly, prepartum non-esterified fatty acids (NEFA) and postpartum beta-hydroxybutyric acid (BHBA) measurements were made to investigate energy imbalances in cows (Duffield 2000).

Blood samples were collected on prepartum day 60 (60 days before parturition) after the animals in the study groups were placed in the dry period. Blood NEFA levels were measured using a commercial kit (FA 115, Randox Laboratories Ltd, United Kingdom) before feeding.

Afterward, those with and without lameness at one month before birth constituted the study groups. Thus, the study material consisted of 40 biparous (3 years old) cows with lameness associated with ID from prepartum day 30 to postpartum day 60 (Study group) and 40 biparous (3 years old) cows without lameness during the specified period (Control group).

Lameness in the study group resulted from ID because it was associated with wet ulcerative lesions located along the coronary band and in the interdigital sulcus (Holzhauer et al. 2008). ID occurs in four forms in terms of the stage of development and the lesions they induce (Döpfer et al. 1997). Based on this classification, the cows in our study group were identified as level 2 (painful ulcerative stage) and level 3 (in which crusts formed on the ulcerated area).

Reproductive records (mode of delivery, litter viability, litter weight, litter membrane expulsion, etc.) were captured during the delivery. Within the first five postpartum days, the presence of infection was investigated by assessment of body temperature, character of vaginal discharge, and general condition.

Weight measurements were taken twice upon delivery, the first on postpartum day 1 and the second on postpartum day 30, to determine postpartum body condition loss. The difference was considered weight loss in the postpartum first month and compared between the groups.

Daily milk yields were recorded in the first postpartum month. The first five days of postpartum milk were not included in the average in the assessment of the daily milk data. Daily milk yield was calculated by dividing the total milk amount of 25 days without colostrum secretion by 25. Milk yields were compared between the groups.

Weekly ovarian examinations were performed to determine when the animals resumed cyclic activity postpartum. Therefore, consecutive examinations were performed until the occurrence of corpus luteum in the ovary. Cystic structures on the ovary were recorded during this process. The groups were compared in terms of these two data.

Blood samples were collected from fresh animals at certain time intervals for reproduction management of the facility. Blood samples for BHBA assessment were collected on postpartum day 10. Blood samples were tested using a ketone meter (Precision Xtra, Abbott Diabetes Care, Abingdon, UK)/commercial kit to assess the levels of BHBA. BHBA level was expressed in mmol/L (Puppel et al. 2019).

Furthermore, animals in both groups were examined for possible abomasal displacements during the postpartum period. Conditions, including hypocalcemia and hypomagnesemia, were also recorded in the first five postpartum days.

Culture and identification. Microbiological swabs were used to collect samples from the interdigital spaces of the animals on the postpartum 30th day to investigate the causative agent. Upon cleaning the lesion area with sterile FTS, samples were collected from 13 cattle with the help of brushes, which were then placed in an anaerobic transport medium and transported to the laboratory. Cellular morphology was investigated using Gram staining in the laboratory. For the isolation of anaerobic agents, inoculations from the transport medium were made onto brucella agar supplemented with hemin (5mg/L) and menadione (1mg/L) and the media were incubated at 37°C for 24-48 hours under anaerobic conditions. The transport medium was also inoculated onto 7% sheep blood agar, and EMB agar and the media were incubated at 37°C for 24-48 hours under aerobic conditions. Upon incubation, colonies in the media with growth were separated and identified by MALDI-TOF

MS. The method suggested by Hijazin et al. (2012) was used for the identification of bacteria using MALDI-TOF MS and an identification score of 2.0 and above was considered reliable.

Statistical analysis. The Statistical Package for the Social Sciences (SPPS 25, IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, IBM Corp., NY) software was used for the data statistical analysis obtained in the study. The variables were first tested to see if they met the normality and homogeneity hypotheses using the Shapiro-Wilk and Levene tests. The Mann-Whitney U test was used to compare the means of two groups when the Student's t-test parametric test prerequisite was not met. Categorical data were analyzed with the Chi-squared test. A *P* level of $P<0.05$ and $P<0.01$ were considered statistically significant.

RESULTS

There was no difference between the groups in terms of assessment of NEFA in blood samples on day 60, when the animals in the groups were taken into the dry period, i.e., prepartum (60 days before parturition) ($P=0.38$).

There was no intergroup difference in reproductive parameters (mode of delivery, litter viability, litter weight, litter weight, expulsion of membranes, etc.) and infection (acute puerperal metritis) ($P>0.05$ for all parameters).

There was no abomasum displacement in the cows from both groups in the postpartum period. There was no hypocalcemia in both groups in the first five postpartum days.

Weight loss during the first postpartum month was compared between the groups. There was a significantly higher weight loss in the ID group ($P<0.001$) (Fig.1, Table 1).

Similarly, ovarian cysts were observed during the postpartum first 60 days of ovarian follow-up. A total of 17 (42.5%) ovarian cysts (14 follicular and 3 luteal) were detected in the ID group, while a total of four (10%) ovarian cysts (3 follicular and 1 luteal) were detected in the Control Group (Fig.2). The intergroup difference was statistically significant ($P<0.001$).

There was a significant intergroup difference in BHBA levels on postpartum day 10 ($P<0.001$). ID reduced body reserves during the first ten-day phase (Fig.3, Table 1).

Upon comparison of milk in the first one-month stage (5-30 days postpartum), excluding colostral milk, the ID group produced significantly less milk compared to the control group ($P<0.001$) (Fig.4, Table 1).

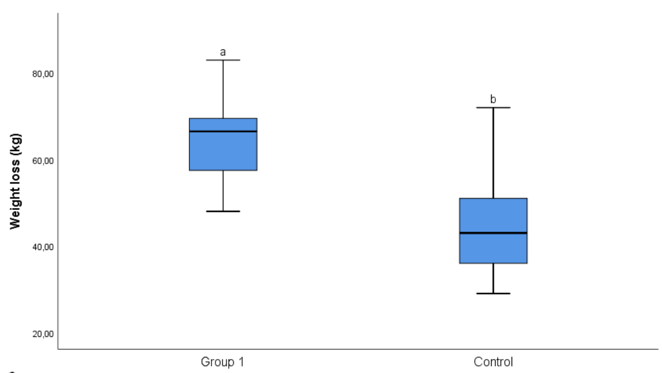

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Fig.1. Intergroup comparison of weight loss during the postpartum first month. Median weight loss (kg) (min-max), Study Group (Group 1) = 66.50 (48-83), Control Group = 43.00 (29-72). Varied characters are statistically significantly different (a, b).

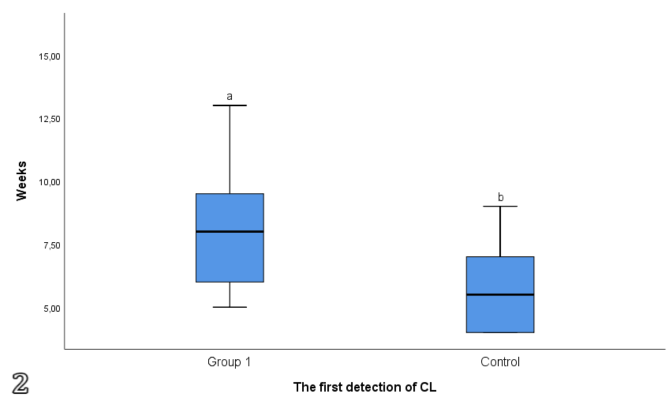

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Fig.2. Comparison of the time (weeks) of first corpus luteum detection as evidence of ovarian cyclic activity during the postpartum period. Median corpus luteum (CL) week (min-max), Study Group (Group 1) = 8 (5-18), Control Group = 5.50 (4-9). Varied characters are statistically significantly different (a, b).

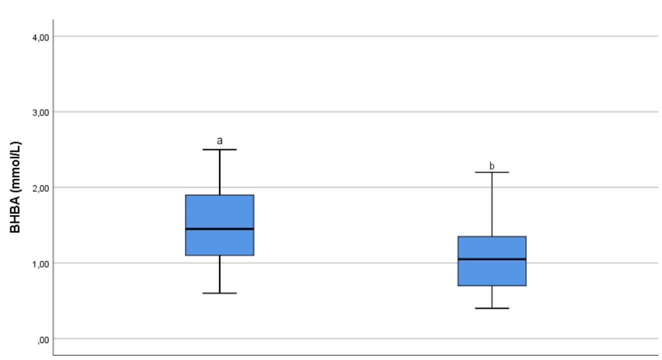

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Fig.3. An intergroup comparison of BHBA levels in blood samples collected on postpartum day 10. Median BHBA mmol/L (min-max), Study Group (Group 1) = 1.45 (0.6-3.20), Control Group = 1.05 (0.4-2.20). Varied characters are statistically significantly different (a, b).

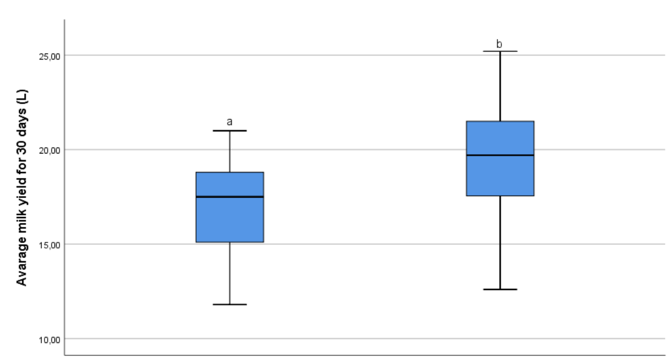

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Fig.4. An intergroup comparison of average daily milk data in the postpartum first month. Mean milk yield (kg) ± Standard Deviation, Study Group (Group 1) = 16.99±2.47, Control Group = 19.37±3.06. Varied characters are statistically significantly different (a, b).

Table 1. Median weight loss (kg), the time (weeks) of first corpus luteum (CL) detection, BHBA levels and average daily milk yield in Study (Group 1) and Control Groups (Group 2)

Groups	Median weight loss (kg) (min-max)	Median CL (week) (min-max)	Median BHBA (mmol/L) (min-max)	Mean milk yield (kg) ± SD
Study (Group 1)	66.50 (48-83)[a]	8 (5-18)[a]	1.45 (0.6-3.20)[a]	16.99±2.47[a]
Control (Group 2)	43.00 (29-72)[b]	5.50 (4-9)[b]	1.05 (0.4-2.20)[b]	19.37±3.06[b]

BHBA = beta-hydroxybutyric acid, SD = standard deviation; [a,b] Varied characters in the same column are statistically significantly different (P<0.01).

Microbiological analysis result

Gram-negative bacilli were detected upon Gram staining and microscopic examination. After culture, 11 of 13 cattle samples were identified as *Wohlfahrtiimonas chitiniclastica* using MALDI-TOF-MS as non-pigmented, shiny colonies with smooth center and rough edges on blood agar.

DISCUSSION

Interdigital dermatitis persists for at least 42 days with accompanying lesions and causes a range of problems in dairy farms. This infection persists for longer periods in cows in the early stages of lactation from (on average 60 days) (Nielsen et al. 2009). In the present study, the lesions took comparatively longer periods to manifest. The infection process was prolonged in the study group because the animals had the infection in a distressed stage, i.e., the periparturient stage.

It is well-established that fertility is lower in animals with lameness during or before mating than in those without (Somers et al. 2015). In this study, lameness in the periparturient stage induced other metabolic conditions and subsequently had an adverse effect on fertility. It has been reported that ovarian cysts were more prevalent in cows with lameness during the postpartum first 30 days, and consequently, pregnancy rates were lower (Melendez et al. 2003). The ovarian cysts were followed up until the voluntary waiting period (postpartum 60 days), and there were more cysts in the group with ID (P<0.001). This was attributed to increased adrenocorticotrophic hormone (ACTH) suppressing pulsatile luteinizing hormone (LH) in lameness. Suppressed LH causes delayed ovulation, thus increasing persistent follicle formation (Dobson et al. 2000).

Painful ID, which may cause lameness, prolongs lying time in animals and shortens the time for feeding. Therefore, it causes metabolic disorders (Hassall et al. 1993, Juarez et al. 2003, Palmer et al. 2012). In the present study, the BHBA levels of the animals in the groups were assessed on postpartum day 10 to determine whether such an effect occurred. There was a significant intergroup difference (P<0.001), and it was concluded that ID exacerbated the negative energy balance. In a study that examined the NEFA values and non-cyclic rates in lame and healthy animals, NEFA values were significantly higher in the lame group, and the number of non-cyclic animals was also higher (Praxitelous et al. 2023). In the present study, ID suppressed the initial corpus luteum formation and delayed the onset of ovarian cyclic activity. Other studies have reported that dermatitis slows down cyclic activity, in agreement with the results of the present study (Humberto Guáqueta et al. 2014, Melendez et al. 2018).

In this study, the ID group (Study Group) lost more weight in the first postpartum month (P<0.001). Although the blood NEFA levels of the two groups were similar during the dry period, it strengthened the hypothesis that those metabolic changes occurred after the formation of ID. Similar to this study, different studies confirm weight loss in cows suffering from lameness (Alawneh et al. 2012). It was hypothesized that the weight loss was due to ID exacerbating metabolic imbalances by reducing feed intake. In a study by Daros et al. (2020), feed intake decreased in animals with lameness; accordingly, live weight loss was seen.

There are varied opinions on the effect of ID as one of the leading foot diseases on milk yield. Previous studies have reported that ID infections did not always reduce milk yield (Palmer & O'Connell 2015). In one study, no decrease in milk yield was seen in cows with ID prior to treatment, but there was a significant post-treatment milk yield increase in cows with ID (Amory et al. 2008). Again, two studies argued that ID decreased milk yield but not significantly (Argáez-Rodríguez et al. 1997, Garbarino et al. 2004). In the present study, the milk yield during the first postpartum 30 days was assessed, and it was found that milk yield decreased significantly in the study group compared to the control group. The reason why this study's result differed from those of other studies is that in other studies, milk yield was assessed throughout the lactation. In contrast, the present study assessed the same only during early lactation (first 30 days). In this study, the milk yield assessment was performed when all the negative effects of ID had occurred. Therefore, the milk decline was dramatic.

Warnick et al. (2001) reported in their study that ID caused decreased milk yield, although not as much as other causes of lameness (Warnick et al. 2001). In addition to all those studies, there are different studies with results similar to this study regarding milk yield. In one of these studies, Pavlenko et al. (2011) reported a significant decrease in milk yield. Gomez et al. (2015) reported that ID caused 199-335kg of milk loss in heifers in their first lactation (Gomez et al. 2015). A study indicated that lameness severe enough to present a clinical manifestation decreased milk yield considerably (Green et al. 2002).

In addition to all of the above points, this study once again demonstrated that ID had an adverse effect on the health and welfare of animals. *Wohlfahrtiimonas chitiniclastica* was isolated from samples taken from ID lesions, which caused such low yields. *W. chitiniclastica* is a facultative anaerobic, gram-negative, non-motile, non-spore-forming bacillus with catalase and oxidase biochemical activities that can survive in a wide pH and temperature range. The causative agent was identified from *Wohlfahrtia magnifica* parasitic fly homogenate in the laboratory and from larvae in infected vulva wounds in Romney sheep in Hungary in 2008 (Tóth et al. 2008). The causative agent has been isolated from a wide spectrum of disease cases ranging from simple wound infection to septicemia (Kõljalg et al. 2015). To date, septicemic or inflammatory cases have been reported in humans and wild animals. A case of septicemia caused by *W. chitiniclastica* was reported in a homeless woman in France (Rebaudet et al. 2009). In animals, it has been isolated from a case of bacterial septicemia following wound myiasis in a wild deer in the USA (Thaiwong et al. 2014) and a case of endocarditis in a whale (Josue et al. 2015), suggesting that it might be a zoonotic pathogen. In recent years, several cases of cellulitis, osteomyelitis, soft tissue, and bone infection of the lower extremities have also been diagnosed in humans. In China, it was isolated from cattle with hoof lesions and reported to be pathogenic in mice due to infection trials (Qi et al. 2016). In Turkey, it was recently isolated in a human case of osteomyelitis and soft tissue infection of the big toe (Karaca et al. 2022). To our knowledge, this is the first isolation of *W. chitiniclastica* from cattle with hoof lesions in Turkey, and the host range of the causative agent appears to extend to domestic animals. It was suggested that transmission occurs when adult *W. magnifica* flies, or larvae come into contact with mucosal surfaces and damaged skin (Fenwick et al. 2019). These flies are found in France, Spain, Korea, Hungary, and Turkey, and it is considered that the fly involved in this case transmitted the causative agent to the hoof lesion in cattle.

CONCLUSION

Interdigital dermatitis (ID) in the periparturient stage, with rapid hormonal and nutritional changes and metabolic load increases, is associated with many severe problems. Besides disturbing the animal's peace of mind, this disease significantly reduced feed consumption during the periparturient stage. It exacerbated the negative energy balance by increasing the tolerable loss of body condition score (BCS) upon delivery. Consequently, the period in which the animal could recover its reproduction and milk yield was excessively prolonged. The bacterium *Wohlfahrtiimonas chitiniclastica* was isolated from ID lesions, which were causing such serious losses.

Conflict of interest statement.- The authors declare that they have no conflicts of interest.

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