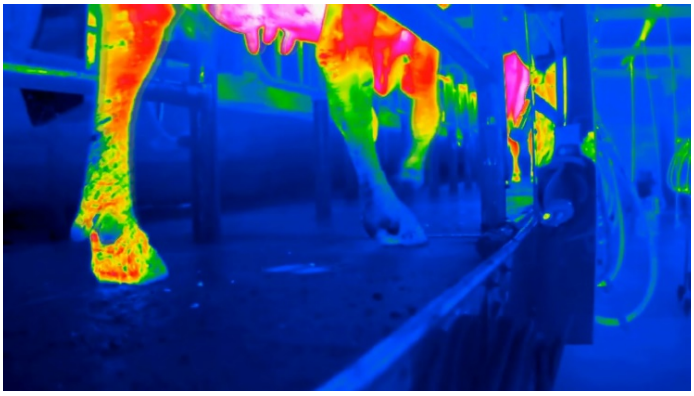
A novel approach to the diagnosis of limb diseases in dairy cows

Zdeněk Havlíček et al.



Brno 2021

Zdeněk Havlíček[1], Petr Řezáč[1], Ivana Novotná[1], Lucie Langová[1], Petra Němcová[1], Oldřich Žďárský[2], Pavel Michalička[2], Václav Straka[3], Pavel Zítek[3]

1) Mendel University in Brno

2) ŽIVA zemědělská obchodní, a.s. Klášterec nad Orlicí

3) "TMV SS" spol. s r.o.

This publication utilises the findings of the Ministry of the Czech Republic PRV project, number 17/005/1611a/453/000114 „Inovace způsobů diagnostiky onemocnění končetin dojnic “.

Oponents:

MVDr. Soňa Šlosárková, Ph.D. – Veterinary Research Institute

Ing. Zdenka Majzlíková – Czech Breeding Inspectorate

ISBN: 978-80-7509-791-0

Contents

1. Objective of the methodology

The principal objective of this methodology is to provide guidelines for the early detection of hoof disease in dairy cows using infrared thermography. The information presented herein will facilitate the utilisation of thermal imaging diagnostics as a tool for identifying alterations in the health of the limbs prior to the full manifestation of clinical symptoms of hoof disease, particularly in cases of lameness in dairy cows. This is of paramount importance for the effective and efficient treatment of dairy cows.

The second phase of the methodology is concerned with the disinfection of the limbs of dairy cows, which is of paramount importance in the prevention of disease. The primary objective is to propose a solution to assist farmers in determining the efficacy of disinfection, particularly during the winter months when disinfectants may be less effective due to low temperatures. Despite the imminent prohibition or significant restriction of formalin for legal reasons, it remains a common disinfectant on many farms. The efficacy of commercial disinfectants is contingent upon a number of variables that must be taken into account by farmers. The inappropriate use of these disinfectants may result in the development of pathogen resistance and a reduction in overall effectiveness.

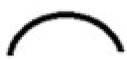
2. Methodology description

The modern precision agriculture approach to livestock breeding is based on the monitoring of individual factors affecting production, life manifestations, and health status. Contemporary society has a multifaceted interest in food safety and quality, as well as sustainable agricultural principles. Additionally, there is a growing interest in the health of farmed animals and the provision of well-being, with an acceptable impact of animal production on the environment. For these and other reasons, there is a growing need to monitor a multitude of variable indicators characterising animals' health status and vital signs.

In the past, decisions regarding livestock farming were primarily based on the farmer's observations, judgement, and experience. This was due to the fact that each animal was known intimately by the farmer, as a result of the daily individual care provided. Nevertheless, the current reality of modern farms, which typically house hundreds of animals or groups of more than 50, renders the aforementioned level of personal care impractical.

In the evaluation of animal limb health, the locomotion score is a crucial indicator of lameness in cows. Lameness is graded on a scale from 1 to 5, with 1 indicating a healthy cow and 5 indicating an animal that does not bear any weight on the limb (Šlosárková, 2016). The characteristics of the locomotion score scale and the back posture of dairy cows are summarised in Table 1, while the back line when walking and standing is illustrated in Appendix No. 4. As outlined by Hulsen (2011), a cow with a locomotion score of 1 is deemed to be in good health, while a score of 2 indicates the need for monitoring. A score of 3 necessitates immediate hoof correction, and the same applies to a locomotion score of 4. A cow with a locomotion score of 5 is considered to be severely lame and requires intensive care and professional hoof trimming. It is imperative that corrective action be taken at a locomotion score of 3 or above (Sprecher et al., 1997).

Table 1: ***Assessment of Locomotion Score (Source: The studies by Sprecher et al. (1997), Flower and Weary (2006), Haskell et al. (2006), and Rajkondawar et al. (2006))***

Score		Description	Back posture	
			Standing	Walking
1	normal walking	- Normal walking - No signs of lameness		
2	mildly lame	- Gait remains normal - Stands with a level-back posture - An arched-back posture while walking - No signs of head bowing when walking		
3	moderately lame	- An arched-back posture is evident both while standing and walking - No signs of head bowing when walking, shortstriding with one or more limbs		
4	lame	- An arched-back posture is always evident - Gait is best described as one deliberate step at a time - In most cases, the head is tilted down		

5	severely lame	- The cow is afflicted with lameness in one or more legs - An inability or extreme reluctance to bear weight on one or more of her limbs/feet - The curvature of the back apparent while standing and walking - The head is tilted down while walking		

On initial observation, the evaluation of the locomotion score appears to be a relatively straightforward process. Nevertheless, the process is time-consuming and laborious, as the evaluator frequently identifies animals that only move after the group has already commenced movement. In these animals, the most evident indications are those of limb discomfort, which manifest as a slow gait with an arched back and an attempt to alleviate the affected limb. It is not uncommon for breeders to be unaware of this condition if the animal is not exhibiting clear signs of limb pain. For successful treatment, however, it is necessary to identify animals in which lameness is not yet fully developed, animals in which the disease is only at the beginning and has not yet caused any deviations in motor skills, or animals that do not yet perceive it negatively.

In the present era, a plethora of automated animal monitoring systems are available, operating continuously or on a regular basis, at varying frequencies, without the necessity for direct operator involvement. This solution, which is based on the principles of "Industry 4.0", has also been applied to the diagnosis of diseases of the locomotor apparatus of dairy cows. In this context, the stationary thermographic system of TMV SS, Ltd. has been employed.

A highly effective diagnostic tool for limb diseases has been developed which is fully automated and non-invasive. The system is capable of collecting data without the need for restriction of the animals being evaluated, and is able to operate independently of human input. The thermographic measurement is automatically initiated when the cows pass in front of the camera following the reading of their radio frequency identification (RFID) identifier. The online measurement is conducted in a manner that does not impede the animals' movement, and it is not associated with any discomfort or stress. The technical tool is designed to replace time-consuming tasks for breeders and to facilitate the daily, often multiple, automatic monitoring of individual animals. The identification of dairy cows in the milking parlour or feed boxes is facilitated by the deployment of responders, who are tasked with this objective.

The evaluation algorithm takes into account the inherent variability in the measured values of individual animals, as is the case with all living organisms. Accordingly, the algorithm takes into account group, individual, and temporal differences when evaluating monitored variables. This approach is necessary because a comparison of individual values to the population average, which is often considered to be in a steady state, is insufficient. This is due to the inherent complexity and variability of the data. **In order to successfully establish evaluation processes, three conditions must be met**.

- It is essential to monitor **the animals' individual factors on an ongoing basis**. This can be achieved through the use of thermographic cameras, which should be installed at each entrance to the milking parlour and used for daily inspections.
- The process of analysing and interpreting data:

- It is imperative that **the result**, which indicates a change in the health status of the limbs, is **available to the breeder within a few minutes**, as any delay could have significant implications for the animal's well-being.
- **The consequence of a modification in the animal's health status is the content of the printout**, which provides information regarding the separation gates and the immediate treatment of individual animals. Such conditions may manifest as dermatitis, necrobacillosis or hoof inflammation, preceded by symptoms of localised inflammation, including an elevated temperature, redness, swelling and functional impairment, prior to the onset of pain. The early diagnosis of this altered health condition will facilitate effective intervention in the pre-clinical phase and the management of the entire situation without impact on productivity. Furthermore, this approach will result in low treatment costs and the overall prevention of the disease. A system that functions in this manner is, by its very nature, one of the primary preventive measures in the daily management of limb diseases, which are a significant concern on many farms, with prevalence rates exceeding ten percent in many cases, and reaching as high as tens of percent in some instances.

➢ The third requisite for the efficacious implementation of the entire thermographic system for the assessment of limb health is a multidisciplinary biological-technical approach, encompassing **the automated collection of defined data and their subsequent online evaluation**.

- All data obtained through measurement are stored for future reference

- In addition to diagnosing limb diseases accompanied by a locally increased temperature, they provide a means of evaluating the efficacy of individual disease treatment based on the course of the temperature values measured on the affected limbs.

The implementation of automated measurement and evaluation processes has the potential to markedly enhance the overall process, rendering it more responsive to the initial indications of emerging discrepancies.

The principal advantages of an automated thermography system are as follows:

- **Uniformity of assessment**, including the setting of condition limit values
- In the event that thermodiagnostics is conducted by an authorised worker, it is imperative that we rely on their level of expertise and ability to perform routine control of both the camera and the evaluation of the thermograms taken (measurement records). In contrast, the automated system employs a **consistent algorithm with the capacity to establish multi-level thresholds**. It is thus feasible to readily generate reports comprising a summary of the prevailing temperature score and a comparison of the measured values between individual herds and farms.
- **Frequency of measurements** up to several times a day
- The automated system is designed to measure each piece of livestock passing through a designated corridor, such as the entrance to the milking parlour. In practical terms, this implies that, in the event that both entrances to the milking parlour are equipped with cameras, **each piece is measured**

on multiple occasions throughout the day. This allows for the initial detection of deviations in the state of health and the subsequent verification of the detection, which may also facilitate the monitoring of disease dynamics or the assessment of treatment efficacy. Similarly, automated data processing enables the straightforward generation of trends and advanced analyses of disease aetiology.

- The service is designed to be **time-efficient**

- From the perspective of the operator, the automated system necessitates only the cleaning of the camera system with water in the event of contamination, such as excrement. The output of the livestock specialist is a tabular representation of the animals, with each animal classified according to the assigned temperature score. Should the authorised operator, typically a livestock specialist, wish to conduct a more in-depth analysis of the data, they may access **the data pertaining to individual records over a longer time period**.

- **Ability to regulate selection elements**

- As the evaluation is **entirely automated and occurs in real time**, the monitoring system enables the automatic separation of suspect animals via the selection gate. Consequently, the monitoring system allows the livestock specialist or veterinarian to verify the monitoring findings.

- **Data archiving** and the evaluation of long-term trends
  - The focus is not only on monitoring the current condition, but also on implementing preventative measures to avert the onset of disease. This necessitates a clear and unequivocal requirement for retrospective, long-term and detailed analysis of the measurements performed, or alternatively, the search for connections with external

influences. In practice, this is a challenging undertaking, particularly when only basic measurements are conducted by an authorised worker. It is impractical for a livestock specialist to analyse hundreds of thermograms at regular intervals, assign them to specific animals, and manually enter them into the system for later analysis. The system employs this functionality.

- From a **financial** perspective, this approach is **more advantageous** than relying on “manual measurements”
  - A comparison of the primary investment costs between an automated system and a mid-range portable thermal camera reveals that the automated system is a more financially demanding solution. However, this is an apparent discrepancy in financial terms, which, in reality, is in the complete opposite direction. In order to obtain a complete picture of the financial implications, it is essential to include variable costs such as operator training, the time required for measurement and, most importantly, the evaluation process.
  - In order to ensure the responsible execution of the aforementioned process, it is necessary to employ the use of a handheld thermal camera. In such a case, the reality is that the authorised worker will be required to work for a significant number of hours each week, which will have a notable impact on the economic balance of labour costs. Furthermore, it is essential to consider the potential reduction in the efficiency of individual capture due to the significantly lower frequency of measurements (in comparison to a fully automated system). Additionally, the ability of measurements to convey meaningful information may be diminished when evaluated by multiple workers.

Thus, when evaluating the efficacy of an automated and a "portable" solution from both financial and performance perspectives, both criteria unequivocally favour the automated solution. In terms of speech recognition, the automated solution is immediately superior, while from an economic standpoint, it becomes increasingly advantageous within a few months, with the advantage growing over time due to the absence of variable costs.

It is important to note that this is an automated system, that is to say, a complete unit whose output is reported with a generated evaluation of the temperature score and the development of specific temperature indicators. A solution that necessitates the manual evaluation of thermograms by an operator or livestock specialist, or one that appears to be an effective solution for exceeding the temperature limit on a limb, cannot be considered an automated system.

Hoof diseases with the manifestation of a locally elevated temperature

Šlosárková and Fleischer (2009) posit that the prevalence of lameness in cows in the Czech Republic may reach 50%. It is estimated that 85-90% of these cases are attributable to disorders of the hoof and surrounding skin.

The various hoof disorders can be categorised according to whether they are infectious or non-infectious. The infectious disorders include interdigital dermatitis and digital dermatitis, while the non-infectious disorders include sole ulcer and white line disease. Each hoof disorder has a direct impact on the cow's production (Charfeddine, Pérez-Cabal, 2017). The sensitivity of the lesion is primarily influenced by the quality of the hoof horn, which is itself influenced by a number of internal and external factors, including hygiene, nutrition, hormonal changes during calving and lactation, animal age, and genetic predisposition (Novotná et al., 2019). In the majority of cases, these are inflammatory diseases that are accompanied by a locally increased temperature.

Digital dermatitis = inflammation of the skin of the hoof = strawberry disease = raspberry

Digital dermatitis represents the most prevalent, highly infectious, and inflammatory disease affecting the hoof skin of cattle. The condition was initially delineated by Cheli and Mortellaro (1974) and has since become commonly designated as Mortellaro's disease. It results in inflammation and damage to the skin. As the disease progresses, the lesion becomes increasingly erosive, and the affected area expands (Peterse, 1992). There is mounting evidence that digital dermatitis may be associated with other limb lesions, such as interdigital dermatitis, which presents a diagnostic challenge (Watson, 1999). It primarily affects the skin of the heels or the coronary area of the skin. The disease is primarily caused by treponemes, which are transmitted to animals with reduced immunity and those with mechanically damaged skin. The disease is more likely to develop in animals bred in unhygienic conditions or with high humidity.

Digital dermatitis is a painful condition that causes lameness in cows. The restriction of movement and subsequent reduction in feed intake result in a decline in milk production. The disease has the potential to persist for extended periods, even up to several months, in individual animals and herds (Peterse, 1992; Bečvář, 2000).

The lesion develops rapidly and has a relatively short lifespan within a given phase. The acute form of the condition is characterised by the development of minor inflammatory lesions, approximately the size of a pinhead (M1), which subsequently increase in size to a diameter of up to 4 cm (phase M2). At this stage, the appearance can be likened to that of a strawberry, with deposits of up to 4 cm in diameter displaying red granulation and exudate, which adheres the hairs on the affected skin (Šterc, 2010). In the chronic form, hard growths gradually form, but the bleeding that was previously observed ceases (Šterc, 2006). The initial manifestation of dermatitis is lameness. The objective is to relieve the affected limbs, which may result in a notable limp when stepping on the hoof. The condition most frequently affects the pelvic limbs.

Stages of the disease:

M0 – healthy limb (evaluated temperature field unchanged)

M1 – lesions smaller than 2 cm, a risk of progression to stage M2

M2 – acute phase, red surface, very painful

M3 – less painful, black wound in the healing process

M4 – Phase M2 that has not undergone adequate treatment. A chronic phase that reverts to an open form. The affected tissue displays hypertrophy of the skin, which may revert to the M2 phase. Additionally, hyperkeratosis, proliferation, and the formation of a hairy wart are observed. The treponemes are able to survive beneath the scab, and the rupture of the scab can result in the contamination of the surrounding environment

M4.1 – new, painful lesions up to 2 cm in size represents a consequence of unsuccessful treatment

In a considerable number of cows, there is a persistent transition between the M0 and M1 phases, which is a significant risk factor as it can rapidly revert to the M2 phase. A treated M3 lesion may then change to M2 or M4. The most problematic scenario on the farm is the constant transition of lesions in stages M4.1 and M2, as other animals are infected by their movement through the corridors. It must be acknowledged that a certain percentage of animals will inevitably be affected by some stage of DD.

Interdigital dermatitis = inflammation of the skin between the claws

Interdigital dermatitis is regarded as a comparatively superficial infection, and as such, it may not be discernible from the condition of healthy dairy cows (Cruz et al., 2005). The disease is most frequently observed in the context of wet weather and unhygienic conditions in stables. Nutritional deficiencies, including those of vitamin A and zinc, can also contribute to the development of the disease (Šterc, 2006). The disease has the potential to impair the overall health of the animal. The temperature rises (frequently to a maximum of 40 °C), accompanied by an increase in heart and respiratory rates. A reduction in feed intake is observed. The animal is observed to spend the majority of its time in a recumbent position. Additionally, a reduction in milk yield is observed. As outlined by Hulsen (2011), the infection begins between the hooves and progresses to the heel, where it causes the formation of small cracks and ridges. The aetiological agent is bacteria, most commonly *Dichelobacter nodosus*, *Fusobacterium necrophorum* and *Prevotella* spp. (Bennett et al., 2009).

Interdigital phlegmon (necrobacillosis)

The term necrobacillosis is used to describe an acute or subacute necrotising inflammation of the skin. The cow presents with lameness and frequently exhibits a fever. The animal's appetite is diminished, and milk production is markedly reduced. Additionally, an odorous discharge is present. Should the disease progress, the only remaining option is to amputate the affected claws or slaughter the animal (Alban et al., 1995).

The aetiology of this disease is attributed to the presence of *Fusobacterium necrophorum* (Novák et al., 2003), *Porphyromonas levii* and *Prevotella intermedia*. The anaerobic bacterium *Fusobacterium necrophorum*, previously designated as *Sphaerophorus necrophorum*, has long been considered a pivotal player in the pathogenesis of the disease. However, other bacteria, including *Porphyromonas levii* and *Prevotella*, have also been identified as contributing factors (Van Metre, 2017). Bennett et al. (2009) demonstrated that *Dichelobacter nodosus* and *F. necrophorum* frequently co-occur. Once established on the surface of the hoof, *D. nodosus* and *F. necrophorum* are the causative agents of disease. It is possible that they may disseminate to other sites and infect different hosts. The uneven surface of stables, which is prone to being trodden on, is a contributory factor in the increased occurrence of necrobacillosis. It has been demonstrated to affect hoof horn and skin injuries (Novák et al., 2003). Nevertheless, a number of factors may be implicated in the aetiology of this disease.

Rusterholz's ulcer (specific-traumatic inflammation of the hoof dermis, sole)

The ulcer in question is a pathological alteration of the dermis in the region of the heel. It is situated in the region of the foot that transitions into the heel. It is most frequently observed in the outer digits of the pelvic limbs (Hofírek, 2009). Rusterholz ulcers are more prevalent on the outer claw of the pelvic limb (Alsaaod et al., 2015) and less common on the front limbs. A predisposition to these diseases is associated with high productivity and metabolic disorders. Unsuitable housing conditions may also contribute to the development of the disease (Hofírek, 2009).

The disease has four stages (Hofírek, 2009; Šterc, 2010), as follows: the hidden stage (characterised by hypertrophy of the digital flexor tendon without clinical symptoms), the non-open stage, and the simple or complicated open stage. In the open, complicated stages, purulent and necrotic dermis or digit inflammations develop. The presence of lameness in dairy cows is contingent upon the stage of the disease.

Additionally, herds exhibiting foot ulceration also exhibited bleeding and an abnormal hoof shape associated with the disease (Manske et al., 2002a). An ulcer may be associated with laminitis and is most frequently observed in farms with slippery floors and uneven surfaces (Hulsen, 2011). In comparison to healthy cows, dairy cows suffering from sole ulcers exhibited jerky head movements, shortened strides and an uneven weight distribution between the limbs (Flower & Weary, 2006).

White line disease

The white line permits a degree of flexibility and mobility in the hoof. The reduced quality and hardness of the hoof horn render the structure more susceptible to damage and vascular disorders (Warnick et al., 2001). The lesion is situated in the region of the white line. A change in colour of the horn or the formation of a lesion filled with impurities may occur (Šterc, 2006). The aetiology of this disease is the incorrect loading of the hooves. Furthermore, it can be a disorder of the blood supply to the wall due to the presence of laminitis in the hoof (Holland, 2006). This condition is primarily observed in older cows and is distinctive in its presentation. It manifests in the lateral claws of the pelvic limbs (Warnick et al., 2001).

The formation of a widening white line has the potential to result in the development of a double wall. In instances where a dermis is affected, the formation of an infection and pus within the resulting double wall is a common occurrence (Kováč, 2001).

Determination of the bactericidal effectiveness of disinfectant preparations for cattle footbaths

The ÚSKVBL methodology is employed for the assessment of the efficacy of disinfectants, a process that has been in place since 1 November 2018 following the implementation of annual revisions. The methodology is based on ČSN EN 1656, which outlines a qualitative test using a suspension to determine the bactericidal effect of chemical disinfectants and antiseptics used in veterinary care. This standard is further supported by the Czech Pharmacopoeia. The test utilises reference standards of bacterial strains, provided by the Czech Collection of Microorganisms, in the form of gelatin discs or lyophilised samples:

Enterococcus hirae CCM 4533

Proteus hauseri CCM 7011

Pseudomonas aeruginosa CCM 7930

Staphylococcus aureus CCM 2022

The culture medium is prepared in the laboratory using dehydrated media for the test. The following nutrient medium is used to determine the bactericidal and disinfectant efficacy of veterinary preparations and drugs: Tryptone Soya Agar (TSA) is employed in this process. Nutrient media are prepared from dehydrated bases and physiological solutions by means of distillation or reverse osmosis, and the sample is diluted with this water. In the event of sample dilution, sterile water is employed. Unless otherwise indicated in the registration or approval documentation, a sterile physiological solution with a pH range of 6.9 to 7.1 is employed for subsequent dilution of the primary suspension. Tryptophan-NaCl is employed as a diluent. The selection of an appropriate neutraliser is dependent on the composition of the disinfectant in question. Polysorbate or sodium thiosulphate are two potential neutralising agents that may be employed in this process.

In order to prepare the bacterial suspension for the test, it is recommended that the solution be prepared to a value of $1.5x10^8$-$5x10^8$ CFU/ml (the optical density is usually around the value of 0.5 McFarland). The culture, which is maintained on an agar slant, is inoculated onto blood agar and incubated for 24 hours at 37°C. Subsequently, a suspension is prepared in a saline solution and diluted to the requisite optical density value. The bacterial suspension is then added to the sample of the disinfectant that is undergoing testing. The mixture is then incubated at the specified temperature. Following the specified contact time, a portion of the mixture is removed and the bactericidal effect of the tested substance is promptly neutralised using an appropriate neutralising agent. The number of surviving bacteria in each sample is determined through the use of TSA inoculation. In the absence of any justification or approval to the contrary, the preparation is deemed to have a bactericidal effect if the defined number of colony-forming units (CFU) has been reduced by at least 5 logs.

Monitoring the effectiveness of disinfection

In optimal circumstances, the verification of a disinfectant's efficacy entails two stages: initial verification in a laboratory setting and subsequent verification under controlled field conditions. In practice, however, verification is often conducted exclusively in laboratory conditions.

In collaboration with the Institute for State Control of Veterinary Biological and Medicinal Products (ÚSKVBL), a series of disinfectant tests were conducted in accordance with the standards set forth in ČSN EN 1656

(665208). This standard outlines a quantitative suspension test for determining the bactericidal efficacy of chemical disinfectants and antiseptics utilized in veterinary care. The techniques employed were dilution neutralisation and membrane filtration.

In both methodologies, a sample of the test product is incorporated into a test suspension of bacteria. Subsequently, the disinfection is neutralised. Should the optimal neutraliser for the neutralisation method not be identified, membrane filtration may be a viable alternative. However, given the time-consuming nature of this approach, the second method was ultimately preferred. In vitro tests were conducted to ascertain the impact of varying disinfectant concentrations on the bacteria enumerated in the standard at specified temperatures (5, 10 and 20 °C). The results obtained in the laboratory were subsequently validated in a field study that was conducted in accordance with the in vitro tests. The impact of diverse disinfectants on specific bacteria was investigated to ascertain the active chemical agent responsible for the observed effect on individual bacteria.

In collaboration with the selected farm, we collected smears **from lame dairy cows using sampling kits for anaerobic microorganisms or AMIES sampling medium swabs**. Subsequently, the field samples were **cultured on a standard growth medium and a selective medium**, and the total number of bacteria and individual bacterial colonies were determined and identified. **The identification of microorganisms was conducted using the MALDI-TOF** method, which is highly accurate, applicable to a wide range of microorganisms, and considerably more rapid than traditional methods.

To facilitate comparison between the field samples and the original laboratory test results, the same temperatures were employed for each disinfectant under examination. The temperature of the surrounding environment was recorded at the time of data collection for the cattle farm bathing procedures. The disinfectant employed, including its concentration, was also ascertained.

Five disinfectants with different active substances or combinations were selected for laboratory testing. The active substances present in the disinfectants were identified as iodine, quaternary ammonium compounds (QAC), formaldehyde, organic acids in combination with other active substances, zinc sulphate and copper sulphate. The disinfectants were subjected to testing at temperatures of 20°C, 10°C and subsequently at 5°C. The efficacy of some disinfectants was reliably demonstrated only for ***Pseudomonas aeruginosa*** (see Table 2). It was therefore **recommended** that the product be subjected **to field tests** at temperatures of 10 °C and 5 °C, which are representative of those encountered in farm disinfection baths, particularly during the winter months.

The results of laboratory tests have demonstrated that the combination of organic acids with another active substance is ineffective against a number of selected bacteria at low temperatures. Table 2 illustrates the efficacy of preparations comprising a combination of organic acids with other active substances, demonstrating their effectiveness even at lower temperatures. Copper sulphate and, similarly, zinc sulphate (the results of which are not included in Table 2) were ineffective at 20 °C. Nevertheless, they may be recommended for the purpose of enhancing the quality of the hoof horn, rather than for disinfection. The efficacy of iodine preparations was found to diminish at lower temperatures, with a three-log reduction in the number of microorganisms observed. A similar outcome was observed with formaldehyde, whereby the efficacy of this preparation was diminished at lower temperatures.

Table 2: The results of the disinfectant effectiveness testing are presented herewith

Copper sulphate 5 %	20 °C (CPM)	10 °C (CPM)	5 °C (CPM)	Field test (10 °C)
Pseudomonas aeruginosa	**10^1**	*$>10^5$*	*$>10^5$*	*$>10^5$*
Proteus hauseri	**10^0**	*$>10^5$*	*$>10^5$*	*$>10^5$*
Enterococcus hirae	*$>10^5$*	*$>10^5$*	*$>10^5$*	*$>10^8$*
Staphylococcus aureus	*$>10^5$*	*$>10^5$*	*$>10^5$*	*$>10^8$*
Formaldehyde 2 %	**20 °C (CPM)**	**10 °C (CPM)**	**5 °C (CPM)**	**Field test (10 °C)**
Pseudomonas aeruginosa	**0**	**$10^3>x<10^5$**	**$10^3>x<10^5$**	**$10^3>x<10^5$**
Proteus hauseri	*$>10^5$*	*$>10^5$*	*$>10^5$*	*$>10^8$*
Enterococcus hirae	*$>10^5$*	*$>10^5$*	*$>10^5$*	*$>10^8$*
Staphylococcus aureus	*$>10^5$*	*$>10^5$*	*$>10^5$*	*$>10^8$*
QAC 0,5 %	**20 °C (CPM)**	**10 °C (CPM)**	**5 °C (CPM)**	**Field test (10 °C)**
Pseudomonas aeruginosa	**0**	10^0	0	*$>10^5$ after 60 dairy cows*
Proteus hauseri	**0**	*$>10^5$*	*$>10^5$*	*$>10^8$*
Enterococcus hirae	**0**	*$>10^5$*	*$>10^5$*	*$>10^8$*
Staphylococcus aureus	**0**	*$>10^5$*	*$>10^5$*	*$>10^8$*
Iodine 0,5 %	**20 °C (CPM)**	**10 °C (CPM)**	**5 °C (CPM)**	**Field test (10 °C)**
Pseudomonas aeruginosa	0	0	0	*$10^3>x<10^5$after 60 dairy cows*
Proteus hauseri	0	*$>10^5$*	*$>10^5$*	*$>10^8$*
Enterococcus hirae	0	*$>10^5$*	*$>10^5$*	*$>10^8$*
Staphylococcus aureus	0	*$>10^5$*	*$>10^5$*	*$>10^8$*
Organic acids 1 %	**20 °C (CPM)**	**10 °C (CPM)**	**5 °C (CPM)**	**Field test (10 °C)**
Pseudomonas aeruginosa	0	*$>10^3$*	*$>10^3$*	*$>10^3$*
Proteus hauseri	*$>10^3$*	*$>10^3$*	*$>10^3$*	*$>10^5$*
Enterococcus hirae	*$>10^3$*	*$>10^3$*	*$>10^3$*	*$>10^5$*
Staphylococcus aureus	*$>10^3$*	*$>10^3$*	*$>10^3$*	*$>10^5$*

Note. *Reduction of CPM from 10^8 min by 5 logs = effective disinfection = coloured blue*

CPM reduction from 10^8 min by 3 to 4.9 log = reduced efficiency = greyed out

CPM reduction from 10^8 by less than 3 logs = ineffective disinfection = no discolouration

The majority of disinfectants demonstrated efficacy in vitro. It was not possible to utilise all of the formulations in the field-testing phase due to the specific conditions present on the farm, including the dimensions of the footbath. Samples were collected directly from the footbath in the field. Samples of the disinfectant solution were taken after each group of cows. The results obtained from the field test differed from those obtained from the laboratory analyses. This indicates the necessity for further testing. The representation of various microorganisms and a range of hygienic factors related to maintaining the cleanliness of disinfectants in footbaths, the cleanliness of limbs, the temperature of disinfectants, and the duration of disinfectant effects on dairy cow limbs differs between individual farms. It is therefore **not feasible to devise a single**, **universal procedure** that can be applied across all farms.

3. Comparison of the novelty of procedures

The methodology represents a system for the diagnosis of diseases affecting the limbs of cattle and for the monitoring of temperature changes in the hooves during the course of the disease and its treatment. The system is automated and is designed to monitor the symptoms of dairy cows by thermographic scanning, thereby replacing the labour-intensive and time-consuming assessment of the locomotion score. In contrast to the current methods of evaluating the locomotion score, the so-called temperature score, which is monitored autonomously every time the dairy cow passes the camera, is an indicator that is worthy of consideration. The system is capable of diagnosing inflammatory changes in the distal part of the limb, which are manifested, among other things, by a local increase in temperature. Such conditions include hoof dermis inflammation, certain stages of dermatitis, necrobacillosis, Rusterholz's ulcer and laminitis.

The aforementioned alterations are evaluated in accordance with thermal phenomena, such as instances wherein the affected extremity exhibits an elevated temperature prior to the emergence of discernible clinical indications and lameness, as discernible by the breeder. The early detection of the disease in monitored individuals allows for the reduction of the length and cost of the subsequent treatment. Early diagnosis enables the prevention of the adverse effects of diseases of the locomotor system, which are characterised by lameness in dairy cows, lower feed intake, reduced productivity, a higher predisposition to mammary gland diseases and metabolic diseases. Consequently, there is a higher demand for individual treatment of dairy cows, which is associated with their treatment.

The TMVSS Veterinary system comprises a thermographic camera and a camera operating in the visible band, both of which are fixedly located in the through corridor. Additionally, the system includes a control unit and extension modules for communication with the operational system, such as the identification of individual components or the control of the selection gate. The thermographic system is housed in a fully waterproof enclosure that is mechanically protected against all external influences and, in general, any mechanical damage that may be caused by the monitored individuals. All other TMVSS veterinary monitoring system components are designed in accordance with the same specifications. The temperatures of individual parts of the limb and the distribution of apparent temperature fields on the specified parts of the limbs are evaluated automatically. Subsequently, the measurements are stored in the internal memory of the control unit and automatically compared with the trend from previous measurements. The system is fully automated, obviating the necessity for the operator to undertake any further data analysis, such as manually browsing thermograms or evaluating temperatures. The software interface is divided into two distinct sections: an operational/operator workplace and a diagnostic workplace.

The system will enable the breeder to monitor changes in the temperature field of the limb and, based on these changes, to identify disease changes from the pre-disease state during the treatment period to the recovery of dairy cows. A further notable advantage is the capacity to monitor the health status of dairy cows' limbs, which constitutes a crucial data source for animal breeding from a health perspective. This encompasses not only the documentation of individual limb diseases but also the duration and efficacy of the treatment itself.

From the perspective of evaluating the efficacy of disinfectants, an indicator of the success of disinfection can be the elevation of temperatures on the limb, which reflects the health status of the limbs of dairy cows. Following the evaluation of the temperature score of the limbs, it is recommended that the veterinary service take samples from the wounds of treated animals using AMIES sampling kits. Subsequently, the samples will be cultured in a microbiological laboratory on both a standard growth medium and a selective medium. Identification will then be conducted using the MALDI-TOF method.

4. Description of the application of the methodology

The camera component of the system, situated within the passageway leading to the milking parlour, has been fitted with RFID identification. In contrast, the camera is programmed to automatically follow each individual as they traverse the corridor (Fig. 1). During the passage, a measurement is conducted, and the recorded data is processed by the control unit, which selects the appropriate section of the recording for temperature measurement and evaluation of temperature fields. Subsequently, the resulting measured images are subjected to further segmentation. The hind limbs are identified through segmentation, thus enabling the desired parts of the limbs to be calculated (Fig. 2).

Figure 1 Camera system for temperature score monitoring

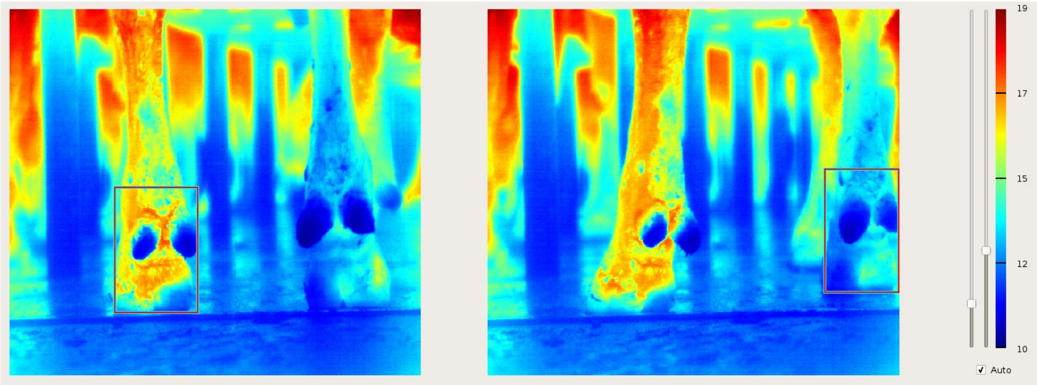


Figure 2: Thermogram of a dairy cow exhibiting signs of illness, with a temperature change evident on the left limb

The resulting data is stored in the control unit's internal database and subsequently processed for presentation to the user. The operator is presented with an automatically generated overview of individuals exhibiting suspicious behaviour, presented in tabular form with a marked temperature score index (Fig. 3). The data may be sorted according to either the temperature score or the dynamics of symptom development.

Should the operator wish to evaluate a specific piece in greater detail, they may mark an individual item in the table. This will then enable them to access the thermograms of that item, including the relevant evaluation parameters (Fig. 4).

The evaluation process is entirely automated, thereby eliminating the necessity for manual review or evaluation of individual records, which is a relatively time-consuming activity. Instead, the operator's role is limited to providing an overview of the data. The operator is not encumbered by other activities; his fundamental premise is an automatically processed and generated overview of individuals, inclusive of the attained temperature score.

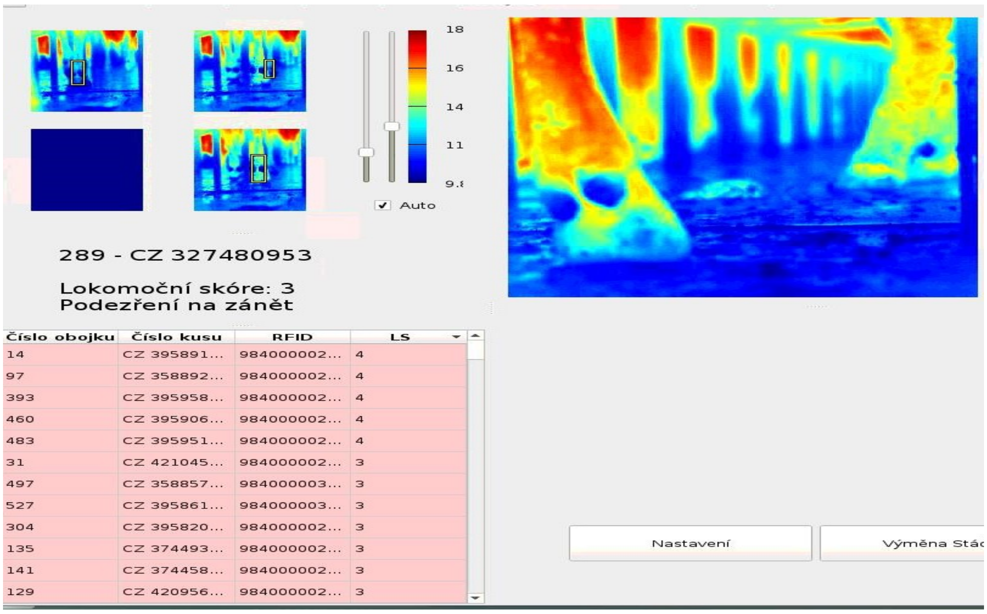


Figure 3: Composition of dairy cows exhibiting elevated temperature scores (intended for examination)

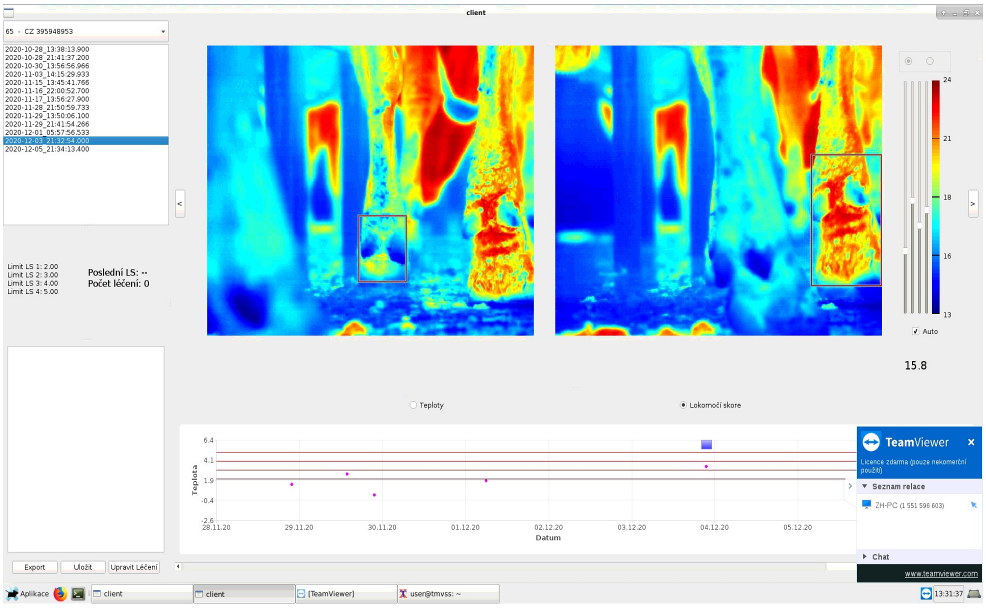


Figure 4 Illustrates the rating of individuals' temperature scores over time

Recommended steps as a result of the methodology:

- **The cornerstone of maintaining optimal limb health is daily monitoring**
  - **Thus far** - the locomotion score has revealed a paucity of possibilities, a high degree of labour intensity, and significant challenges pertaining to registration and data processing in large herds
  - **Innovative approach** - the evaluation of the temperature score, which was conducted using an automated thermography system
- **A post-treatment evaluation of the disease progression and health status of dairy cows**

- **To date** - the approach has been to treat each patient individually and to monitor the disease manifestations
- **Innovative approach** - monitoring temperature changes in the limbs of all animals, enables the capture of animals in the preclinical phase of the disease

- **Evaluating the effectiveness of disinfection**
  - **Thus far** - the efficacy of disinfection has been evaluated on an anecdotal basis
  - **Innovative approach** - recommended for monitoring the effectiveness of disinfection in a given farm. This is due to the fact that farms often differ in terms of their environment, the strains of microorganisms present, the possibility of resistance, and the local conditions, which makes it impossible to establish a uniform universal methodology. The efficacy of disinfection must be evaluated in consideration of the variables that influence it, such as the temperature of the solutions, the potential for impairment due to contamination, and other factors. Based on the findings, a system of preventive procedures for selected limb diseases can be proposed

5. Economic aspects

The economic evaluation is based on an investigation into the specific breeding conditions of 550 dairy cows. The monitoring was based on the locomotion score that has been in use until now, which was evaluated at a prevalence of lameness of 18.7%, which is a situation that many breeders are confronted with in practice. It is not uncommon for the prevalence of lameness to be significantly higher.

The financial implications of treatment and preventive procedures for maintaining the health of dairy cows were monitored for the treated herds. The costs were expressed in monetary units using a cost calculator, with consideration given to the costs associated with the treatment and prevention of limb disease, as well as the impact on productivity and reproductive indicators. The company allocated a budget of 5,632.42 CZK per annum for the treatment of dairy cows with hoof disease. The costs associated with preventive procedures amounted to CZK 525.11, while treatment costs were CZK 5,107.31.

The current prevalence of lameness in dairy cows is estimated to be 3.4%. The aforementioned procedures and the farm's overall management strategy resulted in a 81.81% reduction in the prevalence of lameness in dairy cows. This has the potential to yield annual savings of CZK 4,171,000 on the treatment of limb diseases.

It should be noted that data on economic costs may vary depending on the individual user of the methodology. These costs are primarily based on the current and achieved prevalence of lameness in dairy cows.

6. References

ALBAN, L., L.G. LAWSON a J.F. AGGER, 1995: Foul in the foot (interdigital necrobacillosis) in Danish dairy cows — frequency and possible risk factors. Preventive Veterinary Medicine: 24(2), 73-82. DOI: 10.1016/0167-5877(95)00473-A. ISSN 01675877.

ALSAAOD, M., C. SYRING, J. DIETRICH, M.G. DOHERR, T. GUJAN a A. STEINER, 2014: A field trial of infrared thermography as a non-invasive diagnostic tool for early detection of digital dermatitis in dairy cows. The Veterinary Journal: 199(2), 281-285. DOI: 10.1016/j.tvjl.2013.11.028. ISSN 10900233

BEČVÁŘ O., 2000: Výskyt a zkušenosti s léčbou dermatitis digitalis u dojnic. Veterinářství, 50, 4, 140–142.

BENNETT, Grant, Jon HICKFORD, Richard SEDCOLE a Huitong ZHOU, 2009: Dichelobacter nodosus, Fusobacterium necrophorum and the epidemiology of footrot. Anaerobe: 15(4), 173-176. DOI: 10.1016/j.anaerobe.2009.02.002. ISSN 10759964.

CRUZ, C. E. F., C. A. PESCADOR, Y. NAKAJIMA a D. DRIEMEIER, 2005: Immunopathological investigations on bovine digital epidermitis. Veterinary Record: 157(26), 834-840. DOI: 10.1136/vr.157.26.834. ISSN 0042-4900.

FLOWER F. C. a WEARY D. M., 2006: Effect of hoof pathologies on subjective assessments of dairy cow gait. Journal of dairy science, 89, 1, 139-146.

HASKELL M. J., RENNIE L. J., BOWELL V. A., BELL M. J. a LAWRENCE A. B., 2006: Housing system, milk production, and zero-grazing effects on lameness and leg injury in dairy cows. Journal of dairy science, 89, 11, 4259-4266.

HOFÍREK B., 2009: Nemoci skotu. Brno: Noviko. ISBN 978-80-86542-19-5.

HULSEN J., 2011: Cow signals: jak rozumět řeči krav: praktický průvodce pro chovatele dojnic. Praha: Profi Press. ISBN 978-80-86726-44-1.

CHARFEDDINE, N. a M.A. PÉREZ-CABAL, 2017: Effect of claw disorders on milk production, fertility, and longevity, and their economic impact in Spanish

Holstein cows. Journal of Dairy Science: 100(1), 653-665. DOI: 10.3168/jds.2016-11434. ISSN 00220302.

CHELI, R. and MORTELLARO, C., 1974, October: La dermatite digitale del bovino. In Proceedings of the 8th International Conference on Diseases of Cattle. Piacenza, Milan, Italy (pp. 208-213).

KOVÁČ G., 2001: Choroby hovädzieho dobytka. Prešov: M & M. ISBN 80-88950-14-7.

MANSKE, Thomas, Jan HULTGREN a Christer BERGSTEN, 2002b: Topical treatment of digital dermatitis associated with severe heel-horn erosion in a Swedish dairy herd. Preventive Veterinary Medicine: 53(3), 215-231. DOI: 10.1016/S0167-5877(01)00268-9. ISSN 01675877

NOVÁK, P., M. ŠOCH, S. ŠLÉGEROVÁ a J. ODEHNAL, 2003: Vliv zoohygienických podmínek prostředí chovu na zdravotní stav končetin dojnic. In: ŠIŠKA, B., D. IGAZ a M. MUCHA. Bioklimatologické dny. Ráčková dolina.

NOVOTNÁ, Ivana, Lucie LANGOVÁ a Zdeněk HAVLÍČEK. Risk Factors and Detection of Lameness Using Infrared Thermography in Dairy Cows – A Review. Annals of Animal Science: 19(3), 563-578. DOI: 10.2478/aoas-2019-0008. ISSN 2300-8733.

PETERSE, D. J., 1992: Foot lameness. In Bovine Medicine. Ed A. H. Andrews. Oxford, Blackwell. pp 353-364.

RAJKONDAWAR P. G., LIU M., DYER R. M., NEERCHAL N. K., TASCH U., LEFCOURT A. M. a VARNER M. A., 2006: Comparison of models to identify lame cows based on gait and lesion scores, and limb movement variables. Journal of dairy science, 89, 11, 4267-4275.

SPRECHER, D.J., D.E. HOSTETLER a J.B. KANEENE, 1997: A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology: 47(6), 1179-1187. DOI: 10.1016/S0093-691X(97)00098-8. ISSN 0093691X.

ŠLOSÁRKOVÁ S., FLEISCHER P, 2009: Kulhání jako stádový problém a péče o paznehty. In: Nemoci skotu. Hofírek B., Dvořák R., Němeček L., Doležel R., Pospíšil Z. et al. Noviko a.s., Brno, 1149.

ŠLOSÁRKOVÁ S., 2016: Onemocnění končetin – příčiny, léčba a prevence [online]. 6. Praha 2: Profi Press, [cit. 2019-02-09]. ISSN 0027–8068. Dostupné z: https://www.vri.cz/userfiles/file/Zdravi_zvirat/NasChov2016-Priloha-Jak_na_zdrave_koncetiny.pdf.

ŠTERC J., 2006: Onemocnění paznehtů skotu. Náš chov, 9, 84–86.

ŠTERC J., 2010: Management zdraví pohybového aparátu v chovech skotu. Veterinářství, 60, 5, 294–299.

VAN METRE, David C., 2017: Pathogenesis and Treatment of Bovine Foot Rot. Veterinary Clinics of North America: Food Animal Practice: 33(2), 183-194. DOI: 10.1016/j.cvfa.2017.02.003. ISSN 07490720.

WARNICK, L.D., D. JANSSEN, C.L. GUARD a Y.T. GRÖHN, 2001: The Effect of Lameness on Milk Production in Dairy Cows. Journal of Dairy Science: 84(9), 1988-1997. DOI: 10.3168/jds.S0022-0302(01)74642-5. ISSN 00220302.

WATSON, C., 1999: Lameness in cattle—Lesions and diseases of the skin—Part 1. UK Vet, 1999, 4: 51-60.

7. Previous research and theoretical frameworks

DOSTÁL, P.; HAVLÍČEK, Z.; KRATOCHVÍLOVÁ, L.; 2018. Use of acoustic emission for testing resistance of hooves. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. 66(1), 29-33. ISSN 1211-8516. Dostupné z: https://doi.org/10.11118/actaun201866010029

HAVLÍČEK, Z., LANGOVÁ, L.; NOVOTNÁ, I.; NĚMCOVÁ, P.; ZEMANOVÁ, M.; CHRÁST, V.; 2020. Digitální dermatitida skotu. In: Aktuální otázky bioklimatologie zvířat 2020. Praha-Uhříněves: Výzkumný ústav živočišné výroby v.v.i., s. 20-21. ISBN 978-80-7403-240-0.

HAVLÍČEK, Z.; NOVOTNÁ, I.; LANGOVÁ, L.; STRAKA, V.; ZÍTEK, P.; ŽĎÁRSKÝ, O.; MICHALIČKA, P., 2020. Inovační způsob diagnostiky onemocnění končetin dojnic. Náš chov. 80(7), 56-58. ISSN 0027-8068. Dostupné z: https://digi.profipress.cz/katalog/detail/nas-chov

HAVLÍČEK, Z.; ŘEZÁČ, P.; NOVOTNÁ, I.; LANGOVÁ, L.; STRAKA, V.; ZÍTEK, P.; ŽĎÁRSKÝ, O.; MICHALIČKA, P.; 2020. Inovační způsob diagnostiky onemocnění končetin dojnic. Zemědělský zpravodaj. 26-29. Dostupné z: https://www.zscr.cz/media/upload/zpravodaj/1608711002_zemedelsky-zpravodaj-5-20.pdf.

LANGOVÁ, L.; MACHÁČEK, M.; NOVOTNÁ, I.; HAVLÍČEK, Z.; 2019. Výskyt dermatitis digitalis a dermatitis interdigitalis u dojeného skotu v průběhu roku. In: Aktuální otázky bioklimatologie zvířat 2019. Praha-Uhříněves: Výzkumný ústav živočišné výroby v.v.i., s. 32-33. ISBN 978-80-7403-226-4.

LANGOVÁ, L.; NOVOTNÁ, I.; HAVLÍČEK, Z.; MACHÁČEK, M.; 2019. Occurrence of dermatitis digitalis and dermatitis interdigitalis in dairy cows throughout the year. In: Proceedings of the XIXth International Congress of the International Society for Animal Hygiene: “Animal Hygiene as a Fundament of One Health and Welfare improving biosecurity, environment and food quality”. Vratislav: International Society for Animal Hygiene, s. 38-39. ISBN 0-000-00000-0.

LANGOVÁ, L.; NOVOTNÁ, I.; NĚMCOVÁ, P.; MACHÁČEK, M.; HAVLÍČEK, Z.; ZEMANOVÁ, M.; CHRÁST, V.; 2020. Impact of Nutrients on the Hoof Health in Cattle. Animals. 10(10), 1824. ISSN 2076-2615. Dostupné z: https://doi.org/10.3390/ani10101824.

NĚMCOVÁ, P.; HAVLÍČEK, Z.; LANGOVÁ, L.; NOVOTNÁ, I.; 2020. Výskyt a nákladovost onemocnění končetin dojnic v průběhu roku. In: Aktuální otázky bioklimatologie zvířat 2020. Praha-Uhříněves: Výzkumný ústav živočišné výroby v.v.i., s. 63-64. ISBN 978-80-7403-240-0.

NOVOTNÁ, I.; LANGOVÁ, L.; HAVLÍČEK, Z.; 2018. Hodnocení lokomočního skóre dojnic a vliv kulhání dojnic na mléčnou produkci. In: Aktuální otázky bioklimatologie zvířat 2018. Praha-Uhříněves: Výzkumný ústav živočišné výroby v.v.i., s. 66-68. ISBN 978-80-7403-205-9.

NOVOTNÁ, I.; LANGOVÁ, L.; HAVLÍČEK, Z.; 2019. Risk Factors and Detection of Lameness Using Infrared Thermography in Dairy Cows - A Review. Annals of Animal Science. 19(3), 563-578. ISSN 1642-3402. Dostupné z: https://doi.org/10.2478/aoas-2019-0008.

NOVOTNÁ, I.; LANGOVÁ, L.; HAVLÍČEK, Z.; 2019. Risk Factors and Detection of Lameness Using Infrared Thermography in Dairy Cows - A Review. Annals of Animal Science. 19(3), 563-578. ISSN 1642-3402. Dostupné z: https://doi.org/10.2478/aoas-2019-0008.

NOVOTNÁ, I.; LANGOVÁ, L.; HAVLÍČEK, Z.; 2019. Side preference of dairy cows in the entrance milking parlor. In: Proceedings of the XIXth International Congress of the International Society for Animal Hygiene: “Animal Hygiene as a Fundament of One Health and Welfare improving biosecurity, environment and food quality”. Vratislav: International Society for Animal Hygiene, s. 37-38. ISBN 0-000-00000-0.

NOVOTNÁ, I.; LANGOVÁ, L.; HAVLÍČEK, Z.; 2019. Preferují dojnice jednu stranu při vstupu do dojírny?. In: Aktuální otázky bioklimatologie zvířat 2019. Praha-Uhříněves: Výzkumný ústav živočišné výroby v.v.i., s. 61-63. ISBN 978-80-7403-226-4.

8. Oponents

MVDr. Soňa Šlosárková, Ph.D. – Vetrinary research institute

Ing. Zdenka Majzlíková – Czech breeding inspectorate

9. Dedication

This publication utilises the findings of the Ministry of the Czech Republic PRV project – Inovation 16.1.1. – no.: 17/005/1611a/453/00011 „Inovace způsobů diagnostiky onemocnění končetin dojnic “, resolved through collaboration with the project holder, ŽIVA zemědělská obchodní a.s., Mendel University in Brno, which served as the project's guarantor, and TMV SS Ltd.

Title: A novel approach to the diagnosis of limb diseases in dairy cows

Autor: Zdeněk Havlíček et al.

Published: Mendel University in Brno

Zemědělská 1, 613 00 Brno

Printed: Publishing Center Mendel University in Brno,

Zemědělská 1, 613 00 Brno

Edition: first, 2021

No. of pages: 40

Edition: 50 pcs

ISBN: 978-80-7509-791-0