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BACTERIAL VECTOR-BORNE ZOONOTIC DISEASES AND ONE HEALTH APPROACH. A REVIEW

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ABSTRACT

One Health is a collaborative, multisectoral, and transdisciplinary approach with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environments. The main goal of this paper is to highlight the significance of the One Health concept in relation to the large group of diseases that transmits through arthropods and their worldwide distribution, with a closer look at the zoonoses. The importance of knowledge increases as the globalization and climate changes increases the likelihood of transmission, as both cause favourable conditions for transmission of bacteria and viruses. The increase in reservoir abundance, climate change, changing habitat structure, socio-political changes, and import of animals for welfare reasons, trade and traveling are considered to be potential factors for the pathogen and vector introductions to new areas. This article highlights the selected agents of bacterial zoonoses as sources of human and animal diseases and shows the number of cases of Lyme disease, which is the most common bacterial tick-borne disease in humans in Slovakia and Norway, and the trend in incidence over the past twelve years.

Key words: One Health, Slovakia; Norway; vector-borne diseases; zoonoses

INTRODUCTION

Veterinary and human medicine differed only slightly in the early days. The same healers would take care of both humans and animals. During the middle age, human medicine became part of the medieval universities, while veterinary medicine did not. After the first veterinary school was established in 1762, it wasn't until the 19th century that scientists started considering the link between veterinary medicine and human medicine again, owing to the observation of the same diseases in both species [54].

A One Health approach describes cooperation between different disciplines that together develop strategies for better human, animal and environmental health. Collaboration between disciplines is now recognized as being critical to addressing potential pandemics due to zoonotic agents and the spread of diseases as a result of climate change [5, 45]. As there isn't currently a single, widely accepted definition for One Health, there are many distinct

definitions of the concept. The most common definition, shared by the Centers for Disease Control and Prevention is "One Health is defined as a collaborative, multisectoral, and transdisciplinary approach – working at the local, regional, national and global levels - with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants and their shared environments" [21].

The concept of One Health was proven essential when facing pandemics such as SARS in 2003, and Coronavirus in 2020, as both viruses were zoonotic, spreading from animals, and caused a threat for human health and the world economy. The control of antimicrobial resistance, food and water safety, preventing zoonotic disease transmission, managing pollution and preventing pandemics are all important components of One Health.

The COVID-19 pandemic has caused massive damage to the world economy and imperiled human lives everywhere. The ignorance of the principles of One Health approach in the current health care system has proved to be the Achilles heel of our health policy. Social distancing, lockdown, and hand hygiene are short-term preventive measures imposed by nations worldwide; but are difficult to sustain in the long run. Thus, it is long overdue that we change our unidimensional approach regarding the control and prevention of diseases [18].

One Health – sectors cooperation

As the One Health approach works towards better health for animals, humans and the environments and ecosystems, there is a need for both veterinarians, medical doctors, and researchers with expertise in all three aspects. The three key components in One Health concept are shown in the Fig. 1. In 2010, a tripartite collaboration was formed between the Food and Agriculture Organization of the United Nations, the World Organization for Animal Health and the World Health Organization. The goal of this tripartite collaboration was to provide the multi-disciplinary perspective for an efficient and better management and coordination of the One Health approach [15].

The aim of One Health

The goal of One Health concept is to be able to implement programs, politics, laws and science where more sectors cooperate and communicate to achieve a better public health. The One Health approach aims to improve global food and water safety, reduce disease transmission risks, and ensure that all living beings have sufficient resources of nutrition. Preventing zoonotic diseases from evolving into endemics or pandemics is one of the key goals. When it comes to reaching the Sustainable Development Goals (SDGs), the One Health concept is particularly crucial [26]. The 17 SDGs were jointly developed by the United Nations to strive toward a collaboration in enhancing global health and education, promoting greater equality, protecting wildlife, oceans, and forests. All member nations were required to adopt the goals by 2015, and the aim is for the goals to be reached by 2030 [40].

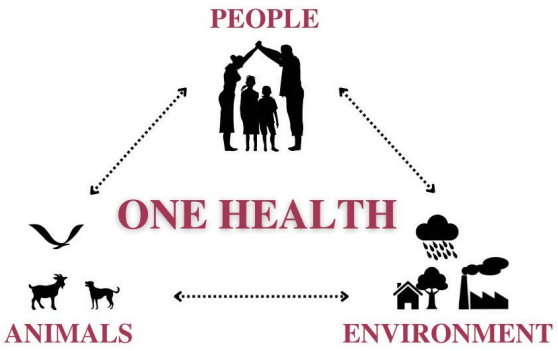

Fig. 1. Three key components in the One Health concept

Ticks as vectors

Ticks are found globally, in various habitats. There are 900 species identified, which are mainly divided into two groups, the hard ticks, Ixodidae, and soft ticks, Argasidae. Ixodidae are the biggest family, with close to 700 species, and is divided into 7 genera, where the main genera are: *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* [3]. Ticks are the most important vectors of human pathogens, leading to increased public health burdens worldwide. The tick-borne pathogens include viruses (e.g. tick-borne encephalitis); bacteria, such as the causative agents of Lyme disease, spotted fever rickettsiosis and human anaplasmosis; and malaria-like protozoan parasites causing babesiosis. Tick-borne diseases are emerging due to the geographical expansion of their tick vectors, especially in the northern hemisphere [34, 52]. Given ongoing climate change, it is desirable to intensify efforts to prevent and control the spread of vectors capable of transmitting infectious disease agents, especially zoonoses [33].

Climate change and zoonotic diseases

Climate change can have a complex impact that also influences human and animal health. For example, climate change alters the conditions for pathogens and vectors of zoonotic diseases. With these changes come new challenges for maintaining human and animal health [20]. Climate change has improved the living conditions of ticks substantially. One of the consequences is an increased distribution of ticks and a rise of the Lyme borreliosis incidence [46].

The influence of global warming and geoclimatic variations on zoonotic disease epidemiology is evident by alterations in the host, vector, and pathogen dynamics and their interactions [14, 37].

The Intergovernmental Panel on Climate Change reported with a high confidence that the prevalence of vector-borne diseases has increased in recent decades and that the prevalences of malaria, dengue, Lyme disease, and West Nile virus infection in particular are expected to further increase during the next 80 years, if measures are not taken to adapt and strengthen the control strategies [16].

Climate change has a major impact on seasonal weather patterns, resulting in marked phenological changes in a wide range of taxa. However, empirical studies of how changes in seasonality impact the emergence and seasonal dynamics of vector-borne diseases have been limited [11].

Lyme borreliosis has an increasing prevalence of infections, as the summers in certain regions are getting longer, and therefore increasing the most active time for the ticks transmitting the disease. The disease is spreading further north, as the weather is getting warmer, with the association of the expansion of ticks for example in Norway and Canada. [48].

Vector-borne diseases

Vector-borne diseases have been the scourge of man and animals since the beginning of time. Historically, these are the diseases that caused the great plagues such as the 'Black Death' in Europe in the 14th Century and the epidemics of Yellow Fever that plagued the development of the New World. Others, such as Nagana, contributed to the lack of development in Africa for many years. At the turn of the 20th Century, vector-borne diseases were among the most serious public and animal health problems in the world [7, 13].

Estimates show that as much as 75 % of emerging diseases affecting human health are due to zoonoses, responsible for 2.7 million deaths annually [9]. Vector-borne diseases account for 17 % of all infectious diseases in humans on a global scale, causing more than 700 000 deaths yearly. The highest occurrence is seen in tropical and sub-tropical regions, where there is a rich biodiversity. The typical vectors for human vector-borne diseases are mosquitoes, ticks, sand-flies, triatomine bugs, tsetse flies, fleas, black flies, aquatic snails, and lice.

The two major vector-borne diseases of the northern temperate regions, tick-borne encephalitis and Lyme borreliosis (LB), show very different epidemiological patterns, but both have increased significantly in incidence since the 1980s [30].

Although there is a summary of all known vector-borne diseases that affect humans, there is still no such list for diseases affecting animals as of 2016. Given that many vector-borne diseases have the ability to infect and inflict disease in a variety of animals, as well as several types of vectors can transmit the same disease, there is a significant potential extent of harm. Reducing the risk of transmission in free range domestic animals as well as domestic animals on pasture can be challenging, as several of the vector-borne diseases can infect and transmit both domestic and wild animals [45].

Bacterial vector-borne diseases

Infections with vector-borne pathogens are a major source of emerging diseases. The ability of vectors to bridge spatial and ecologic gaps between animals and humans increases opportunities for emergence. Small adaptations of a pathogen to a vector can have profound effects on the rate of transmission to humans [35].

Relapsing fever is a zoonotic disease caused by different spp. of the bacteria Borrelia and the main vector is the soft-bodied tick, *Ornithodorus turicata*. The soft-bodied thick differs from the thick-bodied tick with living close to mammalian hosts, and feeding for only 15-90 minutes, causing a risk of not getting detected while biting. Reports of ticks carrying the bacteria has been reported worldwide, with Australia and Antarctica as the only exceptions. The bacteria are naturally found in ticks and a variety of animals, often small rodents. The only distinction between Relapsing Fever and Louse-borne Relapsing Fever is that the latter is exclusively caused by *Borrelia recurrentis*; aside from that, the two illnesses are clinically and microscopically identical. Relapsing Fever was first reported

in 1904 in Africa and has since then spread to all regions except Antarctica and Australia. It is now considered an important public health problem in Western and East Africa, and causes a high mortality among children [17]. Mediterranean Spotted Fever is a zoonotic vector-borne disease of the Spotted Fever group, caused by *Rickettsia conorii conorii*, with the brown dog tick *Rhipicephalus sanguineus* as vector. Typical symptoms in humans are fever followed by a rash. The disease was first recognized in Tunisia in 1910, then detected in the Mediterranean countries in 1927. Today the disease is endemic in the Mediterranean, as well as being present in surrounding countries. The main mammalian hosts for Mediterranean Spotted Fever are dogs, hedgehogs and small rodents. There is still no vaccine to protect humans or animals from the infection. Preventive measures to avoid further spread of the disease are tick-controlling programs, and individual prevention of insect bites [42].

Q-fever is caused by the bacterium *Coxiella burnetii* and was first recognized causing disease in humans in Brisbane, Australia, then shortly after in Montana, USA and in military personnel in Balkan countries. The disease is zoonotic, tick-borne and causes symptoms like the flu in humans. In chronic cases in humans, Q-fever is potentially life-threatening. Infected animals may suffer from infertility, abortions and stillbirths; however, most animals are asymptomatic. There are a diverse range of hosts for the bacteria including humans, ticks, reptiles, birds, fish and ruminants. The bacteria transmit through airborne transmission, consumption of contaminated raw food, direct mucosal contact of contaminated products and ticks as vectors. Preventive measures to prevent outbreaks are vaccination of production animals, strict hygiene on farms, and surveillance programs. As most animals are asymptomatic, the potential for human infection when working at farms, veterinary work etc. is hard to control in endemic areas [50].

Lyme borreliosis (LB) is a multisystemic tick-borne disease that can affect many organs and have various clinical manifestations in animals [8, 24]. LB is the most common vector-borne disease in the Northern Hemisphere, transmitted to humans by the bite of *Ixodes* ticks [10, 23, 29]. Many mammalian and avian species become infected but do not develop overt clinical signs. Humans are at high risk of infection in regions where highly competent reservoirs are the primary hosts for the subadult stages of the tick, in contrast to regions where less competent or refractory animals feed ticks. Human infections are also most frequently associated with spring and summer months when the nymph stage of the tick is active [39]. Lyme borreliosis is primarily caused by the bacterium *Borrelia burgdorferi* in North America and *Borrelia afzelii* or *Borrelia garinii* in Europe and Asia. Infection usually begins with an expanding skin lesion, known as erythema migrans (referred to as stage 1), which, if untreated, can be followed by early disseminated infection, particularly neurological abnormalities (stage 2), and by late infection, especially arthritis in North America or acrodermatitis chronica atrophicans in Europe (stage 3). However, the disease can present with any of these manifestations. During infection, the bacteria migrate through the host tissues, adhere to certain cells and can evade immune clearance. Yet, these organisms are eventually killed by both innate and adaptive immune responses and most inflammatory manifestations of the infection resolve. Except for patients with erythema migrans, LB is diagnosed based on a characteristic clinical constellation of signs and symptoms with serological confirmation of infection [43]. The diagnosis is not always easy, as many patients are not able to recall a tick bite. However, in endemic areas, patients who have the typical rash can be started on treatment without waiting for serology [41]. Vaccination against LB is an effective way to prevent and reduce the number of diseases in endemic areas. Several vaccines have been developed and tested in the past, but no human LB vaccine is currently available on the market. Preventive measures to increase awareness of LB prevention should take place in regions with high LB morbidity [1, 2].

Tularemia is a zoonotic disease, infecting humans, wild animals and domestic animals. It is primarily seen in wild animals, where hares and small rodents are particularly vulnerable. Tularemia is caused by the bacteria *Francisella tularensis*, where the subspecies *Francisella tularensis holarctica* is found in Europe. The bacteria are highly contagious, and transmits directly through contact with infected or diseased animals, through contaminated water or meat, through inhaling of contaminated dust particles, or through blood-sucking midges as vectors. The bacteria are able to survive for a long time in the environment, and mosquito larvae can be infected during the development in contaminated water. Hares are especially sensitive to the disease and will usually die of sepsis a few

days after being infected. Hares in the early phase of infection with no or little clinical signs causes a significant risk of transmission of disease for hunters during hunting [25]. The most recent incidences of tularemia have been reported from Scandinavia, northern America, Japan and Russia, without clear reason. Due to the easy transmission of the bacteria, Tularemia has been classified as a category A biological weapon by the Centres for Disease Control and Prevention [53].

Anaplasmosis is a vector-borne, infectious and non-contagious disease. The different species cause different types of anaplasmosis depending on which cells are infected in the mammalian host. Anaplasmosis has a wide host range, including humans, and it is distributed worldwide. The zoonotic potential of some species is of great importance in regards to public health concerns [19, 31]. Anaplasmosis is caused by bacteria of the genus *Anaplasma*, belonging to the order Rickettsiales, which emerged from the fusion of the families Anaplasmataceae and Rickettsiaceae [6]. Anaplasmas are obligatory intracellular microorganisms and Gram-negative bacteria. They can reside and multiply in vertebrate reservoirs for many years [38]. The main symptoms of anaplasmosis include fever, headache, myalgia, and malaise. Severe illness is more frequently reported in older and immunocompromised patients, but it can also affect immunocompetent individuals and may lead to hospitalization or death if appropriate treatment is not provided promptly. Generally, patients show significant improvement within 24-48 hours after starting antimicrobial treatment with doxycycline [28].

Ticks in Slovakia and Norway

Ticks are blood-sucking ectoparasites of vertebrates. *Ixodes ricinus*, *Dermacentor reticulatus*, *Dermacentor marginatus*, *Haemaphysalis concinna*, *Haemaphysalis inermis* and *Haemaphysalis punctata* are the most common ticks in Slovakia [47].

The most important tick-borne zoonotic pathogens causing disease in humans in Slovakia include *Borrelia burgdorferi* s.l., tick-borne encephalitis virus, *Anaplasma phagocytophilum*, various species of rickettsiae, bartonellae and babesiae.

The prevalence of different tick species in Norway is estimated to be 10–12 species established in the Norwegian fauna. *Ixodes ricinus* is responsible for the majority of spreading of disease. *I. ricinus, I. hexagonus, I. lividus, I. trianguliceps, I. uriae* and *Argas vespertilionis* are considered the most common established tick species. Several more species are observed in Norway, as a result of migrating birds, causing a risk of the establishment of several new tick species. The most important tick-borne zoonotic pathogens responsible for disease in humans in Norway are *Borrelia burgdorferi* s.l., Tick-borne encephalitis virus, *Rickettsia helvetica, Borrelia myiamotoi, Neonehrlichia mikurensis, Spiroplasma ixodetis* and *A. phagocytophilum* [44].

Lyme borreliosis in Europe

Lyme disease is the most common tick-borne disease in the United States and Europe [12, 22, 49]. In both locations, species of genus *Ixodes* ticks transmit the *Borrelia burgdorferi* sensu lato bacteria species responsible for causing the infection. The diversity of Borrelia species that cause human infection is greater in Europe; the 2 *B. burgdorferi* s. l. species is collectively responsible for most infections in Europe, *B. afzelii* and *B. garinii*, are not found in the United States, where most infections are caused by *B. burgdorferi* sensu stricto. Strain differences seem to explain some of the variation in the clinical manifestations of Lyme disease, which are both minor and substantive, between the United States and Europe [22].

Diagnosis of LB is on the rise in some Western European countries, mostly in the northern and central part. Better surveillance in the southern countries is necessary [51].

Despite improvements in prevention, diagnosis and treatment, LB is still the most common arthropod-borne disease in temperate regions of the northern hemisphere, with risk of infection associated with occupation (e.g. forestry work) and certain outdoor recreational activities (e.g. mushroom collecting). Recent surveys show that the overall prevalence of LB may be stabilising, but its geographical distribution is increasing. In addition, much remains to be discovered about the factors affecting gene-specific prevalence, transmission and virulence, although avoidance of tick bite still appears to be the most efficient preventive measure. Uniform, European-wide surveillance programmes (particularly on a local scale) and standardisation of diagnostic tests and treatments are still urgently needed, especially in the light of climate change scenarios and land-use and socio-economic changes. Improved epidemiological knowledge will also aid development of more accurate risk prediction models for LB. Studies on

the effects of biodiversity loss and ecosystem changes on LB emergence may identify new paradigms for the prevention and control of LB and other tick-borne diseases [32].

Lyme disease in Slovakia and Norway

Data presented in review [4] indicate that the incidence of LB disease in Europe is substantial but geographically heterogeneous, both among and within countries. Data reported at the national level can often mask subnational differences, particularly in areas with substantially higher incidences.

During 2021, 621 human cases of Lyme disease were reported in Slovakia. This is 35 % less than in 2020 and 33.6 % less than the 5-year average. The presence of erythema migrans was reported in 551 cases. Arthritis in Lyme disease was reported in 51 patients. Disease was reported mostly in the Trnava region. Disease occurred throughout the year with a peak in June and July [36]. The overview of number of human cases of Lyme disease in Slovakia in years 2010–2021 is shown in Figure 2.

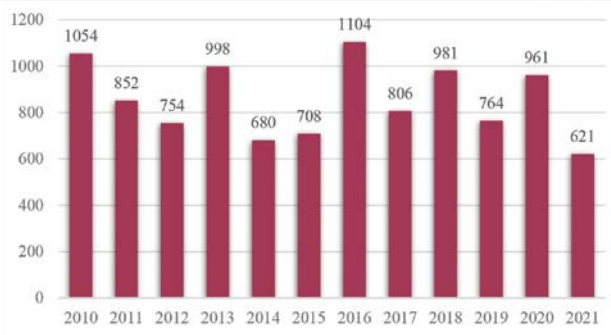

Fig. 2. Number of human cases of Lyme disease in Slovakia, 2010–2021
Source: RPHA, Annual Report, Public Health Authority of the Slovak Republic, 2010–2021

During 2021, 536 cases of Lyme borreliosis were reported in Norway, an increase of 5 % from 2020. The average for the last 5 years is 479, and 2021 represents an increase of 12 % from the 5-year average, and 30 % from the 10-year average. The trend for reported cases of Lyme borreliosis in Norway is on a steady increase. 75 % of the infected were infected in Norway, while 1 % were from other countries, and 24 % had unknown origin. Most cases were reported in August, October, and November, although disease was recorded throughout the year [27]. Figure 3 shows number of human cases of Lyme disease in Norway in years 2010–2021.

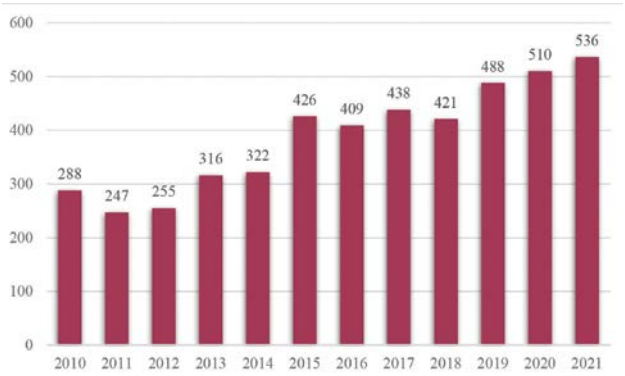

Fig. 3. Number of human cases of Lyme disease in Norway, 2010–2021
Source: Annual Report 2021, Norwegian Institute of Public Health, 2022

CONCLUSIONS

Climate change has a major impact on the transmission and spread of vector-borne diseases. Vectors represent a carrier of the pathogen between different species of organisms. A decrease in the number of cold and an increase in the number of warm days and nights, a reduction in snow cover, and an increase in extreme heat contribute to a significant increase and spread of vectors.

As climate change affects the temperature and humidity, the weather, and consequently changing the habitats of all living beings on earth, the importance of One Health approach is undeniable. A well-functioning cooperation between human health, animal health and environmental health is critical in facing the risk of new pandemics caused by vector-borne zoonotic diseases, to preserve the human and animal health as well as restrict the global economic impact from such pandemics.

LB is one of the major tick-borne diseases in Europe and still requires increased attention, especially due to problems with prevention, complicated diagnosis and treatment, and also due to a possible significant impact on the quality of life of these patients. Tick awareness, appropriate clothing in tick-infested areas, and early removal of attached ticks remain the most important prevention measures.

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PHYSIOLOGICAL RESPONSES IN BROILER CHICKENS ADMINISTERED LYCOPENE DURING THE HOT-DRY SEASON

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ABSTRACT

This study evaluates the effects of lycopene administration on body weight, mortality, cloacal temperature, and haematological responses, in broiler chickens exposed to heat stress. 40 day-old broiler chicks were divided into control and lycopene groups, 20 chickens each. Each bird in the control group received olive oil (1 ml.kg^{-1}), and lycopene at 10 mg.kg^{-1} mixed with olive oil was given to each bird in the lycopene group by oral gavage once daily for 28 days. The dry- and wet-bulb temperature of the broiler chickens' pen was recorded three times daily from days 8 to 28. The body weights were measured weekly. The incidence of mortality was recorded. The cloacal temperatures were measured on days 14, 21 and 28. The blood samples were collected for haematological analyses, on day 28. The results indicated a high overall temperature-humidity index (31.24 ± 0.43 °C) of the thermal micro-environment of the broiler chickens. There was no significant difference (P > 0.05) in body weight. The percentage mortality in the control group was relatively higher compared to the lycopene group. Lycopene reduced the cloacal temperature responses and the daily fluctuations in broiler chickens. The decreased (P < 0.05) heterophil:lymphocyte ratio and percentage erythrocytes haemolysis were recorded in the lycopene group. In conclusion, lycopene administration reduced mortality and improved cloacal temperature and haematological responses without exerting any significant beneficial or negative effects on the body weight in broiler chickens exposed to heat stress.

Key words: cloacal temperature; erythrocytes fragility; heat stress; heterophil:lymphocyte ratio; mortality; temperature fluctuations

INTRODUCTION

Genetic selection of commercial broiler lines has produced birds with fast growth rates, low immune status, and highly susceptible to heat stress [38]. The health and productivity of chickens may be adversely affected by high ambient temperature (AT) and high relative humidity (RH) [32]. The hot-dry season in the Northern Guinea Savannah zone of Nigeria is characterised by high AT and high RH, which causes heat stress in broiler chickens, consequently leading to economic loss including mortality [4]. Heat stress increases body temperature in birds and disrupts body temperature rhythm which may lead to adverse effects on physiological functions [9]. To enable optimal

body functions during stressful conditions, broiler chickens' body temperature, like those of mammals, must be kept within a certain physiological range [22]. Heat stress decreases erythrocytes and immune functions and blood parameters are a useful tool in evaluating the health status of broiler chickens exposed to stressful conditions [13].

Due to climate change, finding feeding and management strategies to lessen the effects of heat stress on poultry production is crucial, not just in tropical countries, but for many other areas of the world as well. The negative effects of heat stress on broiler chickens have been alleviated by the application of a variety of mitigation management methods, including nutritional supplements [17, 40]. One of the potent dietary carotenoids for preventing the damaging effects of heat stress on cells and tissue is lycopene [36]. It is highly effective in removing reactive oxygen species (ROS), particularly singlet oxygen [25]. Lycopene is beneficial for enhancing growth responses in broiler chickens exposed to heat stress [2, 26, 39]. Lycopene's health benefits may be linked to its antioxidant properties, which enhance the numerous physiological reactions in broiler chickens [20, 35].

This study aims to evaluate body weight, mortality, cloacal temperature and haematological responses in broiler chickens exposed to heat stress during the hot-dry season and the ameliorative effects of lycopene.

MATERIALS AND METHODS

Location

The study was conducted in the poultry house of the Veterinary Teaching Hospital, Ahmadu Bello University, Samaru (11^0 10′ N, 07^0 38′ E), Zaria. The experiment was carried out from March to April, during the hot-dry season of the zone [10].

Experimental flock and management

Forty day-old, male, *Arbor acres* broiler chicks with an average weight of 38.65 grams per bird were obtained from a reputable sales outlet. On arrival at the pens, they were assigned to two different groups, that is, the control and lycopene groups. The chicks were raised on a deep litter system. The birds were fed a standard diet from day one to day 28. Proximate analysis of commercial feed was conducted as indicated in Table 1. Clean drinking water was provided *ad libitum.* Biosecurity measures were followed throughout the experiment.

Table 1. Nutrient contents of the broiler chickens feed

Nutrient contents	Amount
Metabolisable energy (kcal.kg^{-1})	2800
Proximate analysis* (%):	
i) Dry matter	97.50
ii) Crude protein	24.74
iii) Crude fibre	4.61
iv) Oil	3.40
v) Ash	4.50
vi) Nitrogen-free extract	62.75

* Analysed in the biochemical laboratory, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

Ethical approval

This study with reference number ABU-CAUC/2021/025 was approved by the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria, Nigeria.

Experimental design and groupings

Forty day old broiler chicks were divided into two groups of 20 chickens each, using simple random sampling. Each bird in the control group received olive oil (1 ml.kg^{-1}), and lycopene at 10 mg.kg^{-1} mixed with olive oil was given to each bird in the lycopene group by oral gavage once daily for 28 days. A gelatine capsule containing 10 mg of lycopene (General Nutrition Corporation, Pittsburgh, U.S.A.) was reconstituted in olive oil (Goya en espana, S.A.U., Sevilla, Spain) to a suitable working concentration [23]. Commencing at 7:00 h (GMT+1), each bird in the groups was administered with either olive oil only (1 ml.kg^{-1}) or mixed with lycopene by oral gavage once daily for 28 days. Lycopene was administered at 10 mg.kg^{-1} [39]. Body weights were measured weekly. The incidence of mortality was recorded. Cloacal temperature of each bird in the groups was measured on days 14, 21 and 28. On day 28, three ml of blood per bird was collected from 7 broiler chickens per group via wing vein into potassium ethylenediaminetetraacetate (K_2EDTA) sample bottles. The blood collected was used for haematological analyses and erythrocytes osmotic fragility test analyses.

Thermal micro-environmental parameters

The dry- and wet-bulb temperatures of the micro-environment of the poultry pen were measured three times daily using a dry- and wet-bulb thermometer (Aura Labtech, India) at 07:00 h, 13:00 h and 19:00 h (GMT+1) from day 8 to 28. From the data, RH on each day was calculated using an online relative humidity calculator (www.1728.org/relhum.htm). The temperature-humidity index (THI) on each day was determined using the formula as described by Tao and Xin [37]:

$$THI = 0.85 \times DBT + 0.15 \times WBT$$

(where DBT = Dry-bulb temperature, and WBT = Wet-bulb temperature)

Body weight measurement

The birds were weighed singly using a weighing scale balance on days 7, 14, 21, and 28.

Cloacal temperature measurement

A digital clinical thermometer (Hartmann Digital Thermometer, Paul Hartman AG, Heidenheim, Germany) was used to take the cloacal temperature. It was placed into the cloaca about 2 cm and in direct contact with the mucosal wall. After the thermometer sounded an alert to signal that the reading had stabilized, the value was recorded.

Determination of haematological parameters

The pack cell volume was determined using the micro-haematocrit method as described by Cheesbrough [7]. The concentrations of haemoglobin (Hb) were obtained by dividing the corresponding pack cell volume values by three. For the white blood cell and differential leucocyte count, blood smears were stained with Giemsa stain as described by Cheesbrough [7]. Briefly, the blood films (methanol pre-fixed) were covered with the diluted stain (1 in 20) for 25 minutes. The slides were finally washed off the stain with tap water, air-dried and observed microscopically under oil immersion. A total of 100 leucocytes were counted on each slide, including heterophils, lymphocytes and monocytes.

Determination of erythrocyte osmotic fragility

Erythrocyte Osmotic Fragility (EOF) was determined according to the method described by Faulkner and King [14]. Briefly, 0.02 ml of blood from each bird in the groups were added to tubes containing phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 at increasing concentrations (0, 0.1, 0.3, 0.5, 0.7, and 0.9 %). After being gently mixed, the tubes were incubated for 30 minutes at room temperature (25–26 °C). After mixing the contents of each tube and centrifuging it at 400 ×*g* for 10 minutes (Hettich Centrifuge, Germany), the supernatant was decanted. The optical density of the supernatant was determined using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, England) at 540 nm. Haemolysis in each tube was expressed as a percentage, with 100 % being the haemolysis in distilled water (0 % NaCl). The percentage haemolysis was calculated using the formula:

$$\text{Percentage haemolysis} = \frac{\text{Optical density of test solution x 100}}{\text{Optical density of standard solution}}$$

Data analyses

The data obtained were expressed as a percentage and mean ± standard error of the mean (Mean ± SEM). DBT, RH and THI were analysed using one-way analyses of variance (ANOVA). The body weights and cloacal temperatures were analysed using repeated-measures two-way ANOVA. The ANOVA tests were followed by Tukey's post-hoc test to compare the mean. The haematological parameters and erythrocyte osmotic fragility were analysed using the Student t-test. Values of $P < 0.05$ were considered significant. These analyses were carried out using GraphPad 5 for Windows (San Diego, CA, USA).

RESULTS

Thermal environment parameters inside the broiler chickens' house

Thermal environment parameters inside the broiler chickens' house during the study period are presented in Table 2. There was a significant increase in DBT and THI values at 13:00 h compared to 7:00 h and 19:00 h. A significantly ($P < 0.05$) higher RH value was recorded at 7:00 h than at 13:00 h and 19:00 h, although the differences between the values recorded at 13:00 h and 19:00 h were insignificant.

Table 2. Thermal environment parameters inside the broiler chickens' house

Parameter	Hour of the day			Overall
	7:00 h	13:00 h	19:00 h	
DBT (oC)	24.61 ± 0.49c (19–29)	39.86 ± 0.67a (32–45)	32.39 ± 0.51b (27–38)	32.29 ± 0.42 (27.33–35.67)
RH (%)	79.10 ± 1.69a (47.40–92.00)	46.86 ± 2.41b (17.90–93.80)	52.27 ± 3.05b (16.70–79.90)	59.41 ± 1.74 (41.40–78.90)
THI	24.21 ± 0.48 c (18.85–28.55)	38.29 ± 0.65a (31.10–43.20)	31.23 ± 0.45b (25.95–35.75)	31.24 ± 0.43 (26.68–34.47)

[ab]Means for the same parameter having different superscript letters are significantly different (P < 0.05).
Values in parentheses are the minimum and maximum. DBT: Dry-bulb temperature; RH: Relative humidity;
THI: Temperature-humidity index; n = 21 days.

Body weight and mortality

Body weights and mortality of broiler chickens administered lycopene during the study period are presented in Table 3. There was no significant (P > 0.05) difference in the body weights, between the control and lycopene groups. Percentage mortality of 15 % recorded in the control group was relatively higher compared to 5 % recorded in the lycopene group.

Table 3. Body weight and mortality of broiler chickens administered lycopene

Parameters	Days	Control	Lycopene
Body weight (g per bird)	1	38.63 ± 0.13	38.67 ± 0.13
	7	87.49 ± 2.19	90.67 ± 0.67
	14	155.50 ± 0.63	154.6 ± 2.51
	21	316.10 ± 3.39	304.80 ± 9.70
	28	701.70 ± 12.61	697.40 ± 23.54
Mortality (%)	-	15	5

Daily fluctuations in cloacal temperature responses

Daily fluctuations in cloacal temperature responses in broiler chickens administered lycopene during the study period are presented in Table 4. In each group, an increase (P < 0.05) in the cloacal temperature values was recorded at 13:00 h compared to 7:00 h and 19:00 h. A decrease (P < 0.05) in the value was obtained in the lycopene group compared to the control group at 13:00 h on days 21 and 28. Whereas an increase (P < 0.05) was observed in the lycopene group compared to the control group at 7:00 h on days 21 and 28, and at 19:00 h on day 28.

Daily range of fluctuations in cloacal temperature responses

A decrease (P < 0.05) was observed in the daily range of fluctuations in cloacal temperature responses in broiler chickens administered lycopene compared to the control group on days 14, 21 and 28 (Table 5).

Table 4: Daily fluctuations in cloacal temperature (°C) responses in broiler chickens administered lycopene

Days	Hour of the day	Control	Lycopene
Day 14	07:00 h	40.46 ± 0.052 (40.10–40.90)	40.54 ± 0.072 (39.90–41.10)
	13:00 h	41.37 ± 0.101 (40.60–42.30)	40.95 ± 0.111 (39.50–41.70)
	19:00 h	40.44 ± 0.082 (39.70–40.90)	40.60 ± 0.062 (40.20–40.90)
Day 21	07:00 h	40.61 ± 0.072b (40.20–41.00)	41.14 ± 0.062a (40.80–41.70)
	13:00 h	41.64 ± 0.051a (41.10–42.10)	41.40 ± 0.031b (41.20–41.60)
	19:00 h	41.41 ± 0.101 (40.00–41.70)	41.00 ± 0.031 (40.70–41.20)
Day 28	07:00 h	40.52 ± 0.083b (39.90–41.00)	41.13 ± 0.063a (40.80–41.90)
	13:00 h	42.05 ± 0.081a (41.60–42.80)	41.72 ± 0.061b (41.10–42.20)
	19:00 h	41.06 ± 0.062b (40.70–41.40)	41.53 ± 0.092a (41.00–42.50)

[abc12] Means for the same parameter having different superscript letters across the row are significantly (P < 0.05) different; Values in parentheses are the daily minimum and maximum cloacal temperatures.

Table 5. Daily range of fluctuation in cloacal temperature (°C) responses in broiler chickens administered lycopene

Days	Control	Lycopene
Day 14	1.05 ± 0.12a (39.70–42.30)	0.64 ± 0.09b (39.50–41.70)
Day 21	1.09 ± 0.08a (40.00–42.10)	0.61 ± 0.08b (40.70–41.70)
Day 28	1.53 ± 0.14a (39.90–42.80)	0.77 ± 0.09b (40.80–42.50)

[ab] Means for the same parameter having different superscript letters across the row are significantly (P < 0.05) different; Values in parentheses are the daily minimum and maximum cloacal temperatures.

Haematological responses

Haematological values in broiler chickens administered lycopene are presented in Table 6. There was no significant ($P > 0.05$) difference in the values of PCV, Hb, white blood cell and differential leucocyte count between the two groups. An increase ($P < 0.05$) in the values of heterophil:lymphocyte (H:L) ratio was recorded in the lycopene group.

Erythrocyte osmotic fragility

At 0.7 %, the percentage haemolysis of 8.80 ± 1.14 % obtained in the control group was significantly ($P < 0.05$) higher than the corresponding value of 3.72 ± 0.45 % recorded in the lycopene groups (Fig. 1). There was no significant difference between both groups in the percentage haemolysis at 0.9 %, 0.5 %, 0.3 % and 0.1 %.

Table 6. Haematological responses of broiler chickens administered lycopene

Parameters	Control	Lycopene
PCV (%)	34.30 ± 1.53	30.50 ± 1.95
Hb (g.dl^{-1})	8.15 ± 0.55	6.64 ± 0.64
WBC (× 10³ µl)	7.86 ± 0.61	6.12 ± 1.57
Heterophil (× 10³ µl)	1.22 ± 0.12	0.65 ± 0.26
Lymphocyte (× 10³ µl)	5.87 ± 0.49	5.38 ± 1.31
Monocyte (× 10³ µl)	0.13 ± 0.07	0.07 ± 0.04
H/L Ratio	0.41 ± 0.09a	0.11 ± 0.03b

[ab] Means for the same parameter having different superscript letters across the row are significantly ($P < 0.05$) different; PCV = Pack cell volume; Hb = Haemoglobin concentration; WBC = White blood cell count; H/L ratio: heterophil:lymphocytes ratio.

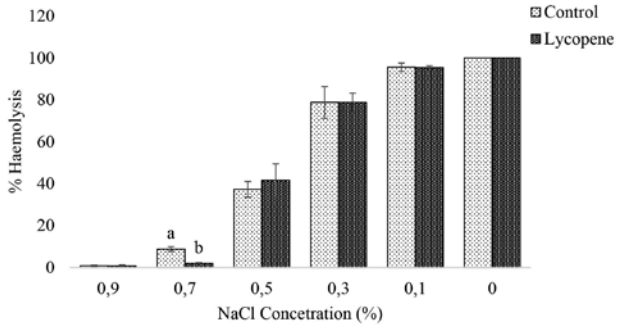

Fig. 1. Erythrocytes osmotic fragility (EOF) in broiler chickens administered lycopene

[ab] Means for the same parameter having different superscript letters differ significantly ($P < 0.05$)

DISCUSSION

Chickens, as homeotherms, have an optimal environmental temperature range which is considered the thermo-neutral zone [3, 24]. The DBT values at 13:00 and 19:00 were 39.86 °C and 32.39 °C, respectively, which were outside the known thermoneutral zones of 18–26 °C for broiler chickens raised in tropical climates [19, 29]. The high RH was between 79.10 % and 46.86 % higher than the standard for broiler chickens grown in tropical climates, which is between 30 and 40 % [18, 40]. The high RH was between 79.10 % and 46.86 % higher than the usual range for broiler chickens grown in tropical climates, which is between 30 and 40 % [24]. Due to the difficulties in heat dissipation through evaporative cooling, broiler chickens suffer from heat stress when the ambient temperature and relative humidity are high [30, 41]. THI is an index of heat stress that is used to assess the combined impacts of both ambient temperature and RH [37]. The overall THI of 31.24 °C obtained in the present study is above 30.10 °C, reported by S u m a n u et al. [33]. The broiler chicks' thermal microenvironment may be uncomfortable given the high THI. The hourly THI values fluctuated between 24.21 ± 0.48 at 7:00 h and 38.29 ± 0.65 at 13:00 h. The fluctuation range is higher than the THI values ranging between 25.55 and 35.30, reported by S u m a n u et al. [33]. The high range of fluctuations in the THI indicates that the birds are more susceptible to the harmful effects of heat stress.

The body weight of birds in the group not administered lycopene was 701.70 ± 12.61 g per bird at day 28. This value was below 873.0 ± 1.0 g per bird at 4 weeks recorded by E g b u n i w e et al. [12] in broiler chickens during the hot-dry season in the zone. The body weight value is also below 783.7 ± 27.2 g per bird reported by S u m a n u et al. [34]. The decrease in body weight may be due to the effects of heat stress which is known to impair productivity in broiler chickens K h a n et al. [17]. In the present study, lycopene administration did not exert any significant beneficial or adverse effects on the body weight of broiler chickens. Lycopene supplementation at 200–400 mg.kg^{-1} of diet has been shown to provide optimal protection in broiler chickens [26, 27]. In the present study, lycopene was administered at 10 mg.kg^{-1} per day based on our earlier study indicating its beneficial effects in apparently healthy Wistar rats [23]. The value was lower than

40 mg.kg^{-1} used by Sun et al. [35], who demonstrated the beneficial effects of lycopene on chick birth weight. The findings indicate that administering 10 mg.kg^{-1} of supplemental lycopene for 28 days may not improve the body weight of broiler chickens. This agrees, in part, with that of Wang et al. [39] who did not observe any significant effect of 10 mg.kg^{-1} of lycopene on the body weight of broiler chickens fed graded levels of dietary lycopene from days 1–21.

Previous reports suggest that such high DBT, RH and THI values may cause increased mortality in chickens [4]. Thus, higher mortality was recorded in the broiler chickens that were not administered lycopene and percentage mortality was lower in the broiler chickens administered 10 mg.kg^{-1} of supplemental lycopene. Although this study was conducted under hot-dry conditions, the result is in agreement with Fathi et al. [13] who observed that lycopene supplementation alleviates adverse effects of cold stress on mortality in broiler chickens fed diets supplemented with lycopene, and attributed it to the ability of lycopene to enhance antioxidant enzyme activities. The beneficial effects of low levels (10 mg.kg^{-1}) of lycopene administration on the mortality of broiler chickens exposed to heat stress require further investigation in a large sample size.

The distinct and similar pattern of daily fluctuations of the cloacal temperature responses observed in both control and lycopene groups indicates that the temperature rhythm of the broiler chickens was maintained for normal physiological processes [9, 22]. Thus, the cloacal temperatures recorded are within the normal reference range of 40.0 to 42.0 °C, established for poultry [21, 31]. This finding suggests that the *Arbor acres* chickens have adapted to the hot-dry season in the zone. The cloacal temperatures were decreased at 13:00 h and increased at 7:00 h and 19:00 h in birds administered lycopene. These findings suggest that lycopene may be stabilizing the body temperature responses by modulating the variation through an increase in the morning and evening and decreasing temperature in the afternoon period. According to Ayo et al. [5], lycopene reduces cloacal temperature responses in broiler chickens, particularly at 12:00 h and 15:00 h. Also, decreased range of fluctuation in cloacal temperature was recorded in broiler chickens administered lycopene. Egbuniwe et al. [11] reported that the wider the range of fluctuations, the more heat stress occurs and the greater the increased chances of negative impacts. By decreasing the range of fluctuations, lycopene may, thus, mitigate the effects of heat stress on the thermoregulatory mechanisms required to maintain homeothermy.

The result shows that lycopene administration reduced the effects of heat stress in broiler chickens as evidenced by decreased H:L ratio. This was due to an increase in heterophil counts and a reduction in lymphocyte counts, causing an increase in the H:L ratio [36]. The findings agree with the report of Hosseini–Vashan [16] who recorded a decreased H:L ratio in broiler chickens fed diets supplemented with dried tomato pomace. H:L ratio is a well-known stress indicator for birds and has been known to rise in response to heat stress [6]. Depressed immune system has been associated with elevated H:L ratio [28]. The decreased H:L ratio observed in the present study suggests that lycopene may ameliorate the damaging effects of heat stress on broiler chickens' immune systems. The decreased H:L ratio seen in the present study implies that lycopene might mitigate the damaging effects of heat stress on the broiler chickens' immune systems.

Because their membranes contain high contents of unsaturated fatty acids, erythrocytes are particularly vulnerable to the damaging effects of heat stress [1, 8]. Lycopene administration significantly enhanced the integrity of the erythrocyte membrane of the broiler chickens by decreasing the percentage haemolysis. The effects of lycopene may be reducing lipoperoxidation of erythrocyte membranes via scavenging of ROS *in-vivo.* The findings of the present study are in line with the observation that increased antioxidant activity in broiler chicken erythrocytes decreased lipid peroxidation and cell membrane fragility [31]. It suggests that the antioxidant effects of lycopene may enhance erythrocyte membrane integrity by decreasing the effects of ROS on erythrocyte membranes [15]. Thus, lycopene exerts an ameliorative effect as indicated by its beneficial role in reducing the impact effects of heat stress on erythrocytes membrane fragility.

CONCLUSIONS

In conclusion, lycopene administration did not exert any significant effects on the body weights of broiler chickens. It reduced mortality and mitigates the impact of exposure to heat stress on cloacal temperature and dai-

ly fluctuations. Lycopene ameliorated the effects of heat stress by decreasing the H:L ratio and enhancing erythrocyte membrane integrity. Thus, lycopene administration reduced mortality and improved cloacal temperature and haematological responses without exerting any significant beneficial or negative effects on body weight in broiler chickens.

CONFLICT OF INTEREST

None.

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VAIRIMORPHA APIS VERSUS *VAIRIMORPHA CERANAE*, REPLACEMENT OR DYNAMIC PREVALENCE?

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ABSTRACT

Nosematosis is currently a frequently discussed disease of bees caused by two species of microsporidia: *Vairimorpha apis,* and *Vairimorpha ceranae*. *Vairimorpha apis* represents the original species of *Apis mellifera* parasite, and *Vairimorpha ceranae,* is a species introduced from Asia. In the last two decades, epidemiological data on the growth of the prevalence of *V. ceranae* infection have increased, which has led to its dominant position at the expense of its congener *V. apis,* practically all over the world. This process is assumed to be the result of asymmetric competition within the host, where *V. ceranae,* showed a better ability to adapt to higher temperatures, which was first manifested by its spread in warmer climate zones. However, several results of studies from recent years somewhat unexpectedly showed that it is premature to talk about the complete replacement of *V. apis* by *V. ceranae*. They pointed to a greater influence of seasonality and average temperatures in winter and summer in a given year on the result of the current prevalence of infection and co-infection of *Vairimorpha* spp., regardless of the climatic zone in which the colonies were located. Considering the different clinical and subclinical manifestations of infection caused by *V. apis,* and *V. ceranae,* and its impact on the defense, survival and productivity of bee colonies, the aim of our work was to analyze the factors affecting the distribution and prevalence of *Vairimorpha* spp.

Key words: nosematosis; prevalence of infection; *Vairimorpha apis*; *Vairimorpha ceranae*

INTRODUCTION

Honey bees (*Apis mellifera*) are one of the most important animal species for human nutrition, as they pollinate up to 78 % of flowering plants on Earth and approximately 70 % of the main agricultural crops. Unlike other pollinators, honey bees do not specialize in certain types of plants, they fly to a distance of approx. 4.5 km from the hive, so one bee colony is able to pollinate an area of up to 6360 ha [64] . Honey bees (*Apis mellifera*) pollinate 9.5 % of agricultural crops intended for human consumption [48], and their economic importance also lies in the production of honey and other bee products, and the contribution of bees to the preservation of biodiversity is also significant. In the last 50 years, the volume of cultivation of crops dependent

on bee pollination has tripled, which places demands on the number of honey bee colonies [48, 26].

According to FAO estimates, there were 22.5 million bee colonies in Europe in 1990. By the turn of the millennium, their number had fallen. Although populations are recovering, in 2021 there were approximately 25.1 million bee colonies. Beekeeping and apiculture have also increased significantly worldwide in the recent two decades. The FAO estimated that the number of bee colonies worldwide to be around 101.6 million in 2021. Compared to 1990, this corresponded to an increase of 47 %. Most bee colonies in 2021 were in Asia (45.3 million). Despite the global increase in the number of bee colonies, fluctuations were recorded in certain periods or regions, such as Eastern Europe, where there was a decrease of 24.9 % in the given period [17].

COLOSS (Prevention of Bee COlony LOSSes) monitors honey bee colony losses on an annual basis, and the last published data from the winter of 2016/2017 showed a total loss of honey bee colonies in 30 countries of the world at 20.9 %, which was an alarming figure [10].

The causes of colony losses can be divided into biotic and abiotic [4]. Important abiotic factors include: uncontrolled or excessive use of pesticides, global climate change, loss of food habitats and deficiency of food for bees [1, 2, 28]. Biotic factors include various types of bacteria, viruses, arthropoda and protozoa [18, 20], Fig. 1 [50]. Collapses rarely have only one cause, they are usually a combination of biotic and abiotic factors, and often a synergistic effect is also observed [52].

The parasitic microsporidia *Vairimorpha apis* (formerly *Nosema apis)* [76] and *Vairimorpha ceranae* (formerly *Nosema ceranae)* [32] responsible for nosemosis in *Apis* spp. represent the second most widespread biotic factor related to the decline of bees [52, 72].

Microsporidia, as is a group of highly divergent fungi, that includes obligate, intracellular, spore-forming parasites, are unable thus posing a threat to generate ATP by oxidative phosphorylation, as instead of mitochondria, they have a simplified form – a mitosome. In the reason for course of evolution, their strong genomes, and proteomes, have been reduced, resulting in metabolic dependence on the hosts, which is also the key to their pathogenicity [8, 69].

From the point of view of taxonomy, *Vairimorpha* spp. among the lower eukaryotes within the kingdom fungi [8, 49], and recently, based on their molecular characteristics,

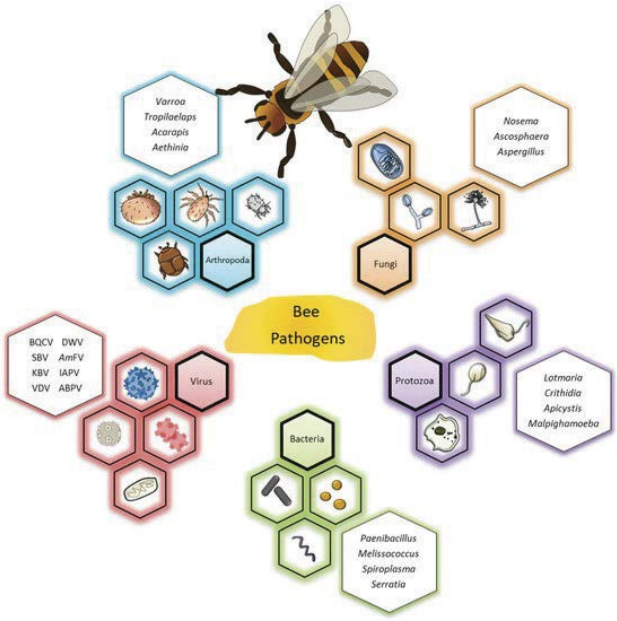

Fig. 1. Main bee pathogens. Schematic representation of the main pathogens affecting *Apis mellifera*, belonging to the arthropoda, fungi, protozoa, bacteria, and virus taxonomic groups [50].

Taxonomy of *Vairimorpha* spp.

their reclassification into *Vairimorpha* spp. was proposed [72]. Historically, microsporidia have had a taxonomy based on unique morphological characters, typical intracellular life cycles, host range, and tissue tropism. These features remain important, but molecular and genomic methods provide much new evidence for revision of microsporidia systematics, species identification, and broader phylogenetic placement [8].

The NCBI taxonomy database currently shows the following lineage for *Vairimorpha apis* and *Vairimorpha ceranae* species: superkingdom: eukaryote; clade: opisthokonta; kingdom: fungi (*fungi incertae sedis*); phylum: microsporidia; suborder: apansporoblastina; family: Nosematidae; genus: *Vairimorpha* (formerly *Nosema*).

Molecular phylogenies based on a single small subunit (SSU) ribosomal RNA gene are not always able to resolve deeper relationships with microsporidia, so it is suggested that future taxonomic definitions of this phylum include additional markers (LSU, ITS) [72].

Distribution and characteristic *Vairimorpha* spp.

Vairimorpha apis has long been known as a pathogen of honey bees (*Apis mellifera*) [29, 47] and was first officially described in 1909 [76], while its congener *V. ceranae* was known as a microsporidian parasite of the honey bee *Apis ceranae* [32], native to South and Southeast Asia.

Vairimorpha neumanni is a newly discovered species of parasite of honey bees (*A. mellifera*), based on molecular characteristics it is close to *V. apis* (97 % similarity) and was first detected in 2016 in Uganda and later it was also discovered in Japan [12].

Vairimorpha ceranae was officially identified as a pathogen in Eastern Europe in 1996 in a diagnosed honey bee [32], and in the early 2000s the pathogen was also found in Western European honey bees (*Apis mellifera*) [41, 44].

After the introduction of the molecular differentiation method [32], working groups around the world, initially focused on the detection of *V. ceranae* in *Apis ceranae*, proved the presence of *V. ceranae* in *Apis mellifera* as well [14, 74]. Retrospective works using the PCR method indicate the existence of *V. ceranae* in the honey bee even before its official discovery of *A. mellifera* infection with this pathogen [27, 60].

The primary factor in the global spread of the pathogen is the globalization of trade in honey bees and bee products [38].

Regardless of the cause of distribution, *V. ceranae* is one of the most common pathogens detected in honey bees [39] and is globally recognized as one of the most common pathogens of intestinal infections in adult honey bees [19, 32]. The huge incidence of *V. ceranae* recorded especially in the USA, while the reason why it became the dominant parasite in the USA remains controversial [14].

It has become a dominant species in Europe as well, and its occurrence in southern countries such as Spain, Italy and Greece is currently even described as the displacement of *V. apis* by *V. ceranae* [30, 53, 57].

This evolution has been interpreted, as a north-south gradient, due to the observed spread of the pathogen in Europe [32], so that this representation does not appear in countries with milder climates [35].

This is also why the influence of climate on the infectious process and prevalence of the pathogen is discussed, especially because experimental results show that *V. ceranae,* is sensitive to cold, compared to *V. apis,* and at the same time reproduces better at higher temperatures [35, 54].

The assumption of clear differences between these two pathogens is also supported by reports from Spain, after which, instead of the seasonality known for the occurrence of *V. apis* infections, a high incidence of spring *V. ceranae* infection could persist from late summer to the following summer [41, 55]. Therefore, the lack of "seasonality" is defined as one of the main characteristics of *V. ceranae* which is more pronounced for countries in warm climate zones.

Epidemiology and life cycle *Vairimorpha* spp.

In the case of nosemosis, the infectious stage and at the same time the only stage that can survive outside the host are spores. Spores contain sporoplasm and an infectious apparatus that injects the sporoplasm into the host cell of the intestinal. Further, several intracellular cycles of asexual reproduction take place inside the host cell, leading to the formation of primarily autoinfectious and secondarily environmental spores, which are released into the intestinal lumen, where enterocyte lysis and cell degeneration occur [32, 36, 40, 50].

The first (primary) spore attacks other intestinal cells of the intestine, while these (secondary enviromental spore) are released into the environment together with feces and spread the infection. Ingestion of food contaminated with spores, trophallaxis and grooming represent the most common ways of transmission of spores [35]. A sexual route of infection was also described, as spores were found in the sperm of drones [62]. It was also proven that SHB (Small Hive Beetle/*Aethina tumida*) can be a biological vector [16].

The intracellular life cycle lasts an average of 72 hours [35]. The developmental course of *V. apis* and *V. ceranae* is generally considered to be similar [15, 32, 40], Fig. 2 [56].

The result of damage to the intestinal epithelium is an immune response in the form of an inflammatory reaction [11].

This, combined with environmental stress and the presence of other pathogens, can weaken bees and, in the long run, the entire hive [38].

A. mellifera infection by *V. apis* is characterized by diarrhea and high winter mortality, while *V. ceranae* infections may progress in a subclinical form. The absence of clinical manifestations prevents early diagnosis and is often associated with high mortality, usually in autumn and spring [33].

Type A nosemosis caused by *V. apis* is opportunistic and often affects already weakened colonies. This disease is often associated with prolonged adverse weather during which bees cannot fly and defecate outside the hive, and

also hibernation and practices that lead to closing the hive [6].

Type C nosemosis caused by *V. ceranae* can be asymptomatic or cause severe damage to bees leading to depopulation [42, 55], often showing a seasonal pattern directly related to increased temperature [37, 43]. *V. ceranae* mainly infects worker bees, can cause high mortality in older bees, which induces early maturation of young bees and consequently causes imbalance in the hive [37, 42, 43].

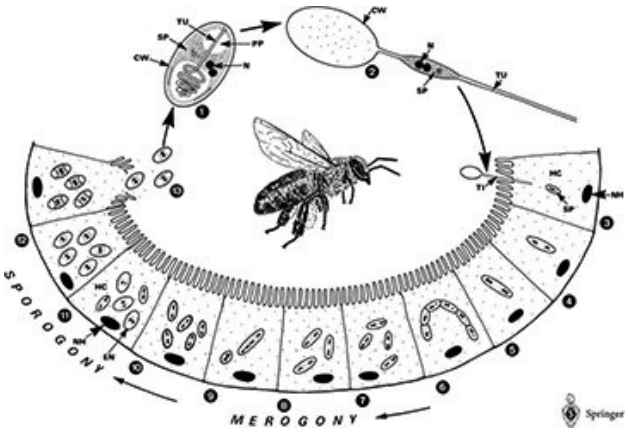

Fig. 2. Life cycle of *Nosema apis*
1 – Infectious spore with a typically binucleate sporoplasm; Bees become infected by ingesting the spores. 2–3 – With the germination of the spore in the intestinal lumen, the infectious apparatus is everted to then close the host cell perforate; the sporoplasm (SP) is injected throughthe tubule into the epithelial cell. 4–12 – Sporoplasm initially divides asexually in the host cell through four-nucleated stages (merogony);this is followed by final division and encystation (sporogony). 13 – release of *Nosema apis* mature spore from lysed host cells into the intestinal lumen. CW – spore wall; EN – encystation; HC – host cell; N – nucleus; NH – host cell nucleus (intestinal epithelial cell); PP – polar plastic; SP – sporoplasm; TI – tubule (injected); TU – tubule *Springer Life Sciences* [56].

Infection with *Vairimorpha* spp.

V. ceranae usually has a asymptomatic course, whereas *N.apis* in its typical form causes dysentery and its presence is visibly manifested by burnt frames, combs, and the outer walls of the hive [33].

The course of the infection depends on the infection load and the treatment threshold is considered to be 10^6 per bee, which is usually associated with serious infections and leads to a significant decrease in the health of the bee colony [24]. *In vitro* it could be determined that a dose of about 10^2 or 10^4 viable *Vairimorpha* spp. spores is necessary, to infect 50 % or 100 % of the honey bee workers in an infection test infect [29].

Spore quantification can be facilitated by a portable device that works on the principle of fluorescence, detects chitin-containing spores, which can detect heavy infections (> 0.5×10^6 spores per bee) caused by *Vairimorpha* spp. in a bee colony [66].

Honey bee colony infected with *V. ceranae* go through a long so-called incubation phase, when there are no clinical symptoms in the colony, as the queen produces eggs, which can compensate for the loss of workers. Colony collapse occurs when more than 80 % of bees are infected with more than 10^6 spores per bee [42].

When comparing species, the decrease in the number of spores produced appears to be generally higher in *V. ceranae* infection than in *V. apis* infection [60, 75], which is reflected in for *V. ceranae* compared to *V. apis* higher, the so-called "biotic potential" (maximum ability of pathogen reproduction under optimal environmental conditions) [54].

However, this aforementioned compensatory mechanism shortens the total lifespan of adult honey bees [63] the time during which they are effective as foragers and also shortens the time each honey bee devotes to colony growth and brood care, as younger bees start foraging earlier in the effort replace the loss of older bees [3].

This leads to a modification of the entire work profile of the colony [73] and when the colony reaches a point where it cannot maintain brood production to compensate for the loss of adult bees, the rate of colony decline accelerates, leading to depopulation [46], which represents the only clear sign of infection described for *V. ceranae* [9, 42].

Changes at the level of endocrine regulatory mechanisms were also observed in colonies infected with *Vairimorpha* spp. An increased level of juvenile hormone has been observed, resulting in accelerated development of honey bees [7, 43], inhibition of gene expression responsible for the production of vitellogenin participating in the immunity of bees [5] and also an increased level of the pheromone ethyl oleate, which delays the maturation of young bees [22].

Changes at the level of endocrinology in infected insects have previously been described as an adaptive mechanism that serves to increase the reproductive capacity of the parasite in an attempt to maximize spore yield [21].

These factors are involved in immunity, the regulation of the division of labor between honey bees, the process of maturation and the transition between the nurse and the feeder, which significantly disrupts homeostasis in the bee

colony and causes significant changes in their biology at the cellular, tissue, organismal, or colony levels [65].

Quantitative and qualitative investigation methods

Recommended laboratory investigation methods for the quantitative and qualitative analysis of Nosematosis are summarized in the Terrestrial Manual of the OIE, 2018 [70].

Methods designed for spore quantification provide an approximate idea of the severity of *Vairimorpha* spp. infection, which helps in deciding the need for treatment. However, these microscopic methods cannot reliably distinguish between the species, as the spores of *V. apis* and *V. ceranae* are minimally different from each other in size and shape [70].

Molecular methods are more sensitive and species specific and can have a high detection sensitivity in the range of 10 to 100 spores per bee for simple PCR and around 1 spore per bee for qPCR or LAMP [25, 51]. Molecular detection of *Vairimorpha* spp. is based on simplex and multiplex PCR, qPCR, RFLP PCR and LAMP, with duplex formats being preferred over uniplex PCR or qPCR protocols. Duplex PCR methods are preferred in cases where a mixed infection is assumed or where it is necessary to distinguish between *V. ceranae* and *V. apis* [34].

Seasonality and prevalence factors

The seasonal course of *V. apis* infection in *A. mellifera* is relatively persistent and is characterized by a low infection rate during the warm summer months, short fluctuations in infection in the autumn, observations show a steady increase in the infection rate during the winter months, with a peak in the spring [31]. In the *V. ceranae* species, the seasonality is not as pronounced as in *V. apis*, the problem is often the absence of symptoms of infection, with the exception of increased mortality during the winter. Severe symptoms have been reported mainly in the south, rarely in temperate climates [53].

However, from the amount of available data, it is clear that the prevalence of *V. apis* infections in different countries is rapidly decreasing in favor of its congener *N. ceranae* [24, 47, 60] and that it even becomes a dominant species in many regions [13, 14, 74]. Based on the amount of epidemiological data, it could appear that *V. ceranae* replaces *N. apis* in honey bee populations worldwide.

This process has been suggested to be caused by asymmetric intra-host competition between *V. apis* and *V. ceranae*, which favors the spread of *V. ceranae* [58, 75], but not all studies have supported this claim [29, 57] and we will try in the next part, based on the available data, to analyze the possible reasons why it happened. Temperature appears to be a key factor influencing the geographic distribution of *Vairimorpha* spp. in different climate zones and the seasonal prevalence of infection.

It was reported that in countries located in warmer climate zones, such as southern European countries (Italy, Spain, Greece), *V. ceranae* practically replaced *V. apis*, while in northern Europe (Ireland, Sweden, Norway and Germany) this was not observed [47], which pointed to climatic conditions as a key factor influencing the establishment, spread and prevalence of *V. apis* and *V. ceranae*. However, the issue will probably be more complicated and it is necessary to approach it more comprehensively, and when trying to objectively evaluate the situation, take into account several factors, such as, for example, average winter and summer temperatures, the frequency of collecting samples, the method of their examination, etc.

The results of experimental studies and also the results of long-term, multi-year monitoring of *Vairimorpha* spp. infection in honey bee colonies within larger territorial units can be the most helpful in understanding the rules affecting the geographical distribution and seasonal prevalence of *Vairimorpha* spp. infection. In our work, we tried to summarize and select the knowledge that we consider the most important from the point of view of the distribution and prevalence of *Vairimorpha* spp., with the aim of creating some space for discussion and further research in this area.

Experimental infection of adult bees showed that the proliferation of *V. ceranae*, unlike *V. apis*, is not affected by temperatures above 33°C [54]. G i s d e r et al. [37] also conducted cell culture experiments that demonstrated that *V. ceranae* has a higher ability to multiply than *V. apis* at 27 °C and 33 °C, which potentially explains the increase in prevalence of *V. ceranae* during summer. These data provide clear evidence of a competitive advantage of *V. ceranae* over *V. apis* in warmer climate zones.

Other experiments have shown that *V. ceranae* spores, but not *V. apis* spores, almost lose their ability to germinate and thus their infectivity when exposed to temperatures near or below freezing [30, 35]. Nevertheless, there is evidence of the spread of *V. ceranae* in the region of Western Siberia with average January temperatures of -18

°C [71], where the dominance of the infection prevalence of *V. ceranae* over *V. apis* was even found in the Tyumen region. Similar results were found in Canada with comparable average January temperatures [23]. In a recent study in Switzerland, it was demonstrated that low ambient temperatures favor microsporidia infection of honey bees [61].

Experiments focused on co-infection of bees in a cage with simultaneous feeding of *V. apis* and *V. ceranae* spores did not provide evidence of intraspecific competition between these two species within the host [29, 57].

In contrast, sequential spore feeding of both species led to competition within the host, the former *Vairimorpha* spp. inhibited the growth of the latter regardless of which species' spores were used to infect first. This should prevent the spread of *V. ceranae* as *V. apis* was present in the bee population before the arrival of *V. ceranae*. However, this effect was shown to be asymmetric and *V. ceranae* showed a stronger inhibitory effect on *V. apis* than *V. apis* on *V. ceranae* [39, 58].

Interesting results were obtained by Gisder et al. [37], who followed 230 honey bee colonies from 23 apiaries over 12 years in a longitudinal cohort study on the prevalence of *V. apis* and *V. ceranae* in northeastern Germany between 2005 and 2016.

Samples were collected twice a year (spring and fall), resulting in a total of 5,600 bee samples that were subjected to microscopic and molecular analysis to determine the presence of *V. apis* and/or *V. ceranae* infections.Analysis of data on the prevalence of infection with *Vairimorpha* spp. showed a significant increase in *V. ceranae* infections over the past 12 years in samples collected in autumn (reflecting summer development) and spring (reflecting winter development). Assuming the gradual replacement of *V. apis* by *V. ceranae* at the population level, it was therefore expected that the prevalence of *V. apis* infection should have decreased simultaneously during the study period.

However, a significant decrease in the prevalence of *V. apis* infections was observed only in autumn, and the results speak against the fundamental displacement of the originally endemic pathogen from northeastern Germany. Moreover, the long-term stability of *V. apis* infection frequency in the spring suggests that any mechanisms acting on *V. apis* during the summer and causing its decline in colony populations are compensated for and reversed during the winter. It was found that there is a balanced ratio of prevalences for both species in the spring period, and in the long-term study, mixed infections in the colonies were found surprisingly often, indicating that there is no interspecies competition at the colony or population level during the wintering of the host. Rather, coinfection levels suggested infection with either of the two microsporidia already existing in the colony, favoring further infection of the colony by a second *Vairimorpha*.

These data do not support a general advantage of *V. ceranae* over *V. apis*, and the total replacement of *Vairimorpha apis* by *Vairimorpha ceranae* in the investigated honey bee population was not confirmed in the climatic conditions of northeastern Germany [37].

Similar conclusions were reached by the authors of a study where the prevalence of *Vairimorpha* spp. in Turkey was monitored for 7 years, despite the fact that this region is located in a warmer climate zone. They found that the number of *V. ceranae* spores significantly decreases at low temperatures (≤ 5 °C), however, statistical analyzes also revealed that there is also a significant relationship between warm weather conditions in winter and high spore levels and high prevalence of *V. ceranae* infection in bee colonies.

The level of co-infection was lowest during the first two years of observation (2009–2010), when average winter temperatures were highest (5.3 °C and 6.5 °C), and at the same time the highest level of *V. ceranae* infection was observed. In the following 5 years (2011 to 2016), the average winter temperatures were lower, ranging between 3.1 °C and 4.3 °C (never exceeding 5 °C), which was reflected by an increase in the level of co-infection (*V. apis* + *V. ceranae*) and at the same time a significant decrease in the infection by *V. ceranae* itself compared to the first two years [59].

They found that the winter season is the main factor influencing the level of *Vairimorpha* infection and the dominance of *V. ceranae* and also that winter temperatures are a significant indicator of co-infection of both species. This study suggests that there is no replacement of *V. apis* by *V. ceranae* under the conditions of Turkey, but there is a dynamic prevalence among *Vairimorpha* species depending on the average winter temperature [59].

In Slovakia, the species *V. ceranae* were confirmed for the first time in 2008 [68] and in 2009–2010 a study was carried out, the aim of which was to monitor the prevalence of mono-infection and co-infection of both species *V. apis* and *V. ceranae* in Slovakia using the polymerase

chain reaction (PCR). Already in 2010, mono-infection with the *V. apis* species was not recorded, and either co-infection of both species or mono-infection of *V. ceranae* prevailed [67].

Thus, a significant increase in the prevalence of *V. ceranae* and a decrease in *V. apis* were confirmed, and this trend was also shown by the results of the examinations of bee colonies, which we carried out 10 years later, in 2020 and 2021, where in none of the examined samples, using PCR examination, we did not detect the presence of *V. apis* [45]. However, this does not mean that this species is completely replaced and displaced by *V. ceranae*. For an objective evaluation of the current epidemiological situation, it would be necessary to examine a larger number of samples and take them twice a year, in spring and autumn. The samples came from bee colonies from queen breeders, they were taken in the spring, all regions of Slovakia were equally represented there, and the examination was carried out on the basis of mandatory periodic control of colonies for the presence of *Vairimorpha* spp. Sampling in the spring showed the development of *Vairimorpha* spp. in the winter period, however, there is a lack of data on the development of the prevalence in the summer, which would be shown during the collection of samples in the fall.

However, these results indicate that the prevalence of *Vairimorpha* spp. in Slovakia is closer to the situation in southern European countries.

CONCLUSIONS

As it turns out, the clarification of the shift in the prevalence of infection in favor of *V. ceranae* will require a more accurate data analysis and further long-term monitoring at the level of the population of bee colonies in different geographical areas. A number of findings obtained from experimental trials cannot be easily extrapolated to the situation at the population level, which was also shown by the results of several long-term observations in different climatic conditions. Factors affecting interspecific competition were experimentally explained mainly at the level of individual bees, while the obtained epidemiological data relate to bee colonies and populations. The concept of intrahost interspecific competition in obligate intracellular parasites such as *V. apis* and *V. ceranae* due to competition for a limited energy resource cannot be easily transferred to the colony level, where a limited resource (in the case of a colony, new hosts) is not yet a problem.

The replacement of *V. apis* by *V. ceranae* at the colony level during the summer, but not during the winter period, points to different mechanisms acting or affecting these two microsporidian parasites and means that there is a variable prevalence between the two species depending on the average winter temperature. The exact mechanisms responsible for replacement at the population level are still not fully explained, but it is clear that climatic factors play an important role. So "replacement" may not be the right term to describe the change in the distribution of *V. apis* and *V. ceranae* in the world, the term "dynamic prevalence" better describes the situation.

Another point of interest when evaluating the results of the studies is the fact that on a purely molecular basis it is not possible to distinguish true infection from contamination, there is no correlation between a positive PCR test result and the presence of infection.

Regarding *Vairimorpha* spp. only a few spores of the pathogen may result in a positive molecular finding, but this evidence is of little or no significance. In principle, only the combination of clinical and laboratory diagnostic findings with expertise in pathogenesis can reduce the likelihood of confusion and minimize incorrect results in interpretation and diagnosis. The aforementioned inconsistencies between studies emphasize the need for standardized protocols for collection, examination of samples and evaluation of subclinical and clinical manifestations of infection. When evaluating the results of the study, the infection of *Vairimorpha* spp. it is necessary to take into account, in addition to climatic factors, above all the sublethal effects of insecticides, the synergistic effect of infection with other pathogens, nutritional conditions, different beekeeping techniques, and possibly also genetic differences. This comprehensive view of the problem of *Vairimorpha* spp. infections can significantly contribute to its control and the design of the necessary measures.

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EFFECTS OF INTRAVENOUS GLUCOSE ON BLOOD POTASSIUM IN CATTLE

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ABSTRACT

The aim of this study was to confirm whether a rapid intravenous glucose administration can lead to a significant drop in blood potassium levels in cattle. For this study, seven cattle were used according to internationally recognized guidelines for animal welfare. Glucose at a dose of 1 g.kg^{-1} body weight was administrated intravenously and then blood samples were taken before and 0.5, 1, 2, 4, and 6 hours after administration of glucose for measurement of potassium and glucose blood concentrations. For statistical analysis of glucose effects on both glucose and potassium levels one-way analysis of variance (ANOVA) for repeated measures was used. ANOVA showed a strong significant effect of the glucose administration on plasma glucose levels ($P < 0.001$). The glucose administration resulted in a mean plasma increase from 4.2 mmol.l^{-1} to 21.2 mmol.l^{-1} within the first minutes after the administration followed by a steady decrease back to the initial values. In contrast, ANOVA showed no significant effect of the glucose administration on plasma potassium levels. In conclusion, the glucose does not have a potassium-lowering effect when administered in a single large intravenous dose.

Key words: cattle; glucose; potassium

INTRODUCTION

Potassium is recognized as an essential nutrient in animal nutrition. It is the third most abundant mineral element in the animal body and the main ion of the intracellular fluid. Most of the total potassium in the body is located in the muscle tissue [6]. The most significant role of potassium is in relation to nerve innervation and muscle excitability, and it is also involved in carbohydrate metabolism [17]. In monogastric animals, higher secretion rates of insulin after a meal largely prevent food-induced increases, not only in plasma glucose but also in plasma K^+ concentrations, because both are taken up by the skeletal muscle cells under the influence of insulin. Cellular K^+ uptake into skeletal muscle cells was stimulated via an insulin-dependent increase in Na/K pump activity [4]. A rise in blood glucose concentration in response to intravenous glucose infusion results in a commensurate release of insulin from the pancreas, which acts to clear glucose by insulin-sensitive tissues to pre-infusion levels. The clearance rate of glucose from blood depends on non-insulin-dependent glucose clearance, the amount of insulin released from the pancreas, and the peripheral response to insulin [8]. The glucose requirements of the dairy cow are dominated by the necessity of the mammary gland for milk synthesis. With greater milk yield comes larger re-

quirements for glucose, which is mainly met through glucose synthesis in the liver [19]. An interesting relationship between hypokalaemia, insulin imbalances and hyperketonaemia was found in high-yielding dairy cows whereby changes in glucose homeostasis and hypokalaemia might, according to the researchers, be a contributing factor to the pathogenesis of abomasal displacement resulting from a decrease in abomasal tone [22]. On the contrary, the glucose administration can be also used in hyperkalaemic conditions in animals. Electrolyte imbalances such as hyperkalaemia are also common in diarrheic calves and can result in skeletal muscle weakness and life-threatening cardiac conduction abnormalities and arrhythmias [20]. It is well known that restoration of potassium homeostasis in diarrhoeic acidemic calves can be achieved by rehydration and alkalinization using intravenous solutions containing sodium bicarbonate [13]. However, a combined administration of insulin and glucose is a well-established treatment for hyperkalaemia [23]. It was hypothesized that intravenous administration of a glucose-containing solution induces endogenous insulin release and thereby exerts a potassium-lowering effect. This might be especially of relevance in the initial treatment of affected calves, where treatment objectives focus on the rapid correction of hyperkalaemia, hypoglycaemia, and profound acidemia [5]. This research question is also of interest because administration of glucose containing infusion solutions to diarrheic calves that require intravenous fluid therapy is popular in ambulatory field practice. Intravenous glucose is administered to counteract negative energy balance and provide a readily utilizable energy source when calves are housed in cold ambient conditions [1]. Although the administration of intravenous dextrose is among the most commonly recommended treatments for hyperketonaemia in postpartum dairy cattle, the evidence for its use and a comparison of its respective metabolic consequences alone or in combination is largely unstudied [15].

The purpose of this study was to confirm whether a rapid intravenous glucose administration can lead to a significant drop in blood potassium levels in cattle.

MATERIALS AND METHODS

The group of the experimental animals consisted of five heifers and two Holstein-Friesian dairy cows which were admitted to the Clinic for treatment of various health disorders. They were clinically healthy at the time of the study. Blood was collected by venepuncture of the jugular vein before, and 0.5, 1, 2, 4, and 6 hours after the administration of glucose. Each animal was given one gram of glucose per kg body weight, which resulted in 1000 ml of 40 % glucose solution for a 400 kg cow on maximum infusion speed. The average time of administration was 25 minutes, approximately. The plasma concentrations of glucose were assayed with the kits supplied by Randox Laboratories Ltd. on spectrophotometer Alizé (Lisabio, France). The serum potassium concentrations were determined by the flame AAS method (Perkin Elmer Analyst 100).

For statistical analysis of glucose effects on both glucose and potassium levels, one-way analysis of variance (ANOVA) for repeated measures was used.

Ethical statement

All procedures concerning the animals were performed in compliance with the national guidelines for animal care.

RESULTS

The glucose administration resulted in a mean plasma increase from 4.2 mmol.l^{-1} to 21.2 mmol.l^{-1} within the first minutes after the administration. From the peak in plasma glucose at 30 minutes, there was a steady decrease in the plasma glucose concentration to 3.36 mmol.l^{-1}. ANOVA showed a strong significant effect of the glucose administration on plasma glucose levels ($P < 0.001$). The serum potassium concentrations tended to decrease within the first hour after the glucose injection and then there was a trend towards a steady increase in potassium serum concentration. ANOVA showed no significant effect of the glucose administration on serum potassium levels (Table 1).

Table 1. Mean plasma glucose and serum K (x ± SD) before and after glucose administration

Collection time (h)	Glucose (mmol.l^{-1})	Potassium (mmol.l^{-1})
0	4.22 ± 0.59	3.56 ± 0.74
0.5	21.2 ± 5.02	3.41± 0.74
1	15.4 ± 3.97	3.30 ± 0.75
2	8.41 ± 3.05	3.52 ± 0.83
4	3.83 ± 0.66	4.03 ± 0.74
6	3.36 ± 1.39	3.75 ± 0.96
ANOVA	P < 0.0001	P > 0.05

DISCUSSION

Potassium is one of the major elements and has an important role together with other elements such as sodium, chlorine and bicarbonate ions. Together they control the acid-base balance and the osmotic regulation of fluids in the body [17] as serum potassium serves as a regulator of aldosterone secretion in which high serum levels of potassium increases plasma aldosterone levels [2]. The most abundant role of potassium is in relation to nerve and muscle excitability, and is also involved in carbohydrate metabolism [17]. Potassium deficiency is rather rare in farm animals under normal conditions, due to a high concentration of potassium in plants, for example, 25 $g.kg^{-1}$ DM in grass. Symptoms of deficiency in calves given milk replacement low in potassium includes severe paralysis [17]. In general, hypokalaemia will increase the membrane potential, resulting in a hyper-polarization block causing weakness of muscles or paralysis, ileus, cardiac arrhythmias, rhabdomyolysis and renal dysfunction [7, 14]. Hypokalaemia is commonly the result of gastrointestinal losses from either diarrhoea or vomiting or excessive renal losses due to diuretics, overload of mineral-corticoids or renal tubular acidosis [14] but also from excessive rapid bicarbonate administration, insulin with glucose administration or catecholamine release [12]. As glucose is used very frequently in the treatment of many bovine diseases it was essential to test the hypothesis of the *in vitro* study [22], as it was stated that when glucose was administered in a single rapid dose of (500 ml, 40 %) the serum concentration of potassium decreased strongly. It was previously shown that an intravenous bolus of glucose led to an increase in insulin concentrations with a peak approximately 10 min after the administration and to a nadir in NEFA concentrations approximately 45 min after the treatment with a return to baseline concentrations within two hours [16].

There is concern that the standard treatment of 500 mL of 50 % dextrose is excessive and may have detrimental effects, including the causation of electrolyte and mineral imbalances following an intravenous bolus infusion [9]. In particular, the physiological decline in plasma phosphate concentration due to an insulin-dependent intracellular shift is of interest as clinical hypophosphatemia may be associated with muscle weakness and recumbency [10]. Bolus infusion of 500 mL of a 50 % dextrose infusion lead to an average decline in plasma phosphate ranging from 1.1 to 1.5 $mg.dl^{-1}$ within 60 min after bolus infusion in 2 separate studies [11]. A prior investigation has demonstrated that the cellular uptake of glucose, potassium, and phosphorus, when stimulated by insulin, operates independently and is not interrelated. There are specific resistance mechanisms in place that decouple impaired glucose disposal, as seen in diabetic patients, from potassium uptake, and vice versa [18]. In an experimental study involving rats, it was observed that even a brief period of potassium deficiency resulting from a potassium-deficient diet (which led to only a 9 % reduction in plasma potassium concentration) caused an 80% decrease in insulin-induced cellular potassium uptake. In contrast, glucose disposal rates remained unaffected [3]. The results presented in this study did not reveal a serum potassium-lowering effect of intravenous glucose administration. However, most of the experimental animals had the serum potassium levels slightly below the physiological range (4.0–5.5 $mmol.l^{-1}$) prior to the glucose administration. The absence of changes in potassium concentrations after treatment with 500 mL of 40 % glucose is consistent with the findings in the study with intravenous administration of 500 ml of 50 % dextrose by Mann et al. [15]. This finding was confirmed in a study on calves where an intravenous bolus of 0.3 g of glucose per kg of BW administered over a period of 1 min resulted in an increase of serum insulin concentrations but did not affect potassium responsiveness [21].

CONCLUSIONS

Based on the results of this study, it can be concluded that a single intravenous administration of the glucose is not associated with a severe risk of metabolic and health impairment in cattle due to the significant drop in blood potassium. Thus, the use of the glucose in treatment of an energy deficiency in cattle can be further recommended.

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DERMATOLOGICAL DISEASES IN DOGS – A SURVEY IN VETERINARY FACILITIES

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ABSTRACT

Skin diseases in dogs are very often the reason for visiting the veterinarian. For the successful management of a dermatological disease, accurate diagnosis, correct setting of the treatment and cooperation of the owner are necessary. This article provides a summary of information related to the diagnosis and treatment of skin diseases in dogs obtained from 50 veterinary facilities in Slovakia. In the monitored veterinary facilities, the most common dermatoses are skin diseases due to immune disorders, followed by bacterial and parasitic skin diseases. For the prevention of external parasites (ticks, fleas), tablets containing fluralaner, sarolaner and afoxolaner are increasingly preferred, even though spot-on preparations, especially those containing fipronil, maintain a constant position in prevention. Among the most commonly used ATBs in the treatment of skin bacterial infections are amoxicillin with clavulanic acid and cephalexin. Currently, the veterinary medicine with the active substance oclacitinib and the medicine containing lokivetmab are coming to the fore in the treatment of allergies in dogs, while veterinarians are trying to limit the use of glucocorticoids.

Key words: dermatologic disease; diagnostics; dog; therapy

INTRODUCTION

The skin is the largest organ system and is often considered an indicator of an animal's health. Skin diseases are not only a health and aesthetic problem, but can significantly worsen the quality of life of the dog and the owner. The basis of successful therapy of dermatological diseases is the correct determination of the diagnosis. Skin diseases are divided into parasitic, bacterial, fungal, viral, immune, hormonal and genetically determined. The most common symptoms of dermatological diseases include pruritus, redness, scaling of the skin and alopecia [13, 16]. The clinical symptoms of many skin diseases are similar to each other, and the diagnosis is often complicated. It depends on the practical skills and experience of the veterinarian, but also on the equipment of the veterinary clinic and the possibilities of diagnostic methods [9, 10, 14]. Therapy of skin diseases in dogs can be influenced by various factors, such as age and breed of the dog, type of disease, other systemic or skin disease, wrong diagnosis or wrong therapy, non-cooperation of the owner, etc. In many cases, the treatment is not only time-consuming, but also financially demanding and can last „a dog's whole life" [17].

The aim of this survey was to obtain and present relevant information about skin diseases in dogs, their diagnosis and treatment from veterinary facilities (ambulances, clinics, hospitals) in Slovakia by means of an anonymous questionnaire.

MATERIALS AND METHODS

For the purpose of this study, an anonymous questionnaire intended for obtaining relevant information about skin diseases in dogs was prepared for veterinarians working in veterinary ambulances, clinics and hospitals in Slovakia. The questionnaire consisted of 25 questions (mostly rating and multiple-choice questions). The questions related to the origin of skin diseases, the most common pathogens causing these diseases, the use of medicinal preparations with specific active ingredients, and therapeutic procedures used by veterinarians in the interviewed veterinary facilities.

The questionnaire was sent to 50 veterinary facilities in Slovakia: 29 ambulances; 18 veterinary clinics; and 3 veterinary hospitals. All addressed facilities responded. There responses are summarised in the text and in Tables 1–3 and Figure 1.

RESULTS

Skin diseases

The results of the survey showed that skin diseases represent on average, up to a third of all diseases, with which owners presented their dogs in the respective facilities. Seventeen facilities indicated that the occurrence of skin diseases ranging from 11 to 20 % and 19 facilities in the range of 21–30 %. Eight veterinarians reported dermatological treatment in 31–40 % of all treated cases, and another two up, to over 40 %.

It is interesting that up to 18 veterinarians from the 50 veterinary facilities identified immune skin diseases (allergies, atopy, etc.) as the most common dermatoses. In thirteen facilities bacterial diseases were the most frequent. Parasitic skin diseases rated as third (10 facilities). Viral diseases, skin diseases due to hormonal disorders and skin mycoses were less common.

1. Parasitic skin diseases

Table 1 shows the occurrence of the causative agents of parasitic skin diseases in dogs. Veterinarians had to determine which of the parasitic skin diseases they treat most often in dogs. *Ixodes* spp. and other species of ticks were identified by up to 26 veterinarians as the most common cause of parasitic skin diseases. *Ctenocephalides* spp. is considered the most frequent causative agent by 17 veterinarians. As many as 21 veterinarians identified demodicosis as the third most common parasitosis, but 12 veterinarians rated it as the second most common skin parasitosis (Table 1).

Table 1. Prevalence of causative agents of parasitic skin infections

Type of parasite	1	2	3	4	5
Ctenocephalides spp.	17	13	10	6	4
Ixodes spp. and other species of ticks	26	11	4	4	5
Cheiletiella spp.	5	5	7	15	18
Demodex spp.	1	12	21	12	3
Sarcoptes spp.	4	9	10	16	11

Representatives of parasitic diseases were rated by participants on a rating scale from 1 to 5 (1 – occurs most often; 5 – occurs the least).

Veterinarians from 34 veterinary facilities recommend oral tablets the most from among antiparasitics, namely Bravecto (84 %) with the active substance fluralaner, Simparica tablets (82 %) with the active substance sarolaner and NexGard (64 %) with the active substance afoxolaner. Comfortis tablets are less used (10 %). A high percentage of use is also achieved by spot-on preparations designed for external parasites or combined, suitable for internal parasites at the same time. Spot-on preparations, with the active substance fipronil have long held the lead in the group of spot-on preparations against fleas and ticks, all types of Frontline, Fyprist and Effipro are most often used.

In the group of spot-ons against external and internal parasites, the most widely used is Advocate with the active substance imidacloprid in combination with moxidectin, which is used in 80 % veterinary facilities. The second most used spot-on preparation is Stronghold (selamectin), or Stronghold plus (selamectin, sarolaner), which are used in 36 veterinary facilities (72 %). The third most used preparation, is Vectra 3D (dinotefuran, pyriproxyfen, permethrin), which was confirmed by 31 veterinary facilities (62 %).

Anti-parasitic collars finished in 3rd place in anti-parasitic protection. Among them, the most frequently used collar is Foresto, with the active substances imidacloprid and flumethrin. Up to 88 % of veterinarians confirmed that the use of antiparasitic tablets is gaining more and more favor among dog owners. According to 86 % of veterinarians, dog owners seek advice when choosing a suitable antiparasitic. In 36 % of cases, dog owners listen to advice, but choose a cheaper preparation.

Medicinal products used for the treatment of whole-body demodicosis in dogs were investigated. The selection of medicinal preparations is shown in Table 2.

Table 2. Medicinal preparations used to treat whole-body demodicosis in dogs

Medicinal preparation	Use for the treatment (%)
Bravecto tablets	88
Advocate spot on	30
Supportive treatment	23
Neostomosan – bath	18
Ivermectin – injection	16
Amitraz – bath	12
Preventic collar	2
Scalibor collar	2

For the therapy of whole-body demodicosis, veterinarians use Bravecto tablets most often (88 %). The bath in Neostomosan, is still used by up to 18 % of veterinarians and Ivermectin in the form of an injection by up to 16 % of veterinarians. According to veterinarians (23 %), supportive treatment in the form of vitamin E, zinc, biotin, and unsaturated fatty acids, is also important for demodicosis. Veterinarians had the opportunity to add other medicinal preparations that they use in the therapy of whole-body demodicosis. They were as follows: NexGard spectra (4 %), Simparica (6 %), and Selehold (2 %).

2. Bacterial skin diseases

Secondary bacterial infections and superficial bacterial skin infections are the most frequently occurring bacterial skin infections in the questioned veterinary facilities. In second place, are hot spots, and bacterial skin infections caused by injury, biting and licking. Interdigital bacterial infections ranked third, and deep bacterial skin infections ranked fourth. Antibacterial agents play a key role in the treatment of bacterial infections.

It was determined how often veterinarians use the option of determining the sensitivity of isolated bacteria to antibiotics (ATB). Thirtysix veterinarians (72 %), use culture methods (creation of antibioticogram) only in case of recurrent infections, and 21 veterinarians (42 %) use these methods only in very severe cases of infection. Four veterinarians (8 %) choose other ATBs, in case of ineffective treatment, with the first antibiotic, and 17 veterinarians (34 %) use ATBs that they believe should be effective for the given infections.

Forty veterinarians (80 %) included aminopenicillins (ampicillin and amoxicillin), and 74 % veterinarians cephalosporins among the most frequently used ATBs in the treatment of skin infections. Cephalosporins belong to bactericidal ATBs, unlike penicillins, they are more effective against gram-negative aerobically growing bacteria [13, 15]. For the therapy of bacterial skin infections, fluoroquinolones are used in 19 veterinary facilities.

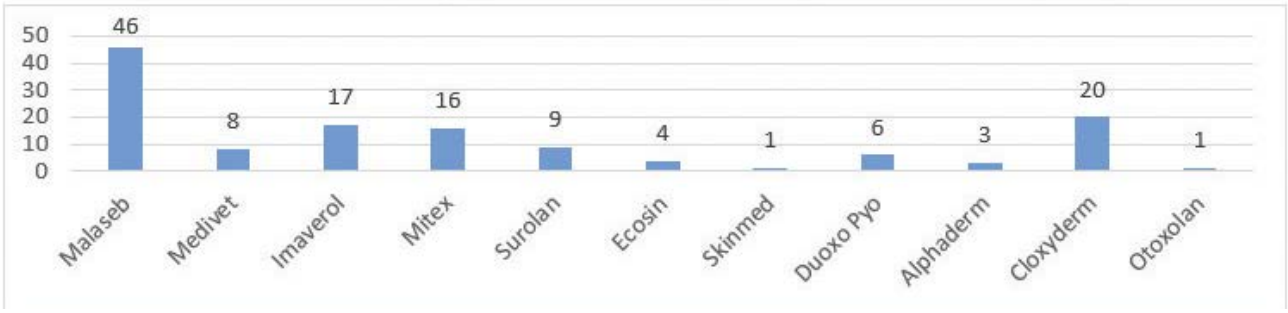
The most common ATBs used for the treatment of bacterial skin infections in the monitored clinics include: amoxicillin with clavulanic acid (31 %), cephalexin (28 %), enrofloxacin (11 %), cefovecin (8 %), doxycycline (6 %) and marbofloxacin (3 %). Up to 8 % of veterinarians use topical ATB (neomycin, bacitracin, polymyxin B and fusidic acid) in the treatment of skin infections.

3. Mycotic diseases of skin

Yeast infections of the skin caused by the genera *Malassezia* and *Candida* account for up to 90 % of skin mycoses, treated in veterinary facilities in Slovakia. Dermatophytoses caused by pathogens *Microsporum canis,* and *Trichophyton* spp. occur less frequently (10 %). For the most common procedures in the treatment of skin mycoses were indicated in the questionnaire. In treatment of fungal diseases, the local form of therapy is used the most (74 %). There are cases when general therapy in the form of tablets (10 %) is also used, but such form of therapy is not used in 16 % of facilities.

In general, azole antifungals, which are divided into imidazoles, and triazoles, are used for the therapy of skin mycoses. Imidazoles include clotrimazole, miconazole, enilconazole, and ketoconazole. Triazoles include itraconazole and fluconazole. The mechanism of action of azole antifungals consists in the inhibition of the enzyme lanosterol-demethylase, which prevents the synthesis of ergosterol, which is an important part of the plasma membrane [15]. Some disinfectants and antiseptics with the active substance chlorhexidine are also used for the treatment of skin mycoses (Fig. 1).

Malaseb shampoo is one of the most widely used preparations in the treatment of skin mycoses, probably due to the content of up to two active substances (miconazole and chlorhexidine). It is used by up to 46 facilities out of 50 (96 %). Popular preparations include Clorexyderm, which is prescribed by veterinarians in 20 facilities (40 %) and Imaverol in 17 of them (34 %). Mitex ear drops are



x axis – veterinary preparations used; y axis – number of participating veterinary facilities

Fig. 1. Overview of medicinal products used for the treatment of skin mycoses and otitis in interviewed veterinary facilities

applied by veterinarians in 16 facilities (32 %). Thirty-five vets (70 %) agree that yeast infections are most common in allergic dogs. About one-third of veterinarians predict that yeast infections will return more than 50 % of the time.

From the results of the questionnaire, it follows that in allergic dogs with skin manifestations, an allergy to food was detected in 56 %, and to mites and yeasts in 46 %. Flea bite allergy with skin manifestation is less frequent (8 %). From practical experience, the most common allergen is chicken meat (78 %), followed by beef (42 %), and cereals (40 %). Less common are allergies to pork (16 %) and lamb (10 %). Turkey, duck, fish and eggs accounted for 6 % and rice for 4 %.

The use of allergy immunotherapy in the form of a specially prepared mixture of allergens (injections or drops) according to the results of the tested panel of allergens of a particular dog is shown in Table 3. The immunotherapy in this form is still not a standard in the treatment of allergies, mainly because of the financial burden for dog owners.

Table 3. Use of immunotherapy in dogs with skin manifestations of allergy

22 % of veterinarians do not use immunotherapy because they do not offer such an option
26 % of veterinarians offer immunotherapy, but the owners refuse it for financial reasons
34 % of veterinarians offer immunotherapy, they use it 3 times a year
12 % of veterinarians offer immunotherapy, they use it 3–6 times a year
4 % of veterinarians use immunotherapy more than 6 times a year
1 veterinarian (2 %) had not heard of this form of therapy

The survey determined the frequency of administration of glucocorticoids in allergies with skin manifestations in dogs. Three veterinarians (6 %) use glucocorticoids in allergy therapy almost always, 14 % of veterinarians less often, and 58 % of veterinarians only in necessary cases. Veterinarians try to choose another alternative of therapy in 22 %. Most veterinarians confirmed the use of human preparations when applying glucocorticoids.

Currently, the veterinary drug Apoquel with the active substance oclacitinib, which acts on pro-inflammatory cytokines or others, that play a role in the allergic reaction – pruritus, is coming to the fore in the treatment of allergies in dogs. It is prescribed by veterinarians in up to 32 veterinary facilities (64 %). In the treatment of allergies, the drug Cytopoint (lokivetmab) is also used to treat pruritus associated with allergic dermatitis. It is administered in the form of a subcutaneous injection. It is a caninized monoclonal antibody expressed using recombinant methods in a Chinese hamster ovary cell line. Fifteen veterinarians also use human medicinal preparations in the treatment of allergies. They are as follows: Zodac and Zyrtec (cetirizine) – 10 veterinarians, Dithiaden (bisulepin) – 8 veterinarians, Equoral (cyclosporine) – 2 veterinarians, and Atarax (hydroxyzine) – 1 veterinarian.

The questionnaire included a question regarding the use of individually prepared drugs for the treatment of skin diseases in dogs. Up to 47 out of 50 interviewed Slovak veterinary facilities do not use the induvidually prepared drugs at all. Two veterinarians do not use this option because they do not have a pharmacy available to prepare these drugs. Only 3 veterinarians prescribe them, of which Mikulič ointment, chloramphenicol ointment with or without glucocorticoid, and cyclosporine eye drops were listed.

4. Dermatoses associated with hormonal disorders

Cushing's syndrome was chosen as the most common hormonal disorder by 24 veterinarians (48 %), while 21 veterinarians (42 %) chose hypothyroidism as the most common disorder of this type. According to as many as 39 veterinarians (78 %) hyperestrogenism rated as third. No veterinarian recommended euthanizing a dog diagnosed with Cushing's syndrome. Dog owners who have a dog with Cushing's syndrome most often choose pharmacotherapy (96 %), but they do not always want to continue

long-term treatment, due to high costs. Only one owner chose euthanasia.

DISCUSSION

In our analysis of 50 veterinary facilities, we found that skin diseases represent up to a third of all dog diseases treated by veterinarians noting that immune skin diseases are on the rise. Within the interviewed facilities, up to 36 % of all veterinarians put in the first place skin diseases caused by immune disorders. In a study in Cameroon, K o u a m o et al. (2021) considered parasitic diseases to be the most common diseases in dogs (31 %) [11], while in Slovakia only 20 % of veterinarians chose parasitic skin diseases as the most common. The analysis of our results in terms of skin parasites, showed that representation of fleas and ticks was the largest. In a study that dealt with the identification of ectoparasites, specifically ticks and fleas in 161 dogs, the genus *Rhipicephalus* was represented the most as it was found in 108 dogs. Of the fleas, the predominant species was *Ctenocephalides felis*, which was identified in 62 dogs [7]. In our study, we did not focus on genus and species identification of representatives of ticks and fleas. Slovak veterinarians recommended mostly the oral tablets (Bravecto, Simparica, NexGard) as a prevention against ectoparasites. They were registered a few years ago as a novelty among the antiparasitic drugs. Various studies confirmed high effectiveness of oral anti-ectoparasitic drugs. The percentage of their efficacy against ticks ranged from 85.2 % to 99.6 % at 24 hours after infestation for NexGard tablets, and from 63.4 % to 99.1 % for Bravecto [2]. Efficacy against *Ctenocephalides* spp. after 24 hours reached 100 % for both products (NexGard and Bravecto) [3]. Spot on and collars keep their place in the prevention against ticks and fleas. The choice of an antiparasitic as part of prevention depends on the dog owner. In most cases, the owners seek advice from a veterinarian, who will evaluate the patient's health and the dog's breed type and recommend the most suitable antiparasitic. For dogs kept in an apartment, e.g. with small children, spot on products and antiparasitic collars are not suitable, and therefore pills that have a monthly or three-month effect should be preferred.

B o n t e m s et al. (2020) claimed that dermatophytes are the most common pathogens of skin mycoses not only in humans but also in animals [4]. Our results showed that yeast skin diseases (*Malassezia, Candida*) are the most common skin mycoses. This statement was confirmed by 90 % veterinarians interviewed in our study, and dermatophytoses (*Microsporum canis, Trichophyton* spp.) accounted for only 10 % of treated animals. From the analysis of our results, local therapy in the form of baths, solutions, emulsions or ointments is the most used in the treatment of skin yeast infections. Preparations containing chlorhexidine and those containing miconazole are most often used for the treatment of dermatomycoses.

The response to one question in our questionnaires showed that to 47 out of 50 interviewed Slovak veterinary facilities do not use the individually prepared drugs for the treatment of skin diseases in dogs. For the sake of interest and comparison, we analysed the prescriptions of individually prepared drugs in pharmacies in the Czech Republic. According to this, veterinarians in the Czech Republic compared to Slovak veterinarians more often use the possibility of prescribing individually prepared drugs. They prescribed the following preparations: Prednisone syrup, Emulsion polysan cum oleum helianthi, Dimethylsulphoxide solution, Mikulič ointment, Solutio Galli-Valerio, RSB solution (resorcinol, salicylic acid, boric acid) in a ratio of 1:2:3, alcohol solution of iodine and shampoo with sulfur.

Within this study, we paid special attention to skin diseases that arise as a result of immune disorders. Atopic dermatitis or food allergy is becoming more and more common in dogs. This fact was claimed by 18 veterinary facilities out of 50. Food allergy (56 %) and allergy to mites and yeast (46 %) were most often confirmed in allergic dogs with skin manifestations. In a study that was aimed at detecting common allergens in dogs with atopic dermatitis using a serological immunoglobulin E-specific allergen test the authors found that the dogs were allergic to the following allergens: 38.3 % corn, 28.7 % potatoes, 22.7 % duck, 24.4 % cod, 95.6 % *Aspergillus fumigatus*, 31.9 % fleas. The percentage of detected allergens in dogs (54.8 %) was higher than in bitches (45.2 %) [1]. Our results showed that dogs in Slovakia were most allergic to meat (chicken 78 %, beef 42 %) and cereals (e.g. corn, rice; 40 %). Glucocorticoids are also used in the therapy of allergic manifestations of the skin. They have a fast onset, and show a strong antiphlogistic effect. However, long-term administration of glucocorticoids is not desirable, because they not only have a number of undesirable effects, but also have a negative effect on the hor-

monal balance. In the interviewed facilities, the most used glucocorticoids were those with the active ingredients prednisone and dexamethasone. Monoclonal antibodies, such as lokivetmabum, which are gaining more and more favour, are more suitable for therapy [8], which was also confirmed by the interviewed veterinarians. The drug oclacitinib has a great future worldwide due to its high effectiveness in the therapy of atopic dermatitis [5, 6, 12]. Up to 64 % of veterinarians confirmed the use of oclacitinib in their practice.

CONCLUSIONS

The prevalence of skin diseases in dogs is constantly increasing, which is closely related to the increasing number of dogs kept in the same household with humans and the higher standard of dog breeding. Unlike other organ systems, the skin and its diseases are directly visible and symptoms such as alopecia, scales, pruritus or even the parasites themselves are unpleasant not only from an aesthetic point of view, but also from the fear of infection. The increase in dermatological diseases can also be related to factors such as climate changes, which cause an increased occurrence of ticks even in the winter season, to the increasing resistance of pathogens to active ingredients, increasing number of allergies in the dog population, but also to more accurate diagnostic options. Dermatology in veterinary medicine currently reaches a high level in diagnosis and therapy, but the final result of the treatment still depends on the appropriately chosen therapy and cooperation of the dog's owner.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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BODY CHARACTERISTIC OF DRONES OF DIFFERENT ORIGIN

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ABSTRACT

Computer-based methods help beekeepers and scientists in apidology and bee research. Using software Optika Vision Pro in this study, three body morphological characteristics has been applied to honeybees' drones (*Apis mellifera carnica*) of different origin in identification of difference among them. All three measured body morphological characteristics (body length, forewing length, and head length) were statistically different between drones originated from colonies with mated queens and drones originated from colonies with unfertilised queen and/or with unfertilised worker bee(s), respectively, thus making them potentially more successful in queen fertilization and secondary colony development and productivity.

Key words: body morphological characteristics; computer-based methods; drones; honeybees

INTRODUCTION

The Carniolan honeybee is globally widespread with a wide diversity of subspecies, which can be classified with morphometric tools [13].

Three major groups of body morphological characteristics are commonly used: Length measurements, colour measurements, and wing venation characteristics, while wing venation characteristics have been studied more intensely than other body morphological characteristics. The measurement of body morphological characteristics can be done for different reasons, but a major reason is to characterize bee races [7, 14] and/or to determine the degree of hybridization with foreign races [12]. Moreover, body morphological characteristics are measured to investigate the impacts of imported queens on honeybee populations, or to check population purity [4, 8].

Commonly, body characteristics are standardly measured according to Ruttner et al. [13], and Ruttner [14], which can be divided according to body parts into: head, thorax, and abdomen characteristics. Head characteristics include: head length (HCL), and capsule width (HCW), antenna length (AL), and number of segments (ANS), compound eyes length (CEL), and width (CEW), and tongue length (TonL). Some methods also include: the mandible length (ML), beside some other characteristics. The thorax characteristics are: forewing width (FWW), and length (FWL), hindwing length (HWL), and width (HWW), number of hooks (NH), thorax width (thW), tibia length (TL), femur length (FL), basetarsus length (BL), and width (BW), brush hair rows number (HN), and pollen basket size (PBS). The abdomen characteristics are: lengths of tergit 3 (T3), and 4 (T4), body size (T3+T4), length of hairs on tergit 5 (HLT5), pigmentation of tergit

2-4, length of sternite 3 (LS3), wax mirror length (WML), and transversal (WMT), and sting shat length (StL).

Honeybees are classified into three basic castes: queens, drones, and workers, while only drones (male honeybees), are developed from unfertilised female eggs. The origin of unfertilised female eggs is different; (i) from mated queen which is physiologically laying them during the season, mainly at the beginning; (ii) from unfertilised queen which was not mated from different reasons, and from (iii) unfertilised worker bee(s), when queen is lost from different reasons. The eggs laid by fertilised queen, are developed in cells with larger diameter, compared to eggs originated from unfertilised queen, and/or unfertilised worker bee(s), which are laid into worker bee´s cells [3].

The body characteristics may be used for indirect prediction of colony productivity, or for selection of productivity, where honeybees with longer legs, and wings, have higher power light, and could gather more pollen and nectar for brood rearing, and consequently colony population [10].

To this time, there is no study aimed at the measurement of body morphological characteristics of drones of different origin. Based on the facts presented above, we focused on the body characteristics of drones of different origin. The use of these characteristics may help classify them according to their origin.

MATERIALS AND METHODS

The study was performed during summer 2021. According to some studies, at least 15 honeybee workers should be collected from each colony during the morphological analysis [4, 7]. In this study, we analysed 256 individuals of drones from six different geographical areas of Slovakia (Fig. 1). The sampled drones were of different origin, all three groups were covered equally; 86 drones originated from colonies with laying queens, 80 individuals from colonies with unmated queens, and 90 drones from colonies with unfertilised worker bee(s). Drones were collected directly from brood comb(s), according to P a d i l l a et al. [11]. The collected drones were preserved in a deep-freezer (-18 °C) and later dissected and analysed in a laboratory.

Table 1. Sample origin details

Drone origin	Sample ID	Site	Number of drones
Mated queen	1	Košice	30
	2	Rozhanovce	30
	3	Kozelník	26
Unmated queen	4	Rozhanovce	30
	5	Rozhanovce	20
	6	Prešov	30
Unfertilised worker bee	7	Zvolen	30
	8	Rozhanovce	30
	9	Kurima	30

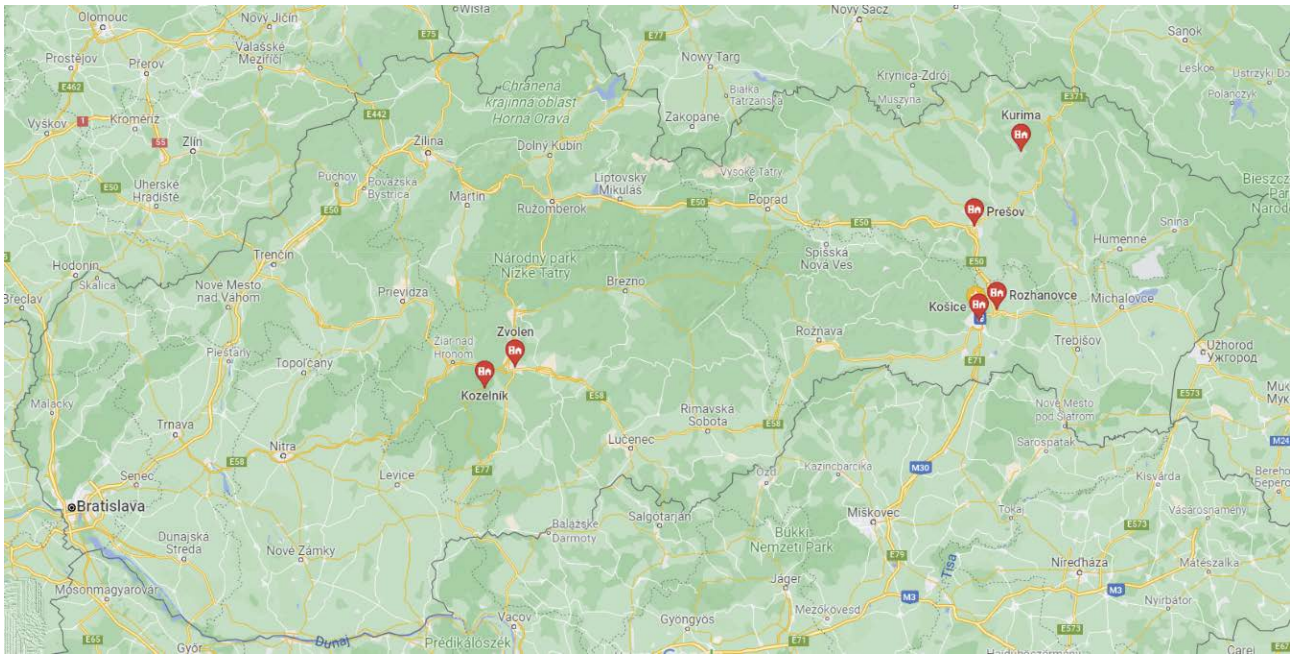

Fig. 1. Sampling sites
https://www.google.com/maps/@48.6635723,19.9533694,8.3z/data=!4m3!11m2!2sQCZwaYWaRt2IHLictE5gHw!3e3?hl=sk-SK)

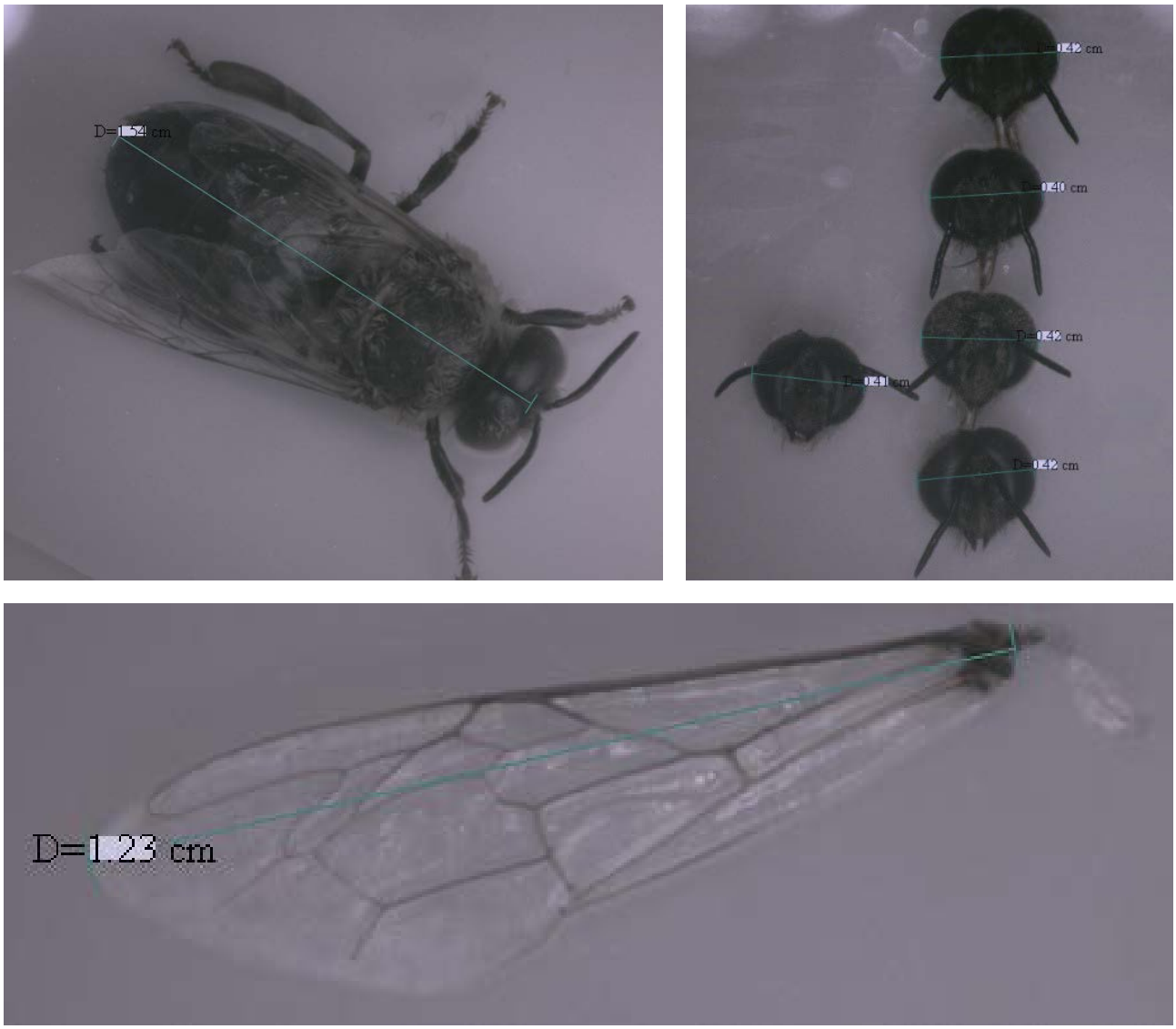

Fig. 2. Examples of body morphological characteristics measurement

The body measurements were obtained using a stereomicroscope (MOTIC, Hong Kong) with an ocular micrometer [3, 6, 14].

The body length, forewing length (FWL) and head length (HCL) were measured using software Optika Vision Pro (OPTIKA SOFTWARE, Italy) (Figure 2).

The measured values of the individual parameters were statistically evaluated using GraphPad Prism 3.0 for analysis of variance (ANOVA), and Tukey's test at a significance level of P = 0.001.

RESULTS AND DISCUSSION

The measurement of body morphological characteristics is done for different reasons, but a major use, is to characterize honeybee races [7, 14], but also to determine the degree of hybridization with foreign races [1, 12]. Moreover, morphological characteristics are measured to investigate the impacts of imported queens on honeybee populations and/or to check populations purity [4, 8]. The obtained results are summarised in Table 2.

Table 2. Body morphological characteristics of drones of different origin

Drone origin	Mean ± SD (cm)		
	Body length	Forewing length	Head length
Unfertilised worker bee	1.44 ± 0.08	1.16 ± 0.05	0.42 ± 0.01
Unmated queen	1.40 ± 0.09	1.16 ± 0.05	0.42 ± 0.01
Mated queen	1.53 ± 0.08	1.24 ± 0.04	0.44 ± 0.02
ANOVA	P < 0.0001	P < 0.0001	P < 0.0001
Tukey test	P value	P value	P value
Unfertilised worker bee vs. unmated queen	P < 0.001	P > 0.05	P > 0.05
Unfertilised worker bee vs. mated queen	P < 0.001	P < 0.001	P < 0.001
Unmated queen vs. mated queen	P < 0.001	P < 0.001	P < 0.001

Mean body length of 1.53 ± 0.08 cm was observed in drones originated from colonies with mated queen, followed by mean body length of 1.44 ± 0.08 cm in drones originated from colonies with unfertilised worker bees. Drones originated from colonies with unmated queen have the mean body length of 1.40 ± 0.09 cm. Using Tukey test, all three groups were statistically significant (P < 0.001) (Table 2). The next two morphological characteristics of drones of different origin (forewing length and head length) showed the same picture; drones originated from colonies with unfertilised worker bee, vs unmated queen have the same length of forewing and head, with no statistical significance (P = 0.001). But comparing these two groups with drones obtained from colonies with mated queen, we may see differences in length of forewing and head with statistical significance (P < 0.001).

Multiple body characteristics, including tongue length, wing length, and width, were used to differentiate between honeybee subspecies [2, 16]. Interestingly, some studies concluded that tongue length was found to be an indicator of geographical variation [6, 15]. Proboscis length was also found to be the most differentiated characteristics between *A. m. carnica*, *A. m. mellifera*, and *A. m. caucasica* [16].

Kolmes and Sam [5] found that honey production was highly correlated to overall size, wing measurements, and corbicular area in Carniolan honeybees. Moreover, newer study showed that honey production was related to tongue length, forewing length and width, hindwing length, leg length, femur length, tibia length and metatarsus width [9]. The body characteristics may thus, be used for indirect selection of productivity where honeybees: with bigger legs, and wings, have higher power light, and could gather more pollen, and nectar, for brood rearing, and consequently colony population [10].

CONCLUSIONS

Nowadays, various computer-based methods are used in morphological measurements used in apidology and bee research. Ongoing evaluation of morphological characteristics may help in understanding the racial fluctuations due to hybridization, beekeeping practices, and environmental factors. Moreover, there is evidence, that body morphological characteristics are very important, and correlated with colony productive characteristics.

This study revealed body morphological differences among drones originated from different origin. The longest mean body length, forewing length, and head length, was observed in drones originated from colonies with mated queen, thus making them potentially more successful in queen fertilization. But it is apparent that still more work is required to provide insights into the impacts on body morphological differences in the future.

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THE HYGIENIC QUALITY OF MEAT PRODUCED IN ALGERIA: META-ANALYSIS

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ABSTRACT

This meta-analysis aims to provide a comprehensive overview of the hygienic quality of meat in the context of Algerian meat production. A systematic literature search was conducted across various databases, yielding a pool of studies assessing the microbial contamination levels in different types of meat, including: poultry, beef, lamb, camel, sausages, and minced meat, within Algerian slaughterhouses and butcheries. A total of 25 studies met the inclusion criteria, with a combined sample size of 550 meat samples. The selected studies employed standardized methods for microbial enumeration: Total aerobic mesophilic bacteria (TAMB). The collected data were synthesized using random-effects meta-analysis models to estimate the pooled mean bacterial counts, as well as to explore potential sources of heterogeneity. The results of this meta-analysis, revealed considerable variation in bacterial counts across different meat types and sources. The overall pooled, mean bacterial count was 5.15 colony-forming units per gram (CFU.g^{-1}), with significant heterogeneity observed ($I^2 = 87.52$ %, $P < 0.001$). Subgroup analyses indicated that bacterial counts were notably higher in butcher shops, highlighting the need for targeted interventions to improve hygiene practices in these areas. Furthermore, sensitivity analyses were conducted to assess the influence of individual studies on the overall results, and publication bias was evaluated through a funnel plot. In conclusion, this meta-analysis provides valuable insights into the hygienic quality of meat in Algerian meat production, emphasizing the significance of TAMB as an informative indicator. The observed variations in bacterial counts underscored the importance of hygiene practices throughout the meat supply chain, from farm to consumer.

Key words: Algeria; bacterial counts; food safety; hygienic quality; meat production; meta-analysis

INTRODUCTION

Global meat production has increased rapidly over the past 50 years, as the total production has more than quadrupled since 1961. At a global level, the dominant livestock types are: poultry, cattle, pig, and sheep and goat to a lesser extent [38]. The last data of the Food and Agriculture Organization of the United Nations (FAO) indicate that since 2018, poultry has become the most widely consumed meat worldwide, surpassing pig production [21]. However, the distribution of meat types varies significantly across the world; in some countries, other meat types such as wild game, horse, and duck can account for a significant share of total production [38].

This occurrence can be explained by the fact that poultry meat is not a subject of culturally or religiously set limitations, and it is perceived as nutritionally valuable foodstuff with a low content of fat, in which there are more desirable unsaturated fatty acids, than in other types of meat [29]. Unlike beef and dairy fat, chicken meat contains no trans fat, which contributes to coronary heart disease [30]. Red meat is a nutrient dense food that is an important source of complete protein with all essential amino acids, highly bioavailable iron, zinc, selenium, and B vitamins, especially vitamin B_{12} in the diet [28]. Fat is an important part of the diet, providing energy, and fat-soluble vitamins, such as vitamin E [42]. The intake of meat in adequate amounts, which supplies the missing essential micronutrients, can reduce nutrition-related stunting and the consequent cognitive impairment [5].

Meat and meat products can serve as vehicles of many pathogenic organisms. Several pathogens, such as: *Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Listeria monocytogenese, Salmonella, Staphylococcus aureus,* and *Yersinia enterocolitica,* are known to produce serious foodborne diseases among the consumers [33, 37]. In addition to foodborne pathogens, bacteria responsible for spoilage may lead to large economic losses. Their growth and metabolic activity during shelf life leading to colour, odour, taste, or texture defects are responsible for waste, and losses of food products [39]. The types of microorganisms present on muscle food products and their numbers depend on the sanitary conditions in the environment of origin of the food, the properties and microbiological quality of any added ingredients, the extent of product processing and handling, and the conditions involved in subsequent storage, handling and distribution [40]. Therefore, the occurrence of pathogens on raw meat can be due to different factors, which include poor farm animal management, improper slaughter practices, processing, storage conditions and lack of meat safety knowledge among meat handlers [37]. The slaughtering and butchering process of animals provides bacteria with an opportunity to colonize meat surfaces. Contamination of meat is a continuing possibility from the moment of bleeding until consumption [35].

Resistant bacteria in animals can be transferred to people usually through the consumption of food [1]. In the case of meat products, chicken, turkey, beef, pork, and resulting products are major vehicles for transmission [1, 32]. In addition to transfer of resistant bacteria in the food chain, exchange of mobile genetic elements among commensal and pathogenic bacteria contributes to the emergence of drug resistance [31]. The emergence of several clones of *Salmonella* resistant to multiple antimicrobials worldwide underscores a significant food safety hazard [24].

In Algeria red meat marketed and consumed consists essentially of mutton and beef. The production of Camlin meat is however marginal. Goat meat production is mainly carried out within the steppe mountains areas [25]. Sheep meat production is estimated at 342,000 tons in 2022 followed by poultry production estimated at 258,000 tons while cattle production is estimated at 146,000 tons (Fig. 1) [19]. The average person consumed around 20 kilograms of meat [38].

Over the last few decades, *Hygienic quality of meat produced in Algeria* has been the subject of several studies. However, the findings vary significantly and have been achieved within a wide range of circumstances, such as different methodologies, sample sizes, types of meat, production practices, geographic regions or time periods.

Overall, the objective of a meta-analysis in this context is to provide a more comprehensive and reliable understanding of the hygienic quality of meat in Algeria by systematically examining and synthesizing the existing body of multiple individual studies.

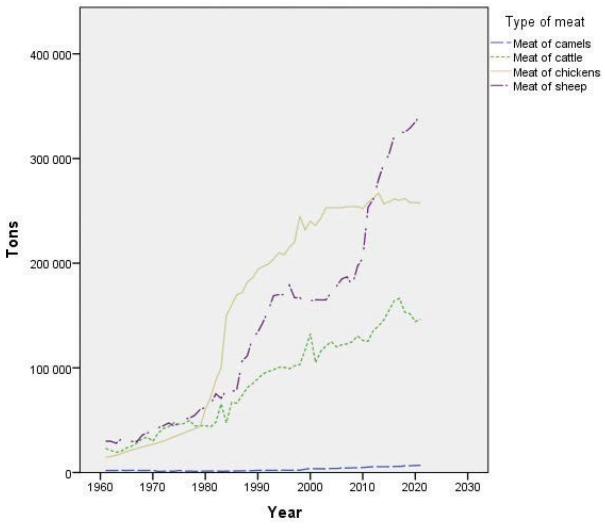

Fig. 1. Annual meat production intended for human consumption, in Algeria (1961–2021).
Source: (FAOSTAT, 2023)

MATERIALS AND METHODS

Defining the search strategy

A comprehensive search strategy was developed using appropriate keywords and database-specific syntax. To conduct this meta-analysis, we adhered to the guidelines provided by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Inclusion criteria

- Studies conducted in Algeria,
- Studies reporting microbial contamination levels (Total aerobic mesophilic bacteria TAMB),
- Studies reporting total bacterial counts in $CFU.g^{-1}$ of meat,
- Fresh meat of ovine, bovine, caprine or poultry,
- Samples of minced meat or sausages,
- Meat samples collected from slaughterhouses or butcher shops.

Exclusion criteria

- Studies from other countries,
- Studies not specifically focused on meat hygiene,
- Studies with insufficient data on microbial contamination,
- Studies focused solely on frozen or imported meat.

Search terms

The key terms related to the hygienic quality of meat were determined: („meat" OR „beef" OR „bovine" OR „cattle" OR „chicken" OR „poultry" OR „lamb" OR „sheep" OR „camel") AND („minced meat" OR „sausage") AND („microbial contamination" OR „bacterial contamination" OR „hygiene" OR „food safety" OR „total bacterial counts" OR „FMAT") AND („slaughterhouse" OR „abattoir" OR „butcher shop").

Search execution

The search strategy was executed in August 2023 in Google scholar and Algerian University databases. The search results were screened and selected based on relevance to the research question and inclusion criteria.

Statistical analysis

Data was extracted for each included study. Overall effect size was calculated using a random-effects model, which considers both within-study and between-study variability. Heterogeneity among the studies was assessed through Cochran's Q statistic, which evaluates whether the variation in effect sizes is statistically significant beyond what would be expected due to chance. Additionally, the I^2 statistic was computed which measures the proportion of total variation across studies due to heterogeneity rather than chance. High I^2 values indicate substantial heterogeneity. A forest plot was generated to visually represent each individual study along with the overall estimate. Each study's point estimate, represented by a square on the plot, was sized proportionally to its weight in the meta-analysis. The 95 % confidence intervals (CIs) for each study's estimate and the overall estimate were displayed. Subgroup analyses were performed based on meat type and source of the meat samples, to investigate potential variations in hygienic quality. Subgroup-specific effect sizes and 95 % CIs were calculated to examine whether variations in total bacterial counts were influenced by these factors. Publication bias was assessed using funnel plots, which illustrate the relationship between observed outcomes and their standard errors. Additionally, Egger's regression test was conducted to detect potential asymmetry in the funnel plot, which might indicate publication bias.R (version 4.3.0) software was used for all analyses.

RESULTS AND DISCUSSION

The present meta-analysis aimed to comprehensively assess the variations in total bacterial counts in meat samples collected from various sources in Algeria, with a specific focus on total bacterial counts as an indicator. By synthesizing data from multiple studies, we aimed to gain insights into the hygienic quality of meat and the potential implications for food safety practices in the Algerian context.

Study selection

From the initial search, 115 studies were identified as potentially relevant to the meta-analysis of hygienic quality of meat. After applying inclusion and exclusion criteria, 25 studies were considered eligible for the analysis. Fig. 2 outlines the study selection process.

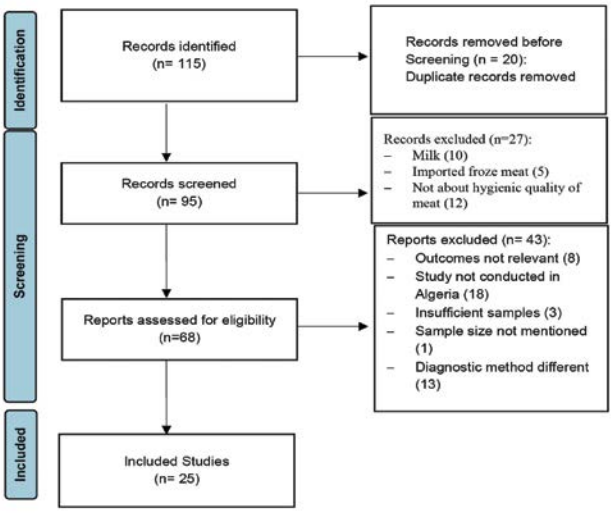

Fig. 2. Flow chart summarizing the process of study selection

Study characteristics

Table 1 presents the characteristics of the included studies. Studies were conducted between 2009 and 2022, with total sample size 550 meat samples. The studies covered various types of meat, including poultry, beef, and lamb, camel, sausages, minced meat from slaughterhouse or butcher shop and were conducted in diverse geographical regions within Algeria (Fig. 3).

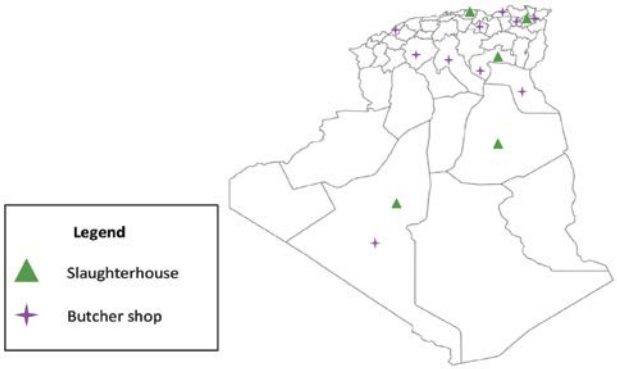

Fig. 3. Map of locations of studies included in the meta-analysis.
Algerian map was downloaded from Vemaps.com.

Heterogeneity

Significant heterogeneity was observed among the included studies (I^2 = 87.52 %, P < 0.001).

Publication bias

Egger's test indicated no publication bias (P = 0.80). The Funnel plot showed a relatively symmetrical distribution of studies, suggesting no significant publication bias (Fig. 4).

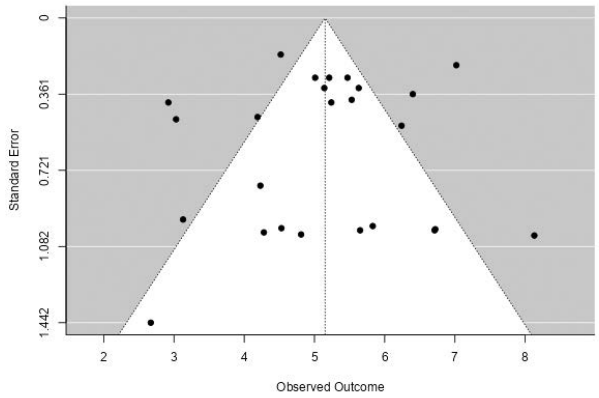

Fig. 4. Funnel plot for the total aerobic mesophilic bacteria (TAMB) level in meat

Overall hygienic quality of meat products

The overall weighted mean total bacterial count was 5.15 $\log_{10}$ CFU.g^{-1} (95 % CI: 4.66–5.64).

Forest plot

Figure 5 displays the forest plot of individual study along with the overall random-effects estimate. The studies are labelled numerically [2], [3], [4], [6], [7], [8], [11], [12], [13], [14], [15], [16], [17], [18], [20], [21], [23], [26], [27], [36], [41].

Table 1. Summarized results of the meta-analysis

Variable	Level	Number of Studies/Samples	I2 (%)	TAMB (log10 CFU.g^{-1})	IC 95 %	P- value
Type of meat	Cow meat	5/38	58.16	5.42	4.08–6.77	0.620
	Camel meat	5/110	83.56	4.19	3.36–5.03	
	Sheep meat	2/29	–	–	–	
	Poultry meat	5/227	60.85	5.60	4.09–7.09	
	Minced meat	4/112	0	5.28	4.81–5.74	
	Sausage	4/34	86.55	5.81	4.67–6.95	
Source of meat	Slaughterhouse	10/184	86.20	4.27	3.61–4.94	0.001
	Butcher shop	15/366	69.04	5.81	5.33–6.29	

I^2 – Heterogeneity; TAMB – Total aerobic mesophilic bacteria; IC 95 %–95 % confidence intervals; P- value – probability value

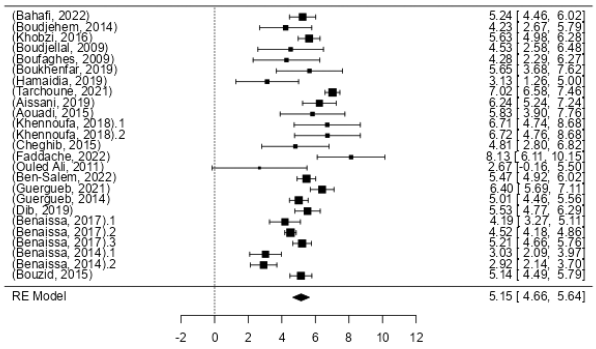

Fig. 5. Forest plot of the overall studies

Subgroup analysis

The subgroup analysis on meat type (poultry, beef, lamb,…), revealed no significant differences in the hygienic quality of meat samples from different sources (P = 0.615). The subgroup analysis by slaughterhouse or butcher shop revealed significant differences in the hygienic quality of meat samples from different sources.

Slaughterhouse: Meat samples from slaughterhouses exhibited a lower weighted mean total bacterial count (4.27 $\log_{10}$ CFU.g^{-1}) (Fig. 6).

Butcher Shop: The samples from this butcher shop had the highest weighted mean total bacterial count, indicating a potential need for improved hygienic practices (5.81 $\log_{10}$ CFU.g^{-1}) (Fig. 7).

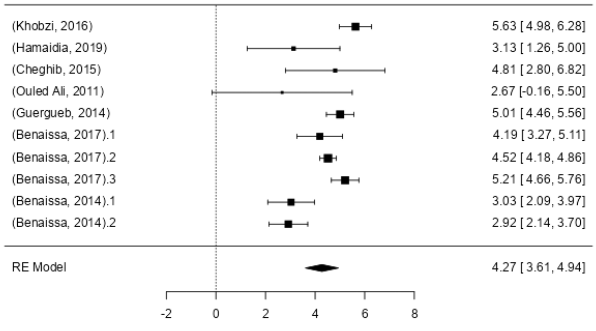

Fig. 6. Forest plot of the bacterial load of meat at slaughterhouses

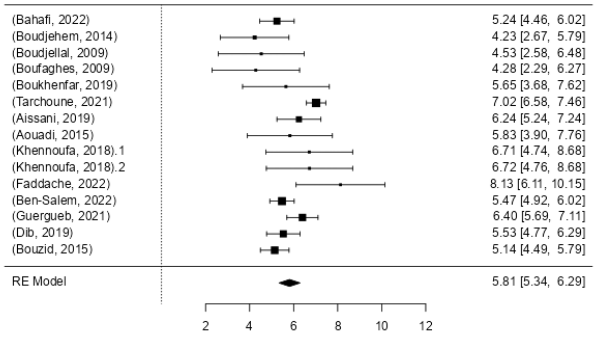

Fig. 7. Forest plot of the contamination level in meat at butcher shops

Variability in total bacterial counts

The results of our meta-analysis reveal notable variability in total bacterial counts across the included studies. This variation suggests that the hygienic quality of meat in Algeria is not uniform, with some samples exhibiting higher bacterial counts than others. The aggregated weighted mean total bacterial count of 5.15 $\log_{10}$ CFU.g^{-1} underscores the need for continued vigilance in the implementation of effective hygiene measures throughout the meat production and distribution chain.

The criteria for TAMB contamination of meat are similar in Algeria and in Europe. However, there are some differences in terms of quality control and monitoring. In Europe, Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs sets a maximum limit of 100,000 CFU.g^{-1} for TAMB in fresh meat. This limit is also in force in Algeria, in accordance with the provisions of Executive Decree 10-2005 of 17 January 2005 on the hygiene of foodstuffs.

Sources of heterogeneity

Significant heterogeneity was observed among the studies included in this meta-analysis (I^2 = 87.52 %). This heterogeneity could be attributed to several factors, including variations in sampling methods, processing practices, and origin source. The variations observed in meat types, such as beef, chicken, lamb, and sausage,… could also contribute to the observed heterogeneity. Subgroup analyses were conducted to explore potential sources of heterogeneity. Subgroup analyses provided valuable insights into the variations in bacterial counts among different meat types and sources. For instance, higher bacterial counts were noted in butcher shops, suggesting that this particular segment of the meat supply chain may warrant special attention to improve hygienic practices. This finding aligns with previous research [9, 10, 22, 34] highlighting the impact of various factors, including inadequate sanitation, cross-contamination, and suboptimal processing conditions, on microbial contamination levels in specific meat sources.

Implications for food safety

The findings of our meta-analysis have important implications for food safety practices in Algeria. The observed variability in total bacterial counts highlights the need for consistent adherence to proper hygiene practices in slaughterhouses, butcheries, and throughout the supply

chain. High bacterial counts in meat samples pose a potential risk to consumers, as consumption of contaminated meat may lead to foodborne illnesses. Addressing this issue requires a concerted effort to implement and enforce stringent hygiene standards at all stages of meat production and distribution.

Limitations

Several limitations of our meta-analysis warrant consideration. First, the availability of data from studies specifically focusing on total bacterial counts in Algerian meat samples was limited, which could have impacted the comprehensiveness of our findings. Additionally, variations in study methodologies and reporting practices could have introduced bias and contributed to the observed heterogeneity. The availability of relevant studies specifically focusing on total bacterial counts in Algerian meat samples was limited, potentially affecting the comprehensiveness of our analysis. Future research should aim to address these limitations by conducting well-designed studies with standardized methodologies and transparent reporting.

CONCLUSIONS

In conclusion, this meta-analysis highlights the variability in total bacterial counts in meat samples obtained from various sources in Algeria. The findings underscore the need for continuous efforts to improve hygiene practices in slaughterhouses and butcheries, thereby enhancing the overall hygienic quality of meat products. Implementing and enforcing rigorous food safety measures is crucial to safeguarding public health and meeting international standards. Addressing these challenges will require collaboration among stakeholders, including regulatory authorities, meat producers, and consumers, to ensure the provision of safe and wholesome meat products in Algeria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SERUM BIOCHEMICAL PROFILE DURING EARLY, MID, LATE PREGNANCY, AND CALVING PERIODS IN DAIRY COWS IN ALGERIA

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ABSTRACT

This study aimed to assess the impact of pregnancy on biochemical indices of dairy cows raised in a semi-arid region of western Algeria. A private farm provided forty dairy cows for this study. The cows were divided into four groups based on their pregnancy stages: G1 included pregnant cows at 3 months, G2 included pregnant cows at 6 months, G3 included pregnant cows at 9 months, and G4 included lactating (calving) cows for 1–2 months. All of the cows were raised under the same environmental and management conditions, following the regional customs. Twelve biochemical parameters were measured, including cholesterol (CHO), triglycerides (TG), total protein (TP), albumin (Alb), globulin (Glob), urea (Urea), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), and magnesium (Mg). The results of the study showed no significant difference (P > 0.05), in the concentrations of various biochemical constituents, among the four groups of dairy cows. The mean value of Glob slightly increased in early pregnancy, compared to late pregnancy, and non-pregnant cows. AST exhibited a significant difference, between non-pregnant, and early-pregnant cows. ALT levels significant decreased in late pregnant cows, compared with early-pregnant cows. Additionally, Ca levels showed a slight increase, during late pregnancy, compared to non-pregnant cows. As this is the first study of its kind on dairy cows in Algeria, the data generated during this research demonstrated the good management of the herd and can serve as valuable reference values for the scientific community.

Key words: Algeria; biochemical parameters; dairy cows; pregnancy; Tiaret

INTRODUCTION

Since the 1960s, Algeria has implemented measures to promote the breeding of dairy cattle by importing pregnant heifers, with low customs duties, and subsidizing their acquisition, by farmers. In Algeria, dairy cattle breeding, play a crucial role, in the economy, as reported by ONIL [27], with the per capita milk consumption rising from 30.1 litres in 1964, to 145 litres in 2018, surpassing the global average. However, the official statistics show a much lower number of "modern" dairy cows, than expected, if the cows imported since the 1960s and their offspring, had been appropriately bred [19].

Some researchers hypothesize, that the breeding performance in this context, has been poor. The observed discrepancy, seems to be largely explained, by the low reproductive performance, which is attributed to the inadequate maintenance of the herd, in terms of food, and animal health [19]. Additionally, while importing pregnant heifers, has contributed to increased milk production, certain studies indicate, that its full potential, has not been realized [23].

Using blood biochemical characteristics as a pre-symptomatic diagnostic tool, can help in detecting, preventing, and treating infectious diseases, nutritional and metabolic health issues, as well as other disorders, like reproductive, and productivity problems in cattle [1]. However, it's important to consider that factors, such as age, breed, sex, pregnancy, lactation, diet, and health status, can all influence these parameters in cows [24]. In the same context, T u r k et al. [32] indicated that all animal species undergo significant changes in blood variables during pregnancy. For dairy cows, the pre-parturient period, is associated with a high incidence of metabolic and reproductive disorders.

Managing the performance of herds in semi-arid environments during this critical period is often: challenging, particularly concerning their well-being, health, and productivity [7, 15].

Developing an animal blood metabolic profile test has proven to be a challenging, due to the variations influenced by factors such as: breed, farming practices, and environmental conditions. As a result, establishing physiological baseline values for different breeds and specific environments has become necessary.

To investigate and determine the specific biochemical parameters in dairy cows during various stages of pregnancy, and the calving period, in the semi-arid region of Tiaret, we conducted the current study. It's noteworthy that, as far as we know, this is the initial research of its kind conducted in Algeria. The primary objective was to compare the different pregnancy stages with cows that were not pregnant.

MATERIALS AND METHODS

Ethical statement

The use of animals was conducted in accordance with worldwide animal welfare standards (Terrestrial Animal Health Code 2018, section 7, Art 7.5.1), as well as the national executive decree No. 95–363 of November 11, 1995 (Algeria).

Study location

This study was carried out in Tiaret. This region is located in the Centre West of the high plateau region of the country. Its space is heterogeneous, with a mountainous zone in the north, high plateaus in the centre, and semi-arid spaces in the south (35° 23′ 17″ nord, 1° 19′ 22″ east). Its morphology and geographical position give it an agro-pastoral character. The town covered around 20.086.63 km² and is located at 1,143 m (altitude of the Pass), on the flanks of Jebel Guezoul, which is part of the Tell Atlas chain. The Köppen-Geiger classification of the climate is Csa. Tiaret sees an average annual temperature of 14.7 °C (58.5 °F). Each year, there is about 529 mm (20.83 in) of precipitation.

Animals and diet

A total of 40 clinically healthy dairy cows (17 Holstein, 23 Montbeliard), were selected from a private farm. These cows were imported as pregnant heifers. The animals were comparable in age (3 and 4 years), body condition score (3.13 ± 0.70), and parity (1.34 ± 0.47). Based on their stage of pregnancy, they were divided into four groups: G1 included pregnant cows at 3 months. G2 included pregnant cows at 6 months, G3 included pregnant cows at 9 months, and G4 included lactating (calving) cows. The study spanned 8 months, from January 2020 to August 2021.

All of the cows, were raised under the same environmental, and management conditions, following regional customs. They were kept in a straw-bedded, free-moving barn, with access to the pasture allowed, only during spring and autumn for a minimum of 6 hours, during daylight (from 08:00 to 14:00 CEST). Milking occurred twice daily, at 5 a.m. and 4 p.m. The cows were fed the same diet, consisting of a total mixed ration (TMR), given twice daily at 08:00 and 14:00 CEST. The diet included a combination of 6 kilograms of bran and concentrates, 8 kilograms of maize ensilage, and 2 kilograms of dried alfalfa per cow. Additionally, the animals had continuous access to fresh water and salt lick.

Collection of blood samples

Aseptically collected blood samples were obtained from each animal in the morning using jugular vein punc-

tures. A 22-gauge needle, coupled with a 10 ml syringe (KD-JECT® III, KDM®, Germany), was inserted into the jugular vein, and the blood was collected in a vacutainer anticoagulant-containing tube (Vacutube®, Algeria). This process was repeated for each animal in each of the four groups.

Biochemical analyses

We conducted the analysis of thirteen blood biochemical metabolites (CHO, TG, TP, Glob, Alb, Urea, AST, ALT, ALP, Ca, P, and Mg) using a split-beam UV/Vis spectrophotometer (OPTIZEN 2120UV Plus, Korea). These blood tests were carried out at the laboratory of the Institute of Veterinary Medicine (Department of Animal Health), University of Tiaret.

Statistical analyses

The data obtained, was recorded in Microsoft Excel 2016. The descriptive analyses were carried out with IBM SPSS, version 21, and all results were presented as a mean ± standard deviation. Before analysing the data on pregnancy groups, the normality of the data was tested using the Kolmogorov-Smirnova test. The Kruskal-Wallis test and one-way analysis of variance test were used. To evaluate the significance of differences between groups, the Mann-Whitney and Tukey tests were used. The data from the samples of pregnant and calving cows underwent t-tests and Mann-Whitney tests, with a significance level set at $P < 0.05$.

RESULTS

Table 1 reveals that there is no significant difference, in the values, of cholesterol (CHO), triglycerides (TG), total protein (TP), albumin (Alb), Urea, phosphorus (P), and magnesium (Mg) between the groups ($P > 0.05$). However, early pregnancy showed a slightly higher mean value of globulin (Glob) compared to late pregnancy or non-pregnant cows. Aspartate aminotransferase (AST) exhibited a significant difference, between non-pregnant, and early pregnancy, whereas it decreased substantially during late pregnancy. Alanine aminotransferase (ALT) levels significantly decreased, in the late pregnant cows, compared with early pregnant ones. Additionally, the calcium (Ca) levels increased slightly during late pregnancy, compared with non-pregnant cows.

DISCUSSION

The average total plasma cholesterol recorded in our study was similar to the findings reported by Bruger–Picoux [5]. However, our results did not align, with those obtained by Al-Miahy and Saleh [1], where cholesterol concentrations in the serum of lactating cows were significantly decreased compared to pregnant cows. Other researchers, such as Hagawane et al. [14], found that the cholesterol level increased in cows during lactation. According to these authors, the rise in plasma cholesterol in lactating cows

Table 1. Results of biochemical analyses (Mean ± SD) at different stages of pregnancy

Parameter	Mean ± SD	Max–Min	Mean ± SD	Max–Min	Mean ± SD	Max–Min	Mean ± SD	Max–Min
CHO $g.l^{-1}$	1.17 ± 0.39	1.86–0.30	1.05 ± 0.18	1.330.84	1.10 ± 0.32	1.66–0.61	1.02 ± 0.38	1.58–0.37
TG $g.l^{-1}$	0.23 ± 0.09	0.38–0.12	0.26 ± 0.08	0.36–0.16	0.20 ± 0.04	0.29–0.14	0.27 ± 0.08	0.39–0.16
TP $g.l^{-1}$	68.03 ± 9.66	80.40–53.57	63.65 ± 13.24	86.77–40.76	61.87 ± 14.03	72.92–29	61.34 ± 10.178	80.57–46.72
Alb $g.l^{-1}$	13.56 ± 2.32	16.10–9.10	14.05 ± 3.41	19.60–8.85	14.60 ± 2.27	18.05–10.35	13.81 ± 1.79	17.60–9.80
Glob $g.l^{-1}$	54.47 ± 10.79	69.70–40.07	49.59 ± 15.49	78.12– 22.39	47.27 ± 13.84	58.30–15.40	47.53 ± 10.67	67.02–29.12
Urea $g.l^{-1}$	0.07 ± 0.01	0.10–0.05	0.08 ± 0.01	0.09–0.05	0.08 ± 0.01	0.10–0.06	0.06 ± 0.02	0.10–0.04
AST $IU.l^{-1}$	59.50 ± 34.02*	117.8–19.83	43.17 ± 2.77	79.92–1.17	40.19 ± 32.55	97.42–2.33	38.01 ± 30.88*	86.33–4.08
ALT $IU.l^{-1}$	41.80 ± 16.78**	73.50–9.33	29.82 ± 7.29	47.83– 24.50	22.63 ± 10.92**	36.75–8.75	32.26 ± 11.51	55.42–15.17
ALP $IU.l^{-1}$	129.31 ± 31.52	172.7–86.9	124.44 ± 42.77	191.4–68.02	112.64 ± 59.42	279.4–53.9	113.05 ± 76.67	361.9–55
Ca $mg.l^{-1}$	87.93 ± 10.17	104.67–79.10	97.51 ± 26.65	165.03–74.70	99.94 ± 12.74*	120.6–72.48	82.21 ± 16.86*	117.9–54.84
P $mg.l^{-1}$	87.72 ± 16.82	126.14 75.24	84.51 ± 15.25	98.52–50.65	79.48 ± 13.69	107.01–1.53	79.98 ± 14.99	99.25–55.93
Mg $mg.l^{-1}$	52.97 ± 25.81	108.67–29.01	53.48 ± 28.51	98.12–13.14	43.19 ± 12.87	57.03–20.18	45.63 ± 29.36	102.58–7.62

Mean ± Standard deviation (SD), and Maximum – Minimum (Max–Min) values are reported.
Significance of differences: *$P < 0.05$; **$P < 0.001$;

might be a physiological adjustment to meet the demands of lactation. The fluctuations in serum cholesterol levels in cows during the peripartum period could be attributed to a physiological adaptation to meet the requirements of lactation, similar to animals on a restricted diet [11].

In our study, the average triglycerides (TG) obtained was higher, compared to the reference values of Radostits et al. [29]. Despite this difference, we found no noticeable variation in plasma triglyceride levels among the different cow groups, which may be attributed to the feed consumed by the cattle.

Interestingly, the plasma triglyceride (TG) concentration in the pregnant group did not show any significant difference, compared to that of the lactating group, contrary to the findings of Kristanto et al. [20]. In a similar vein, Ashmawy [2] observed higher TG concentrations in pregnant buffaloes than during lactation, and [10] noted that TG concentrations were elevated in the three weeks prior to parturition compared to the lactation phase. These authors associated these noteworthy fluctuations in TG concentration in cows with increased energy requirements during lactation. Conversely, the study by Chillar et al. [8] suggested that TG concentrations were influenced by the cows' body condition score (BCS), with higher levels recorded in cows with a BCS above 3.5.

Based on the protein content of the animal's feed, the total protein (TP) content in cows' blood plasma can serve as a hidden indicator of the animal's nutritional status, as it reflects nitrogen metabolism in the organism [21].

The TP levels observed in the studied cows were similar to the values reported for healthy cattle, ranging from 6.2 to 8.2 $dg.dl^{-1}$, as documented by Khahn and Line [18]. Throughout the research period, no significant changes in total protein concentration were observed in these cows. However, the mean plasma TP was slightly decreased in late pregnancy, non-pregnant, and lactating cows, which is consistent with the findings of Al-Miahy and Saleh [1], who also reported no variation in serum total protein, between pregnant, and non-pregnant cows.

For Holstein cows, Yaylak et al. [34] recorded lower protein values during the dry and early stages of lactation. Conversely, the study by Pouroucholtamane et al. [28] showed an increase in serum total protein in non-lactating yaks.

Regarding the mean concentration of serum albumin (Alb) in the cow groups of our study, no significant difference was observed, but the mean concentration of serum Alb was lower than the physiological level for cows, as reported by Radostits et al. [29]. This finding contrasts with previous studies on dairy cows [10, 24]. The lower serum Alb levels found in our study could indicate that the animals in these groups had a suboptimal digestible crude protein status. According to Herdt [16], the long-term circulation levels of Alb can serve as an indicator of the quantity of digestible crude protein.

In the first trimester of pregnancy, the plasma globulin (Glob) concentration in our cows was higher than the physiologically normal amount described by Radostits et al. [29]. These findings differ from those obtained by Gianesella et al. [13] and Mohammed [26], who found that the breastfeeding stage significantly affects Glob concentrations. Our findings align with those reported by Aziz and Mujalli [4], who discovered that in dairy cows, lactation was accompanied by a decrease in Glob concentration compared to the non-lactation phase.

In our study, no statistically significant differences in blood urea nitrogen concentration were observed in the cows, and the urea values recorded fell within the normal limits as reported by Radostits et al. [29]. This indicates that the feed protein was efficiently utilized by the rumen microflora [3].

These results are in line with those of Celeska et al. [6], who found no variations in urea levels between prepartum and postpartum Frisian-Holland cows. Additionally, our findings differ from those of Djokovic et al. [10], who reported that urea levels in pre-partum and late pregnancy were higher than during the lactation period.

Urea is a valuable and accurate measure of protein or energy imbalance and protein utilization efficiency. Blood or milk sera can be used to assess the relationship between dietary protein levels and reproduction [31].

Liver function significantly influences the levels of numerous blood parameters and various physiological systems. Liver enzymes, such as AST and ALT, are commonly used in medicine as indicators of liver function [10].

In our study, the enzyme activity of AST was found to be lower than the reference range, with a significant difference between non-pregnant and pregnant cows in the first trimester ($P < 0.05$). On the other hand, the mean activity of ALT in our research remained within the limits of normal values, as reported by Radostits et al. [29], with a significant difference between early and late pregnancy ($P < 0.001$).

Das et al. [9] observed no significant alteration in the concentration of AST and ALT among the three groups at different stages of lactation. In contrast, Khadim and Ali [17] reported that ALT activity decreased during the latter three months of pregnancy until delivery, and increased during the lactation period. Higher levels of AST during cows' lactation may indicate the presence of fatty liver syndrome, ketosis, or a lower dry matter intake. Additionally, AST activity in dairy cows can change throughout early lactation and gestation due to metabolic events [17].

According to Kupczynski and Chudoba–Drowdowska [21], variations in ALT activity do not provide enough information to assess the nature, scope, or reversibility of hepatic alterations. An increase in the activity of these enzymes is often associated with disorders of energy metabolism.

According to Radostits et al. [29], the alkaline phosphatase (ALP) activity observed in the cows under investigation remained within appropriate limits, with no statistically significant variations in blood levels between the groups as reported by Kadhim et al. [17]. These researchers observed heightened ALP levels in pregnant cows, suggesting a potential association with the presence of the corpus luteum, responsible for progesterone production and the establishment and maintenance of pregnancy. Conversely, Sharef et al. [30] noted a significant increase ($P < 0.05$) in ALP activity during late pregnancy compared to other periods. In contrast, Das et al. [9] reported no significant alteration in ALP concentration among the groups at different stages of pregnancy. Regarding calcium levels, our results were within the normal range recorded for healthy cattle by Kahn and Line [18], with an increase in late pregnant cows, a significant difference was observed between mid and late pregnancy ($P < 0.05$), which is in agreement with Mohammed et al. [26] in crossbred dairy cows. They demonstrated that there was no significant variation in blood calcium levels throughout pregnancy. The study of Fadlalla et al. [12] also reported no appreciable differences in calcium content in dairy cows at various physiological phases. On the other hand, Djokovic et al. [10] found that the concentration of calcium in female cattle in the lactation group was significantly higher than in pregnant cows.

The mean value of phosphorus (P) did not show a significant difference between the groups of pregnant and non-pregnant cows. Our investigation's findings demonstrate that P levels were within the acceptable ranges reported by Latimer et al. [22]. These results are consistent with those of Djokovic et al. [10] and Mohammed et al. [26], who found no variations in P concentration between pregnant and lactating cows. The amount of phosphorus in the diet is believed to be reflected in the blood phosphorus levels [33], and the analysis of our study's results suggests that the cows' diet strongly influenced blood phosphorus concentration.

Regarding blood serum magnesium, the mean concentration in all groups was higher than the normal range, indicating an adequate daily supply of magnesium and good absorption of magnesium in the cattle's digestive tract. As mentioned in the literature, the concentration of magnesium in livestock blood is influenced by the magnesium content of the feed or magnesium intake and its absorption in the rumen [10].

However, our results contradict the findings of Mohammed et al. [25], who showed that magnesium levels in lactating animals were higher than those in pregnant animals. Fedlalla et al. [12] also stated that the concentration of magnesium in the prepartum period was higher than in the postpartum period, with no significant change during lactation.

Based on the study's findings, all groups of cattle exhibited mean blood serum magnesium concentrations that were higher than the considered normal range. This suggests that the cattle have a sufficient daily intake of magnesium and that their digestive systems absorb magnesium well. As supported by the literature, the amount of magnesium present in the feed or the amount consumed and absorbed in the rumen can influence the concentration of magnesium in the blood of cattle [10].

However, our results are in contrast to those of Mohammed et al. [26], who demonstrated that magnesium levels are higher in lactating animals compared to pregnant ones. Additionally, Fadlalla et al. [12] reported a higher concentration of magnesium during the prepartum phase.

CONCLUSIONS

In Algeria, dairy cattle breeding play a crucial role in the agricultural economy. To improve this sector, the government has recommended importing heifers with high genetic poten-

tial. However, production and reproductive performance in the country are currently below their true potential. Moreover, genetic merit is significantly influenced by non-genetic factors and can vary across different environments.

Western Algeria, with its focus on agriculture and cattle breeding, has chosen to prioritize dairy farming. To promote successful breeding, it is essential to manage and control reproduction and production according to local conditions. In our study, we did not find significant variations among the biochemical profiles during different stages of pregnancy, and most values fell within the reference intervals. These findings are indicative of the herd's good management in terms of feeding and breeding conditions.

Nutritionists may find value in this study as blood biochemical analysis is commonly used to identify dietary factors affecting productivity in dairy cows. This research contributes valuable insights that can aid in optimizing feeding practices and overall herd management.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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COMPARISON OF NDVI-2 VACCINE EID_{50} TITRE VALUES AFTER THREE WEEKS OF FREIGHT UNDER COLD MONITORED STORAGE CONDITION

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ABSTRACT

The EID_{50} titres per dose values of three batches of NDVI-2 were determined as log_{10} EID_{50} 7.7, 7.6 and 6.8 after production, against reference EID_{50} 5.5 per dose. These values were log_{10} EID_{50} 2.2, 2.1 and 1.3 above the reference EID_{50} for NDVI-2. These vaccines were re-evaluated at PANVAC laboratory Debre-Zeit, prior to release. The initial EID_{50} values were compared with EID_{50} values obtained by the certifying laboratory, after three weeks of cold monitored freight. The packaged vaccine cold storage temperature of +1.9 °C prior to the shipment, was recorded with the aid of a temperature logger. The temperature logger was set to record and retain packaged vaccine cold storage temperature at hourly intervals during the freight. At the end of three-week freight, the temperature logger readings were analysed, and temperature range of -2.48 °C to 7.7 °C was observed throughout the freight. However, sudden rise in packaged vaccine storage temperature from 7.7 °C to 22.28 °C was noticed on the last day of freight. The initial EID_{50} titre per dose values, when compared with EID_{50} log_{10} 6.5, 6.8, and 6.6 per dose obtained by the certifying laboratory after the cold monitored freight and re-evaluation, showed EID_{50} titre loss of log_{10} EID_{50} 1.2, 0.8 and 0.6, respectively. The observed EID_{50} loss could not invalidate these vaccine batches, considering that these had an initial EID_{50} titre per dose values that were well above the reference EID_{50} titre for NDVI-2. In Nigeria, and to our knowledge, this is the first recorded monitored cold storage temperature transit for NDVI-2.

Key words: monitored cold storage conditions; NDVI-2 compared EID_{50} potency value; three weeks of freight

INTRODUCTION

Newcastle disease (ND) aetiologic agent is the Newcastle disease virus (NDV), also known as type 1 avian paramyxovirus, a member of the genus *Avulavirus* of the *Paramyxoviridae* family [5, 8, 13]. This is a single stranded, enveloped, negative sense RNA virus [8, 13]. Newcastle disease affects all avian species, however, outbreak of ND in unvaccinated poultry flock results in severe losses [4, 6]. Mortality rate of 75 % to 100 % in unvaccinated poultry flock has been reported [16].

Due to poor cold keeping conditions in developing countries such as Nigeria, as a result of inadequate electricity supply especially in the rural communities across

the country, the NDVI-2 vaccine was developed to bridge this gap [9, 17]. The avirulent thermostable Newcastle disease vaccine virus strain (NDVI-2) was tested in several laboratories across the world and has proven to be efficient against the local virulent strains of the ND virus [2,14]. Thus, it has been recommended for use in developing countries for the protection of rural backyard poultry flock against severe consequences of Newcastle disease infection [9]. Vaccination against ND has been shown to be an efficient control strategy against ND outbreak in susceptible poultry flocks [3, 7, 14, 16]. Strategic Newcastle disease control through vaccination require the use of wholesome, safe, efficacious and potent ND vaccine [10]. Therefore, careful handling of vaccine according to the required standard method in regards to cold storage condition of vaccine after production till the time of use, is critical in effective ND vaccination control strategy, especially in Newcastle disease endemic regions. The strategic Newcastle disease control programme in endemic regions will involve series of vaccinations and re-vaccinations in accordance with stipulated NDVI-2 vaccination schedule [10]. Thus, vaccine cold storage and cold keeping condition during transit, till the time of use is critical for an efficient Newcastle disease eradication programme. Poor vaccine cold storage and handling can significantly contribute to vaccine EID_{50} titre loss. Therefore, this study was carried out to examine the potential EID_{50} titre loss for NDVI-2 vaccine after an extended transportation under monitored cold keeping conditions with minimal alterations in packaged vaccine cold storage temperature.

Our study revealed that within a monitored cold storage transportation with evidence of alterations in vaccine cold storage condition as recorded by the temperature logger, EID_{50} titre drop or loss is possible for the NDVI-2 thermostable vaccine. Thus, cold storage condition at all time is critical and essential in vaccine EID_{50} titre longevity irrespective of vaccine thermostable and thermolabile nature.

MATERIALS AND METHODS

Vaccine source

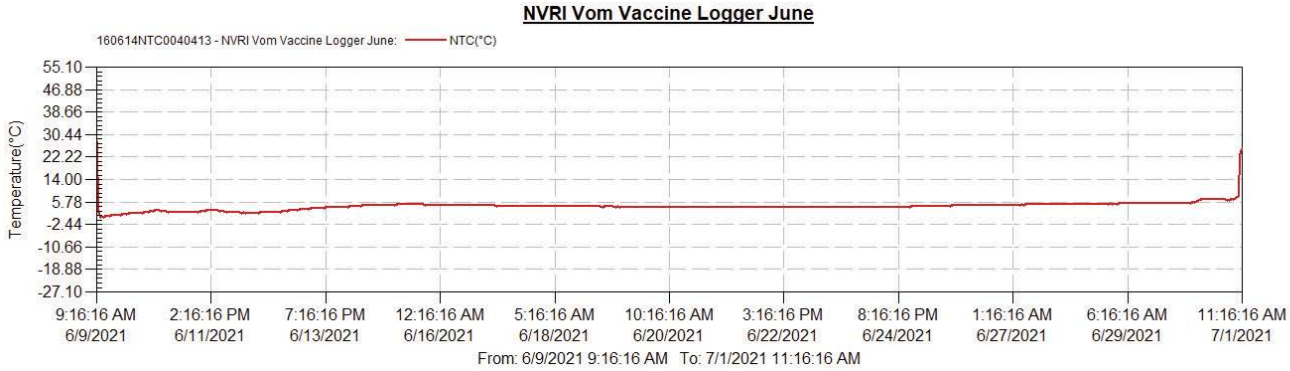
Samples of three batches of NDVI-2 vaccine produced at the National Veterinary Research Institute, Vom, Nigeria, were titrated to determine their EID_{50} titre per dose values after production. The vaccine is lyophilized live attenuated, packaged in 50 doses per vial and administered through the intra ocular route.

Determination of EID_{50} values

The EID_{50} titre per dose values of three batches of Newcastle disease (NDVI-2) vaccine of 50 dose produced by the National Veterinary Research Institute (NVRI), Vom, were determined after titration and inoculation into 10-day old embryonated chicken eggs. The inoculated eggs were incubated in a humidified incubator at 37 °C following the World Organization of Animal Health (WOAH) Newcastle disease live vaccine titration protocol. The NDVI-2 vaccine EID_{50} titre per dose values (virus concentration per dose) were determined according to Karber method [12]. The original vaccine EID_{50} titre per dose values obtained after production were consequently compared to EID_{50} titre per dose values obtained after re-evaluation or re-validation by the Pan African Vaccine Centre (PANVAC) laboratory, Debre-Zeit, Ethiopia, which is the certifying laboratory, prior to release for field veterinary deployment.

RESULTS

The temperature monitor logger was set to monitor, record and store the packaged vaccine cold storage temperature at an hourly interval during the vaccine freight to the certifying laboratory. The temperature logger readings showed steady temperature maintenance throughout the vaccine three-week transit, with minor temperature variations. The temperature logger recorded temperature of 27.2 °C (Fig. 1) (the laboratory room temperature) prior to insertion into the cold storage packed vaccine shipment box. The vaccine cold storage condition from the temperature logger reading range between -2.48 °C to 7.7 °C throughout the duration of the vaccine three-week freight (Fig. 1); from June 9, 2021 to July 1, 2021. The cold storage temperature of the vaccine during the freight was not adversely altered. At the time of the initial temperature reading on the day of freight (June 9), the temperature logger recorded +1.9 °C and the final reading on July 1 was +7.7 °C. However, a sudden rise in temperature on July 1 (last day of the vaccine transit) was equally observed with the packaged vaccine temperature rising from 7.7 °C to 22.28 °C (Fig. 1). This sudden rise in temperature was



Temperature in °C monitored during the freight is indicated vertically while, freight time duration is indicated horizontally

Fig. 1. Three-week monitored temperature logger chart from June 9 to July 1

attributed to possible custom clearance handling at the port of entry, and probably the vaccine transportation from the airport at Addis Ababa to Debre Zeit. Though the full event at this stage that triggered the significant rise in temperature from 7.7 °C to 22.28 °C was not understood or studied. Thus, the use of temperature logger, should where possible be incorporated in vaccine storage and transport as it will reveal true vaccine cold temperature keeping condition prior to NDVI-2 vaccine use.

The NDVI-2 vaccine batches showed EID_{50} titre per dose value of $\log_{10}$ EID_{50} 7.7; 7.6; and 6.8 per dose after production (Table 1); which is 2.2, 2.1 and 1.3 logarithm above the recommended standard reference EID_{50} 5.5 titre per dose value for NDVI-2 vaccine. However, logarithm titre loss of 1.2, 0.8 and 0.6 titres were equally observed after the extended monitored transit (Table 2). The temperature logger showed evidence of alteration or variations in the vaccine cold keeping temperature (Fig. 1). Furthermore, despite the obvious titre loss of $\log_{10}$ EID_{50} 1.2, 0.8 and 0.6 respectively (Table 2) after comparison of EID_{50} per dose titre values obtained by both laboratories, the vaccine batches EID_{50} titre per dose value after transit, were well above the minimum reference $\log_{10}$ EID_{50} 5.5 titre value for NDVI-2 intended for field veterinary use. Thus, the EID_{50} titre per dose value loss was not enough to invalidate these NDVI-2 vaccines batches, after the extended transit and re-testing for certification. This finding further demonstrates that a naturally thermostable vaccine is prone to EID_{50} titre value drop, with evident minimal alterations in temperature.

Table 1. NDVI-2 vaccine EID_{50} titre per dose value after vaccine production

EID_{50} titre per dose value after vaccine production	EID_{50} titre per dose value above recommended standard reference value after vaccine production	Reference EID_{50} titre per dose value for NDVI-2
$\log_{10}$ 7.7 per dose	$\log_{10}$ EID_{50} 2.2 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 7.6 per dose	$\log_{10}$ EID_{50} 2.1 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 6.8 per dose	$\log_{10}$ EID_{50} 1.3 per dose	$\log_{10}$ EID_{50} 5.5 per dose

Table 2. Vaccine *EID_{50}* titre per dose value after three-week freight and re-evaluation by the certifying laboratory

EID_{50} titre per dose value after vaccine re-evaluation	EID_{50} titre per dose value loss after cold monitored freight and re-evaluation	Reference EID_{50} titre per dose value for NDVI-2
$\log_{10}$ 6.5 per dose	$\log_{10}$ 1.2 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 6.8 per dose	$\log_{10}$ 0.8 per dose	$\log_{10}$ EID_{50} 5.5 per dose
$\log_{10}$ 6.2 per dose	$\log_{10}$ 0.6 per dose	$\log_{10}$ EID_{50} 5.5 per dose

DISCUSSION

The vaccination of immunodeficient poultry flock with Newcastle disease vaccines especially NDVI-2 has been established and demonstrated to be a major control strategy against ND pillage in poultry population in endemic region [3, 7, 14, 15, 16]. Thus, the use of various forms of ND vaccines at various stage of poultry flock growth has been inculcated towards achieving this strategic Newcastle disease control objective.

The thermostable nature of the NDVI-2 vaccine has an additional edge over other Newcastle disease thermolabile vaccines such as the intra-ocular, Komarov and Lasota. Thus, the use of ND thermostable NDVI-2 vaccine has

been suggested by [9, 11] in situations where ND vaccine cold chain maintenance is difficult. The use of the NDVI-2 thermostable vaccine under poor cold storage conditions does not entail or encourage complete or absolute negligence of standard vaccine cold storage condition; despite the NDVI-2 vaccine thermostable nature. This is also in agreement with the findings of [10] where it was reported that the thermostable nature of the NDVI-2 vaccine does not exempt it from EID_{50} titre value drop. It is therefore important to note that a thermostable vaccine must be handled with same care accorded to other thermo-sensitive biological products; thus, thermostable vaccine such as NDVI-2 cannot be expose to sunlight and frequent alterations in temperature and still be expected to remain potent [1]. It has also been observed and reported that variation in temperature can alter NDVI-2 vaccine viability especially if the vaccine cold storage temperature is altered [1]. Thermostable and thermolabile vaccines require good vaccine cold storage condition from the time of production to the period of vaccine veterinary use. In many cases this cold keeping conditions are not strictly adhered to; especially in regions of the world where electricity supply is grossly inadequate; for appropriate vaccine storage especially in rural settings.

Furthermore, efficaciousness of NDVI-2 vaccine in field situation depends on its wholesomeness, safety, potency, with standard, and acceptable EID_{50} titre per dose value, thus, vaccine cold keeping condition and handling from the time of production to the time of veterinary field application; especially where the vaccine use is geared toward Newcastle disease strategic control, and eradication initiative cannot be overemphasized.

The findings of our study revealed that at a temperature range of +1.9 to 22.28 ^{o}C under monitored cold storage condition and with limited observable temperature alterations, the EID_{50} titre drop is most likely; based on the readings of the temperature data logger (Fig. 1) and the compared EID_{50} titre values obtained by both laboratories. Table 1 shows the NDVI-2 vaccine EID_{50} titre value after production while (Table 2) shows the NDVI-2 vaccine EID_{50} titre value after extended transit and re-evaluation by the certifying laboratory. Table 2 shows observable EID_{50} titre value loss of $\log_{10}$ 1.2, 0.8 and 0.6 EID_{50} per dose respectively, after three weeks of freight. The record of the temperature logger analysis, showed that the vaccine cold storage condition was fairly maintained throughout the duration of the freight with minimal temperature variations, but a significant rise in temperature was equally observed on the day the vaccine was moved from the port of entry to the certifying laboratory (Fig. 1).

The re-validated vaccine final EID_{50} titre per dose values were well within the acceptable EID_{50} potency titre value for NDVI-2 (Table 2), despite the observable EID_{50} titre loss of $\log_{10}$ 1.2, 0.8 and 0.6 respectively. The vaccine potency EID_{50} per dose titre values were well above the recommended $\log_{10}$ 5.5 EID_{50} titre per dose reference for NDVI-2. The event of July 7, 2021, showed a sudden and significant rise in temperature based on the temperature logger data, although, the full event that led to this rise in temperature was not studied; however, it was attributed to possible clearance delay by customs service at the point of entry and probably to delay during the road trip from Addis Ababa to Debre Zeit.

The extra EID_{50} titres per dose value of $\log_{10}$ 2.2, 2.1 and 1.3 per dose (Table 1), incooperated into these NDVI-2 vaccines batches while being compounded as observed in this study, were able to carter for the vaccine EID_{50} titre loss observed (Table 2). Our finding and that of [10], is a further confirmation of the robustness, and integrity NDVI-2 vaccine, produced by the National Veterinary Research Institute Vom, Nigeria. It is thus suggested that for all vaccines, be it thermolabile or thermostable, that extra EID_{50} value of about 1 to 2 EID_{50} logarithm values be in-cooperated to carter for possible EID_{50} titre loss that might occur due to poor cold vaccine storage conditions, especially in regions of the world with limited, or poor electricity supply.

CONCLUSIONS AND RECOMMENDATIONS

Our study revealed that irrespective of the Newcastle disease vaccine thermostable nature, its cold storage condition from the time of production till time of use, should be accorded recommended vaccine cold storage condition. Therefore, having demonstrated EID_{50} titre loss in thermostable NDVI-2 vaccine under limited variations in vaccine cold storage conditions; the need for in-cooperation of extra EID_{50} titre per dose value during vaccine production cannot be overemphasized, since vaccine poor handling, and possible temperature alterations during transit in the field situation, cannot be ignored, or treated with levity

by the Newcastle disease vaccine end users. The vaccine producers must insist on strict adherence to vaccine cold keeping conditions at the point of purchase.

Based on our findings, being the first reported NDVI-2 vaccine transportation under cold storage monitored condition in Nigeria; we recommend the use of temperature logger for vaccine cold storage monitoring prior to NDVI-2 vaccine veterinary field deployment and use.

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COMPETING INTEREST

Authors declare no competing interest.

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MONITORING OF REPRODUCTION ACTIVITY ON ALGERIAN DAIRY CATTLE FARMS

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ABSTRACT

This study aimed to evaluate the reproductive monitoring activity in Algerian dairy cattle herds. A participatory survey was conducted with 75 veterinarians and inseminators affiliated with the National Centre for Artificial Insemination and Genetic Improvement (CNIAAG). Reproduction monitoring represents a primary activity for 12 % of the veterinarians. Only 10.8 % of veterinarians earn more than 50 % of the yearly global revenue from reproductive activities. 85.3 % of the veterinarians fix the herd monitoring objectives, 64 % use a paper notebook to manage breeding data, and only 22 % establish a reproduction statute inventory. The most requested information before the visit is the last mating (80 %), oestrus (80 %), and calving (90.7 %) dates. Monthly routine fertility visits were practiced by 32 % of the interviewed veterinarians, and only about half of them (52 %) checked the cows systematically at post-partum. The pregnancy diagnosis is practiced by 72 % of veterinarians at 56.71 ± 22.82 days postpartum and confirmed at 91.85 ± 26.94 days postpartum. Furthermore, 73 % of the respondents systematically examined non-pregnant cows after 3 matings; however, only 48 % simultaneously recorded the cows' body condition score. Regarding postpartum genital pathology control 38.7 %, 22.7 %, and 26.7 % of veterinarians always perform manual transrectal exploration of the genital tract, vaginoscopy, and ultrasonography, respectively. While, 73 % of them examine the cows' cyclicity when evaluating uterine involution. A substantial proportion of veterinarians included monitoring feed, milk quality/mammary disease, and livestock diseases, in their regular visits. The dairy farmers must be advised of the economic benefits of routine fertility monitoring to achieve good herd reproductive traits.

Key words: dairy cattle; fertility; population medicine; reproduction monitoring; veterinary practices

INTRODUCTION

One aspect of the globalization of dairy production and the unrestrained competition between giant dairy producers, and individual producers of "popular milk", is the control of dairy herd reproduction [25]. Technical management issues affect Algerian dairy production, as seen by the low milk output, which falls short of demand,

and the large importation of milk powder, which cost the nation 800 $ million in 2016 [11]. Milk production cannot be optimal without reproducing, due to physiological interactions, between lactation, and reproduction [2]. Performances in reproduction, and productivity, significantly impact dairy farmers' economic success [34]. These tasks require proper management and monitoring [22]. Therefore, population medicine in dairy cattle herds offers valuable tools, to enhance dairy-cow-herd health and reproductive management. Monitoring is a program planned, and coordinated between the breeder, and the veterinarian, to allow intervention at the most appropriate time, while limiting factors that affect health, preventing problems, and improving reproduction [28]. This includes assessing fertility metrics, such as the calving-to-first-service period, a crucial period ideally lasting fewer than 70 days. Monitoring days open, as an extended period negatively impacts calving intervals, and leads to economic losses. The length of the calving interval, a key aspect of reproductive performance, significantly influences livestock production, with an optimal duration targeted at 12 to 13 months. Additionally, monitoring the conception rate, at the first artificial insemination, is crucial for reproductive success [16]. The present study's aim was to carry out a survey, with veterinarians, and cattle inseminators, to investigate current reproduction monitoring organizations, and practices, in dairy cattle herds, and to explore the factors limiting the development of this activity on Algerian cattle farms.

MATERIALS AND METHODS

Study design

The present research was based on a questionnaire containing 32 questions. The survey was first delivered by hand on paper to veterinary practitioners during professional meetings about the Brucellosis Health Statute, at the Institute of Agricultural and Veterinary Sciences of Souk Ahras University, Algeria. From 80 veterinarians who received the survey manuscript during this phase, 32 veterinarians (37.5 %) participated in the survey. For the second time, the survey was distributed on an online platform (Google Forms), during the period between September 2021 and August 2022. Also, veterinarians affiliated with, the National Centre for Artificial Insemination, and Genetic Improvement (CNIAAG) were notified about the survey by e-mail to get more accurate results. Indeed, a total of 75 practicing veterinarians, and cattle inseminators, belonging to twenty-four Algerian departments were effectively involved in this study.

Survey description

These data were collected, after approval was received from competent authorities, using a standardized semi-structured questionnaire by an individual, or focus group participatory surveys with the participants in the six questionnaire sections: veterinary office activity, reproduction monitoring, animal examination, post-partum pathologies, reproduction monitoring organization, and future development of reproduction monitoring in dairy herds.

The first section checklist, contained different questions, including the number of practitioners, and those who just do the reproductive monitoring, Likert-scale questions about the bovine reproductive activity as a percentage, the annual proportion of global income from reproductive activities, and the main activity (milk, feedlot, reproduction, or milk/ reproduction activity).

The second section checklist contained questions about the experience in veterinary practice, the number of farms with regular and occasional interventions, as well as their evolution (increasing, stagnating, and decreasing). Furthermore, Likert-scale questions were asked about time spent on reproduction monitoring, as a percentage of overall time, and income from reproductive activities as a percentage. Multiple-choice questions were used to investigate requested information, before the farm visit, (calving dates, oestrus dates, insemination dates, and individual dairy production) and tools used to perform reproduction data recording, (papers, documents, software, personal tools).

The examination of animals was the subject of the third section. Multiple-choice questions were asked about animal selection (veterinarian, breeder, or both), categories of bovine animals examined, as part of breeding monitoring, pregnancy diagnosis, and confirmation (yes, no, delay), use of ultrasonography (yes, no, costs), examination of non-pregnant cows after three matings, (systematically realized, not realized), and simultaneous scoring of body condition when examining non-pregnant cows, after three matings (always, sometimes, never).

The fourth section asked a multiple-choice question about diagnosis tools, manual genital exploration, vagi-

noscopy, ultrasonography (systematically, sometimes, or never used), or other techniques. This section also included the question about examining cow cyclicity during uterine involution control (Yes, No).

In the fifth section, questions about activity billing, variability of a flat rate, the frequency of the average intervention per farm (monthly, bimonthly), closed questions about the determination of objectives, modalities of monitoring work collaboration, the establishment of reproductive assessment, and integration of feed monitoring, milk quality/mastitis monitoring, and livestock pathologies during routine visits.

The last section contained questions about the evolution of monitored dairy farms during the last five years, veterinary tendencies, prospecting for the development of reproductive monitoring activity (remain in the state, could develop a little or a lot), and finally, Likert scale question about constraints related to reproduction monitoring activity development (1–5).

Statistical analysis

These data were validated, after an initial exclusion of incongruent declarations, before data processing and analysis. Statistical analyses were conducted on collection using SPSS® Statistics 26 (IBM Corp, Armonk, NY). Descriptive statistics, including the average, percentages, and standard deviation, were carried out. The categories defined in each variable are considered to occur in an equitable manner. The asymptotic p-value was calculated using an approximation to the true distribution. We retained this hypothesis at $P > 0.05$ and rejected it at $P < 0.05$.

RESULTS

Veterinary office activities

The results of our study indicate that in Algerian veterinary clinics, a single veterinarian works in 57 % of clinics, and 80 % of these veterinarians perform reproductive activities. Reproduction activity constitutes 40 % of the interventions in 16 % of the veterinary offices. 21.3 % of the participants have an income rate based on reproduction activity greater than 50 %. Additionally, 69.3 % of veterinary clinics perform mixed activities, and only 12 % dominant reproductive activities (Table 1).

Table 1. Characteristics of veterinary offices involved in cattle reproduction activity

Variables	Modalities	Frequency	Percentage	P
Number of practitioners per veterinary office (%)	1	43	57.3	**
	2	25	33.3	
	3	6	8.0	
	4	1	1.3	
Number of practitioners engaged in the reproductive activity (%)	1	60	80	***
	2	15	20	
Income percentage by reproduction activity per year (%)	50%	13	17.3	***
	60%	8	10.7	
	70%	1	1.3	
	80%	4	5.3	
	90%	3	4.0	
Type of bovine activity (%)	Dominant reproduction	9	12.0	**
	Dominant milk activity	10	13.3	
	Dominant meat activity	4	5.3	
	Mixed activity	52	69.3	

Significance: The categories defined in each variable occur in an equitable manner: $P > 0.05$ retains the hypothesis; $P<0.05$ rejects the hypothesis; *P <0.05; **P <0.01; ***P <0.001.

Reproduction monitoring

The average number of cattle farms where the veterinary office carries out breeding monitoring, a regular and occasional activity is 14, 8, and 13, respectively. Inquired veterinarians had a variety of experiences ranging from 1 to 33 years (Table 2). The percentage of activity time occupied by the breeding monitoring is very often limited between 10 and 50 % (93.4 %). The dates of the last mating (80 %), the dates of oestrus (80 %), and the dates of calving (90.7 %)are the pieces of information that veterinarians most frequently inquire about before a visit. 64 % of veterinarians use paper media to manage reproductive data (Table 3).

Table 2. Reproduction practice of veterinary offices and their experience in the routine reproduction monitoring activity

	N	Minimum	Maximum	Mean	SD
Number of cattle farms where the veterinary office intervenes for reproduction failure	69	1	300	14.57	38.50
Number of farms with occasional intervention	48	1	100	13.33	20.40
Number of farms with regular intervention	58	1	30	8.20	7.45
Veterinary experience in reproduction monitoring activity (years)	75	1	11	3.41	2.772

SD – standard deviation

Table 3. Importance and practice of reproduction monitoring activity by the veterinary offices

Variables	Modalities (%)	Frequency	Percentage	P
Percentage of activity time occupied by reproductive monitoring	10	14	18.7	Ns
	20	8	10.7	
	30	24	32.0	
	40	12	16.0	
	50	12	16.0	
Percentage of income in bovine activity occupied by reproductive monitoring	10	17	18.7	Ns
	20	7	9.3	
	30	18	24.0	
	40	8	10.7	
	50	17	22.7	
Type of information requested before the visit	Individual dairy production	36	48	*
	Last mating dates	60	80	
	Oestrus dates	60	80	
	Calving dates	68	90.7	
	Other information	29	38.7	
	No information	1	1.3	
Tools used to perform reproduction data management	Software	5	6.7	***
	Personal tool	17	22.7	
	Paper documents	48	64.0	
	Any tools	5	6.7	

Significance: The categories defined in each variable occur in an equitable manner: P > 0.05 retains the hypothesis; P < 0.05 rejects the hypothesis; *P < 0.05; **P < 0.01; ***P < 0.001; Ns – not significant

When it comes to the examination of animals during visits, the breeder and veterinarian jointly decide which animals will be checked, only half (52 %) of veterinarians routinely examine postpartum cows, 82.7 % consult problem cows before the systematic check-update, and 73.3 % systematically evaluate cows treated during the previous visit. When diagnosing pregnancy, 80 % of clinicians perform a pregnancy report on mated females, and 72 % confirm it (Table 4), at 56.71 ± 22.82 and 91.85 ± 26.94 days, respectively (Table 5). Additionally, 25 % of vets use ultrasonography, which may be done for between 1500 and 3000 days (between $10 and $20) (Table 5).

On the other hand, 73 % of respondents routinely examine non-pregnant cows after three copulations, but only 48 % concurrently record the cows' body condition. In cases of pathological anoestrus, only 48 % of veterinarians always note body conditions when examining cows not seen in the postpartum oestrus (Table 4).

Table 4. Reproduction monitoring activities related to animals' examination and management and pregnancy diagnosis

Variables	Modalities	Frequency	Percentage	P
Selection of females for examination during breeding monitoring: decision making	Veterinarians	10	13.3	**
	The breeder	18	24.0	
	Both	47	62.7	
Cow's categories examined as part of the breeding monitoring	Systematic postpartum cows' control	39	52	Ns
	Cows with reproductive problems before systematic control	62	82.7	
	Systematic control of cows treated at previous visit	55	73.3	
Practice of a pregnancy diagnosis on females after mating	Yes	60	80	***
	No	15	20	
Practice of pregnancy confirmation	Yes	54	72	***
	No	21	28	
Examination of non-pregnant cows after three mating (infertile cows)	Systematic	55	73.3	***
	Not realized	20	26.7	
Simultaneous examination of BCS when examining non-pregnant cows after three mating	Always	36	48.0	**
	Sometimes	34	45.3	
	Never	5	6.7	

Significance: The categories defined in each variable occur in an equitable manner. P > 0.05 retains the hypothesis. P < 0.05 rejects the hypothesis. *P < 0.05; **P < 0.01; ***P < 0.001; Ns – not significant

Table 5. Pregnancy diagnosis and conception confirmation

	N	Minimum	Maximum	Mean	SD
Days post-mating for first pregnancy diagnosis	68	20	120	56.71	22.826
Days post-mating for first pregnancy confirmation	54	45	180	91.85	26.940
Costs (AD) for pregnancy diagnosis made by ultrasound	19	1500	3000	2136.36	636.039

SD – standard deviation

Post-partum pathologies

Veterinarians still use the manual transrectal exploration of the genital tract, vaginoscopy, and ultrasound to diagnose postpartum genital pathology in cows, with percentages of 38.7 %, 22.7 %, and 26.7 %, respectively. However, 73 % of them also searched the cyclicity of the cows when examining uterine involution (Table 6).

Table 6. Modalities and tools of dairy cows' examination for post-partum pathologies

Variables	Modalities	Frequency	Percentage	P
Simultaneous scoring of BCS when examining cows not seen in heat postpartum	Always	36	48	**
	Sometimes	34	45.3	
	Never	5	6.7	
Using manual exploration as a tool of diagnosis for cows with genital pathologies	Always	29	38.7	***
	Sometimes	44	58.7	
	Never	2	2.7	
Using vaginoscopy as a tool of diagnosis for cows with genital pathologies	Always	17	22.7	**
	Sometimes	25	33.3	
	Never	33	44.0	
Using ultrasound as a tool of diagnosis for cows with genital pathologies	Always	20	26.7	**
	Sometimes	18	24.0	
	Never	37	49.3	
Using other gynecological examination techniques	Yes	8	10.7	***
	No	67	89.3	
Examination of cows' cyclicity during the control of uterine involution	Yes	55	73.3	***
	No	20	26.7	

Significance: The categories defined in each variable occur in an equitable manner. P > 0.05 retains the hypothesis; P < 0.05 rejects the hypothesis; *P < 0.05; **P < 0.01; ***P < 0.001

Organization of reproduction monitoring

The main factor of variability in flat-rate reproduction monitoring pricing is the number of cows examined (Table 7). Monthly and bi-monthly monitoring visits are carried out by 32 % and 21.33 % of veterinarians. 85.3 % of veterinarians set monitoring objectives, and 44 % collaborate with other vets on farm visits. Only 22 % of veterinarians responsible for monitoring animals establish a reproductive assessment. During routine visits, a significant portion (64 to 80 %) of these veterinarians combine feed monitoring, milk quality/mastitis monitoring, and livestock disease monitoring during regular visits (Table 7).

Table 7. Organization of reproduction monitoring activities

Variables	Modalities	Frequency	Percentage	P
Variability of flat rate pricing in reproduction monitoring activity	Number of examined cows	40	53.3	***
	Number of presented cows	2	2.7	
	Number of calved cows	5	6.7	
	Number of inseminated cows	3	4.0	
	Number of pregnant cows	11	14.7	
	Other	11	14.7	
	No opinion	3	4.0	
The average frequency of intervention per farm during monitoring	Monthly	24	32	***
	Bimonthly	16	21.3	
	Other	32	42.67	
	No opinion	3	4	
Determination of monitoring objectives	Yes	64	85.3	***
	No	11	14.7	
Collaboration during breeding monitoring	Yes	33	44	*
	No	42	56	
Establishment of a reproductive report by the veterinarian responsible for breeding monitoring	Systematically	17	22.67	*
	Sometimes	38	50.67	
	Never	20	26.67	
Integration of feed monitoring into the livestock visit	Yes	57	76.0	***
	No	18	24.0	
Integration of milk quality/mastitis monitoring into the livestock visit	Yes	48	64.0	***
	No	27	36.0	
Integration of the livestock pathologies monitoring into the visit	Yes	66	88	***
	No	9	12	

Significance: The categories defined in each variable occur in an equitable manner. P > 0.05 retains the hypothesis; P < 0.05 rejects the hypothesis; *P < 0.05; **P < 0.01; ***P < 0.001

Future development of reproduction monitoring in dairy herds

A reduction in the number of farms monitored over the past five years is considered by 53 % of veterinarians. 88 % of veterinarians tend to develop the activity, and 52 % of veterinarians think the activity can develop a little. The main constraint for the reproduction monitoring activity development is the breeder's cost (Table 8).

Table 8. Veterinarians' tendency, prospects, and the constraint about reproduction monitoring activity development

Variables	Modalities	Frequency	Percentage	P
Evolution of the number of monitored farms over the last five years	Increasing	15	20	
	Stagnating	16	21.3	**
	Decreasing	40	53.3	
	No opinion	4	5.3	
Veterinarian's tendency to develop reproductive monitoring activity	Yes	66	88.0	
	No	2	2.7	***
	No opinion	7	9.3	
Prospects of veterinarians for the development of the reproduction monitoring activity	Will remain as it is	18	24.0	
	Could develop a little	39	52.0	**
	Can develop a lot	14	18.7	
	No opinion	4	5.3	
Constraint to the development of the reproductive activity related to the availability of the breeder	Very weak	28	37.3	
	Weak	16	21.3	
	Medium	12	16.0	*
	Important	7	9.3	
	Very important	12	16.0	
Constraint to the development of the reproductive activity related to the availability of veterinarian	Very weak	30	40.0	
	Weak	24	32.0	
	Medium	7	9.3	***
	Important	5	6.7	
	Very important	9	12.0	
Constraint to the development of the reproductive activity related to the lack of motivation of breeders	Very weak	30	40.0	
	Weak	12	16.0	
	Medium	8	10.7	
	Important	12	16.0	**
	Very important	13	17.3	
Constraint to the development of the Reproductive activity related to the lack of veterinarians' motivation	Very weak	29	38.7	
	Weak	27	36.0	
	Medium	5	6.7	***
	Important	6	8.0	
	Very important	8	10.7	
Constraint to the development of the reproductive activity related to the lack of veterinarian's technical level	Very weak	24	32.0	
	Weak	24	32.0	**
	Medium	9	12.0	
	Important	10	13.3	
	Very important	8	10.7	
Constraint to the development of the reproductive activity related to organizational aspects	Very weak	25	33.3	
	Weak	18	24.0	**
	Medium	12	16.0	
	Important	5	6.7	
	Very important	15	20.0	
Constraint to the development of the reproductive activity related to high cost for the breeder	Very weak	28	37.3	
	Weak	11	14.7	**
	Medium	9	12.0	
	Important	10	13.3	
	Very important	17	22.7	
Constraint to the development of the reproductive activity related to competition from other contributor	Very weak	32	42.7	
	Weak	18	24.0	
	Medium	12	16.0	
	Important	4	5.3	***
	Very important	9	12.0	
Constraint to the development of the reproductive activity related to the risk of failure to improve outcomes	Very weak	30	40.0	
	Weak	21	28.0	
	Medium	11	14.7	***
	Important	5	6.7	
	Very important	7	10.7	

Significance: The categories defined in each variable occur in an equitable manner. $P > 0.05$ retains the hypothesis; $P < 0.05$ rejects the hypothesis; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

DISCUSSION

The relationship between the frequency of genital pathologies and the welfare of dairy cattle underscores the crucial role of veterinary monitoring activities on farms. The frequency of genital pathologies directly reflects the overall well-being of dairy cattle [30]. Regular veterinary monitoring proves instrumental in identifying and addressing these issues promptly. By closely observing and assessing the reproductive health of the cattle, veterinarians contribute significantly to mitigating genital pathologies. This proactive approach not only enhances the health and comfort of the animals but also has broader implications for the overall productivity and sustainability of dairy farms [15]. Therefore, emphasizing the importance of veterinary monitoring activities becomes pivotal in resolving and preventing genital pathologies, ultimately promoting the welfare and optimal functioning of dairy cattle [30].

Our survey involving 75 veterinarians showed that Algerian veterinarians prefer to operate alone; 57.3 % of offices have just one veterinarian, and 80 % of these clinicians act in reproduction. For 16 % of the veterinary offices, reproduction activity constitutes 40 % of the inter-

ventions. Only 21.3 % of the veterinary offices surveyed may earn more than 50 % of their total income from this activity. The clients of these veterinary clinics are primarily mixed bovine, which may be explained by the fact that most farms in Algeria are dairy operations that also produce meat.

Reproduction monitoring activity is minimal in Algerian offices, where the average number of breeding has a reproduction activity of 14. From the viewpoint of the time spent on reproduction monitoring (30 % for the third practices), as well as from the perspective of revenue, which accounts for 30 % for 24 % of the veterinary office, the reproduction monitoring activity continues to be a limited activity and constitutes a negligible source of income. It appears that experience is not a deciding factor for participation in this activity because the veterinarians who participated in the study had experience ranging from 1 to 33 years.

Before each visit, it is advisable to obtain information facilitating access and analysis to improve the visitor's progress. The exciting data to consider are the results of milk recording (production, protein, and butterfat levels, individual cell counts, etc.), as well as insemination and the breeding record (dates of calving, dates of mating) [3, 13].

According to our findings, 64 % of vets record farm information on paper, while just 6.7 % do it electronically. This is generally caused by a lack of technicalities and limits the exchange of data or the establishment of a database to enhance farm performance.

When it comes to the examination of animals during visits, in contrast to veterinarians, who are primarily affected and directed by the breeder's decisions, it appears that the breeder plays an essential role and actively participates in selecting animals to be examined.

Cows should be examined frequently during the postpartum period since it is a sensitive and important period. Reported results demonstrated that 52 % of surveyed practitioners routinely control uterine involution, which takes three weeks of calving [33], indicating that it is a frequent technique. It was also mentioned that surveyed veterinarians frequently (82.7 %) performed early checks of cows with a risk of calving, cows having had dystotic calving, retained placenta, or acute metritis; this demonstrates that breeders are very conscious when it comes to the selection of cows to be examined.

We inquired about veterinarians' attitudes toward pregnancy diagnosis, which is crucial to an efficient dairy cattle management strategy [17]. Pregnancy diagnostic is used to identify non-pregnant animals so they can be inseminated again or culled rather than pregnant ones [22]. During our research, veterinarians initially notice pregnancy between 20 and 120 days after AI; theoretically, the first observation could be performed between 30 and 60 days after mating [5]. For positive animals, further confirmatory diagnosis is recommended to identify cows that have lost their pregnancy, which can occur at any time [18,42]. Pregnancy confirmation increases farmers' income and the potential for high-yielding dairy cattle to reproduce [19]. A confirmation approximately 60 days after mating is necessary [27]. According to our survey, about two-thirds (72 %) of veterinarians recheck positive cows within 45–180 days.

To determine the alert threshold of infertility considered in cows who need three artificial inseminations/ mating or more, a question on the examination of non-pregnant cows after three matings. About two-thirds of practitioners seemed to practice this (73 %). Early detection of infertile cows is essential. It is a relatively common issue on farms and results in significant economic losses due to the costs of inseminations, veterinary services, treatments, culling losses, and decreased productivity [10, 41]. The best way to increase cow productivity is to minimize fertility problems [1].

A total of 48 % of veterinarians use simultaneous BCS scoring, whereas 45 % do so occasionally. In the monitoring of reproduction, this is an essential element. Good body condition management leads to improved reproductive performance, including fertility. According to several research, the body condition score can decrease the insemination rate, influence the success rate of first insemination, affect the resumption of cyclicity, reduce oestrus activity, worse ovarian activity, and raise the chance of early embryo mortality / non-fecundation [5, 6, 10, 35, 40]. Similarly, decreasing BCS impairs fertility and profitability, causing a rise in disease appearances such as subclinical endometritis [36].

Following calving, the uterine lumen almost automatically becomes contaminated with germs, leading to infections that may impair function. The appropriate choice of pharmaceutical items to be employed, including their associations, and ultimately the success of therapy, depends

on the choice of diagnostic techniques [26]. In our study, we found great variability in the use of the different tools of diagnosis, where the choice of a diagnostic method is mainly made according to precocity; the sooner the diagnosis is made, the sooner the implementation of treatment will be, and therefore ultimately the greater the risk of its effectiveness and practicability; it conditions the implementation of tools and therefore of very different investments, accuracy; it determines the choice of the most appropriate treatment at the individual level, and at the herd level, it determines the precision of the quantification and therefore of the risk analysis [24].

Transrectal palpation is commonly practiced by 38.7 % of veterinarians who participated in this study. Although it is the simplest and most affordable method, it appears to be the least sensitive and specific among the diagnostic techniques [21]. The method lacks accuracy in identifying cows infertile due to endometritis [43]. According to some [33] the diameter of the horns at their base does not appear to be a reliable diagnostic criterion; only the cervical diameter, in conjunction with another clinical symptom (the existence of unusual uterovaginal discharge), is related to the presence of chronic metritis. This technique facilitates the exteriorization of secretions; it helps to evaluate the disease's severity [44]. Ultrasound is a rapid, practical, and less invasive technology for diagnosing endometritis 4 and 5 weeks postpartum, especially when combined with the detection of intrauterine fluid accumulation and measurement of cervical diameter thickness [8]. In particular, at week fourpostpartum, S a l a h and Y i m e r [43] found a low correlation between this approach and the cytological examination; this technique demonstrated 83.3 % sensitivity and 73.3 % specificity. We find that the veterinarians in the study systematically (26.7 %) or occasionally (24 %) rely on ultrasonography to diagnose endometritis.

Vaginoscopy is a helpful procedure for checking the cervix and vagina, spotting and identifying the source of discharges (mucus, pee, pus...), describing discharges in terms of appearance, and identifying the presence of trauma and/or intravaginal scars [7]. When to diagnosing endometritis, the vaginoscopy has a sensitivity (Se) of 54 to 72 % and specificity (Sp) range of 87 to 96 %; it is a highly accurate technique [38, 39]. The inquired veterinarians have not used this tool frequently, with utilization rates ranging from 22.7 % (systemically) to 33.3 % (sometimes); this may be due to the potential for contaminating healthy cows, the obligation to use the appropriate tools, and the requirement to clean the material and the vulva before each examination [48]. Combining different diagnostic techniques may result in more accurate diagnoses than using only one [31].

The condition of the uterus and ovarian activity are strongly correlated in research; the location and selection of ovarian follicles are altered by uterine bacterial infection, which also impairs follicle development and function [39]. They also show that therapies can only be functionally beneficial if they decrease uterine infections and restore ovarian activity, necessitating an ovaries-with-uterine-control evaluation [37]. This was in line with our findings, which showed that 73 % of practitioners simultaneously examine the uterus and the cow's cyclicity.

Reproduction monitoring involves the breeder and the veterinarian working together to develop the best possible observational conditions for the breeder's animals and the shortest possible clinical examination times and anamnesis for the animals. To ensure the animals are performing correctly and being monitored effectively, it is advised to arrange monthly inspections with a frequency suitable to breeding and to evaluate every individual and every case [43]. Indeed, 32 % of the veterinarians surveyed selected this activity as their regular rhythm.

Setting goals is important because it shows the breeder where the deficit is coming from, highlights issues, and offers a plan of action. More than two-thirds of the veterinarians in the survey set visit objectives. Alternatively, it is necessary to discuss objectives to assess if the goals have been achieved. Incomplete problem solutions might reveal areas that want improvement, whether in terms of visits or the manner advised treatments were administered. This is finished by performing a reproduction assessment, which gives the breeder control over the treatments that will be used, the cows that will be exposed at the following visit, and the creation of a final bill for the monitoring season. This activity was chosen to be applied by 22.67 %.

Most veterinarians (56 %) involved in our study preferred to work individually because they considered other veterinarians' competitors rather than collaborators. A significant majority of veterinary surveys also include additional items for routine reproduction monitoring (feeding, mastitis, and lameness) because of their effects on production, reproductive performance, and economics. These pathologies are known as a reason for culling cows [14,

47]. Numerous studies have demonstrated the critical impact nutrition plays in the stability and efficiency of bodily functions. Any nutritional or dietary imbalance has commonly been associated with pathological problems that result in infertility and infecundity, and any weight loss might affect reproduction and milk production even later [12, 20, 44]. Nutrition acts at the brain to exert control of the reproductive endocrine system and also influences the amounts of metabolic substrates that act directly at ovarian follicles, oocytes, and embryos [9, 14, 20].

The study by L o g r o ñ o et al. [34] showed a poor correlation between lameness and milk production, a reduced probability of service and pregnancy, and prolonged calving to conception delay. This is mostly caused by intense discomfort and pain, which reduces the severity of agitation symptoms and releases pro-inflammatory mediators that alter behaviour, resulting in an energy deficit [4, 8, 9, 23].

There is still debate on how mastitis affects fertility. Mastitis is a serious disease that has cost the dairy industry millions of dollars; it causes delayed oestrus, a drop in pregnancy rates, increases the number of services per conception, and a rise in abortion risk [29, 32, 45, 46].

Overall, the practitioners and inseminators reported that the monitoring of reproduction in dairy cattle farms has decreased during the past five years. They mostly attributed that to the competition from other players, the lack of motivation among breeders and veterinarians, and ultimately costs for breeders. The veterinarian must know what the client wants and provide him with services according to his real needs and the veterinarian must resist competition. For motivation to occur, the breeder has to feel invested in the procedure. He must be alerted of his problems, confronted with them, and given the necessary resources to address them. It is considered crucial to create and elaborate a professional guide that calculates the breeder's losses and illustrates the profitability when issues are resolved. The commitment to improving the practice of livestock monitoring was highlighted by 88 % of veterinarians. Finally, a significant percentage of respondents (52 %) are optimistic about the evolution of monitoring in the future.

CONCLUSIONS

Even though reproduction is the cornerstone of cow productivity and the success of modern farms, the present study showed that it only represents a minor portion of practices and is significantly declining activity. The veterinarians are primarily depending on primitive diagnostic and recordkeeping techniques that limited information diffusion and monitoring activity. However, specific procedures were consistent with those described in the literature. An associated effort between animal owners and veterinarians should consider a well-reasoned reproduction monitoring in individual and group dimensions for problem resolution and prevention within a large prospective development of the population medicine approach.

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