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Contents

Front Cover: **Hoopoe** ***(Upupa epops)***

HOOPOE The bird is brown and yellow in color with black and white wings. Its legs are short with relatively large wings. The bill is narrow and thin. The male and female birds look alike. The Hoopoe has a consolidated tassel feather on its head which it can spread at will. The females nest in holes, spaces and crates, generally close to the ground. To protect her nest, the female secretes a repugnant substance from the Uropygial gland, which smells of decaying meat. The secretion is thought to help deter predators as well as parasites. The Hoopoe is found in Europe, Asia and Africa. In Israel there is a stable population of Hoopoes, mainly in the coastal area but there has been a tendency for the birds to spread inland due to the increase in cultivated fields and lawns. The bird feeds on insect larvae which are extracted from the soil with the aid of its long beak. The Hoopoe call is typically a trisyllabic oop-oop-oop, which gives rise to its English and scientific names. The Hoopoe is mentioned in the Bible as being impure (Leviticus: 11) and the G'mara refers to it as the bird that brings dill worms for cutting the Temple stones. The bird is also mentioned in the Koran regarding King Solomon and the queen of Sheba (Sura 27). In medieval times, mystical attributes were ascribed to the Hoopoe. In May 2008, in conjunction with the country's 60th anniversary and following a national survey of 155,000 citizens, the Hoopoe was chosen as the national bird of Israel, outpolling the Eagle, lesser kestrel, barn owl, sunbirds, warblers, bulbul, finch, and the white breasted kingfisher. The Hoopoe was chosen for the cover of this journal in honor of the celebration of the 75th Year of the creation of the State of Israel, on the 14th May 1948.
Mr. **Moshe Tachnai**, generously provided the pictures for the covers.

Editorial

Dear Readers,

The world seems in turmoil. To add to all the worldwide events affecting millions of people, a particularly severe earthquake has affected our neighbors in Türkiye and Syria on 6 February 2023.

I am frequently in contact with Veterinarians and Scientists from Türkiye who contribute to the Israel Journal of Veterinary Medicine by publishing high quality articles documenting their findings and research relating to Veterinary Medicine in Türkiye. As the "scope" of our journal covers the "**Mediterranean Basin**", articles from this geographic region are important and relevant to veterinarians living and working in this part of the world and are welcome contributions to our Journal. These countries, including Israel, have similar climates, fauna, flora, therefore binding all of us living in the region of the Mediterranean Basin.

As Editor of the IJVM I have written to some Turkish authors, expressing our sincere condolences and sympathies as a result of this tragedy, which has befallen the Turkish people.

In reply, three letters from authors from Türkiye are presented in the "Letters to the Editor" section.

On behalf of the Israel Veterinary Medical Association,
we send our sincerest sympathies, support
and prayers to the Turkish People.

Sincerely,

Dr. Trevor (Tuvia) Waner
Editor-in-Chief, Israel Journal of Veterinary Medicine

Letters to the Editor

To Dr. Trevor Waner,
Editor-in-Chief, Israel Journal of Veterinary Medicine.

From: Dr. Suheyla Turkyılmaz, Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Türkiye.

Unfortunately, we experienced the worst disaster of the last century in southern Türkiye. Yes, we have lost so many people; I cannot find words to describe our current sadness and pain.

However many of our people were rescued by search and rescue teams. This is our only consolation. One of the teams that first went to the disaster area and saved many of our people under very difficult conditions at the cost of their lives and showed exemplary solidarity with the Turkish people was the Israeli rescue team. When our children see such beautiful movements of solidarity, they will take an example and the world will become a livable place.

After seeing the efforts of the Israeli team to save lives on television, my son said that "he wanted to go to Israel when he grew up and that he wanted to get to know the Israeli people". I hope he can do that one day.

I hope we can talk about much better things with you in the future. We would like to express our endless gratitude to everyone who served in the Israel Rescue Team and all the Israeli people for their outstanding efforts.

Stay safe and strong.
Best regards,
Suheyla

• • •

From: Dr. Recep Kalin of the Department of Microbiology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye.

"Thank you very much for your warm and sincere wishes for us. This is the biggest disaster to occur in Türkiye in a century. I would like to thank your country and government for sending rescue teams immediately, as well as the other 70 countries. God bless all people in the world after this big disaster."

• • •

From: Prof. Dr. Özkan Aslantaş, Microbiology Department Veterinary Medicine Faculty, Mustafa Kemal University 31034-Antakya/Hatay/ Türkiye

Dear Dr. Trevor Waner,
Thank goodness, my family and I are in good health. We had a great disaster. We left Hatay and came to Ankara. Thank you very much for your interest and sincere condolences.

Sincerely yours.

First Report of *Pennella balaenopterae* Infestation in a Fin Whale (*Balaenoptera physalus*) Carcass Washed Ashore on the Israeli Coastline

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ABSTRACT

This case report describes the first identification in Israel of the mesoparasitic copepod *Pennella balaenoptera*, in the carcass of a marine mammal. A Fin whale (*Balaenoptera physalus*) washed ashore on the Israeli coast was autopsied in contiguity to a large oil spill that occurred along Israel's coastline. At the external examination, a large number of *Pennella balaenopterae* were embedded within the whale's blubber on its ventral and lateral sides. Further toxicological analyses of lung and bronchus samples, revealed the presence of higher-than-acceptable levels of toluene without any evidence of other above-normal petroleum components, suggesting the inhalation of the substance. This paper proposes to consider toluene toxicity as a possible etiological factor affecting the host's immunity and consequently increasing the parasitic pressure.

Keywords: *Pennella balaenoptera*; Toluene toxicity; Fin Whale; Parasitic Copepod.

INTRODUCTION

Pennella balaenopterae is a member of the order Siphonostomatoida, family Pennellidae, and genus *Pennella* (1, 2) . It was initially described by Koren and Danielssen in 1877(3), and is the only mesoparasitic copepod known to infest marine mammals (1, 4, 5). This integument-semi-buried, pan-globally distributed (5), parasite was previously identified in dolphins (Delphinidae) (4, 6), baleen whales (Balaenopteridae) (1, 7-10), and occasionally in pinnipeds (11) and porpoises (12).

P. balaenoptera is the largest copepod (1, 4, 5), and appears as a filament hanging from the skin. Although this parasite has been recognized for more than a century, its exact life cycle remains greatly unknown (4, 8), and currently, only the adult female and the first naupliar stage have been firmly recognized (2, 5, 9, 13). The external morphological regions of the adult female are: cephalothorax, holdfast horns, neck, trunk and abdomen (5).

Under normal conditions, pennellid infestation is rather limited to solely a few organisms per host, yet, an elevated number of *P. balaenopterae* could be seen as indicators of more severe underlying health conditions (e.g. immune suppression) (4, 6).

In his review article, Hogans (2017) validated or potentially validated 15 of 44 members of the genus *Pennella* according to their morphological character and their non-morphological character based on the infested host (5). However, a study conducted by Fraija-Fernández *et al.* (2018) found that *P. balaenopterae* and *P. filosa* (a species known to

Figure 1: Fin whale (Balaenoptera physalus) washed ashore Israeli coastline on February 18th, 2021, prior to necropsy

infest fishes) did not present with significant molecular differences between both, suggesting a con-specificity (14, 15).

CASE REPORT

On February 18th, 2021, a carcass of a young, male Fin whale (*Balaenoptera physalus*) was washed ashore at Nitzanim beach (34.5882:31.7255) (Figure 1). Earlier that week a large oil spill was caused by the leaking of more than 1000 tons of tar, from a tanker sailing to Syria, resulting in numerous dead marine animals and seabirds (16-18). The proximity of the events raised questions regarding the cause of death.

The whale's necropsy was carried out on February 21st by Kimron Veterinary Institute (KVI) pathology department team and the Israel Nature and Parks Authority (INPA) veterinarian.

GROSS PATHOLOGY

The whale's carcass measured around eighteen meters (59 feet) long and the cadaver's condition was estimated between 3 and 4 according to the "Right whale necropsy protocol" (19).

The carcass was positioned on its dorsal side. At the external examination of the body, on the ventral and lateral parts of the integument, numerous, long tubular structures, consistent with a pennelid copepod neck were embedded within the whale's blubber. When gently extracted, a parasitic cephalothorax consisting of a small, rounded, and bulbous head with two to three long anchoring, holdfast horns (Figure 2) was noticed. These morphological features were consistent with *P. balaenopterae* (4), and the current article describes the first report of this parasitic crustacean along Israeli shores.

The presence of the parasites was the only significant finding observed during the necropsy due to the advanced autolytic state of the carcass.

LABORATORY EXAMINATIONS

A dozen partial parasites were sampled and submitted for further morphological and molecular investigation by

Figure 2: A Pennella balaenoptera with its cephalothorax (composed of a small bulbous head and three horns) and a long tubular neck, positioned on top of the ventral integument.

the KVI parasitology department. The parasites were preserved in a 10% neutral buffered formalin (NBF) and a PCR test targeting the mitochondrial cytochrome c oxidase subunit I gene *(COI)* of metazoan invertebrates (20) was performed.

Samples of lung, bronchus, urine, and blood were submitted for targeted toxicological analyses for identification of traces of volatile organic compounds (VOCs) related to oil spill. The laboratory evaluated the following markers: hexane, heptane, octane, decane, undecane, dodecane, tetradecane, benzene, toluene, m-xylene, o/p-xylenes, and ethylbenzene, cyclohexane and, naphthalene.

RESULTS

The parasitology department of the KVI identified the mesoparasitic copepod *P. balaenopterae* by means of morphological identification (4, 5, 7) and host specificity (5) since molecular analyses identified only whale's DNA (14, 20).

Morphologically, the anterior a of the parasite began with the cephalothorax, which consisted of a small, bulbous head followed by two to three holdfast horns (Figure 3), two lateral, cylindrical, unbranched, and variably-sized and a single, partially present, short dorsal horn. The last visible segment was the neck, which in some cases measured about 14 cm. Based on their morphological appearance and host (marine mammal), the parasites were identified as *P. balaenopterae* (Figure 4) (1, 5).

As for the toxicology results, the levels of all targeted VOCs were similar, comparable and below 0.1 ηg/g in whale and control samples (commercial fish samples) with

Figure 3*: Parasite fragment containing the cephalothorax and holdfast horns.*

Figure 4: Three Pennella balaenoptera analyzed by the parasitology department at the KVI.

exception of toluene. Higher concentrations of toluene were detected in the whale's bronchus (0.781-1.417 ηg/g) and lungs (0.154-0.157 ηg/g), in four samples. This finding could indicate the inhalation of petroleum vapors by the whale.

DISCUSSION

This mesoparasitic copepod has been previously described in marine mammals such as Fin whales *(Balaenoptera physalus)* (7, 10), Striped dolphins *(Stenella coeruleoalba)* (4, 6) and one Harbour porpoise *(Phocoena phocoena)* (12) in the Mediterranean Sea coast lines of Italy (10), Spain (4, 6, 14), Türkiye (7, 12), and Croatia. However this is the first time that this mesoparasitic copepod, *Pennella balaenopterae*, has been identified off the coast of Israel.

During the external examination of the whale's necropsy, numerous parasites were observed; an exact number could not be obtained due to technical limitations. However, previous studies have assessed and established the relation between the mental status of a host and the degree of infestation with *P. balaenopterae* (4, 6).

In this case elevated toluene levels were detected. Toluene is a clear, colorless and low to moderate water-soluble component that is found in different solvents and gasoline (21). Usually, it is rapidly weathered but in stagnant and chronically polluted waters it becomes toxic (22). In both humans and animals, the primary target organ for toluene toxicity is the central nervous system (21, 23). Since crude oil contain a mixture of different hydrocarbons and liquid organic compounds, usually an elevated toluene concentration is detected along with the excess of other similar mono-aromatic hydrocarbons (benzene, xylenes, and ethylbenzene) (24), which in this reported case, were not above the regular levels.

In light of these findings, we recommend further research regarding toluene toxicity as a possible underlying etiological factor, able to affect the mentality and immunity of marine mammals, and consequently increase the parasitic pressure.

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The authors wish to thank Julius Ben-Ari for his thorough toxicological report.

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The Effect of Melatonin Administration on Colostrum IgG Levels and Oxidative Stress in Advanced Pregnant Awassi Sheep

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ABSTRACT

The aim of the present study was to investigate the effects on oxidative stress markers and colostrum quality levels of subcutaneous implantation of melatonin in the 4th month of pregnancy in Awassi sheep by considering the sex of offspring born. The animals consisted of 60 healthy Awassi female sheep. Progesterone-based oestrus synchronization was applied to the sheep during the breeding season and pregnancy examinations were performed by the transabdominal route. The pregnant sheep were divided into 2 groups: the melatonin (n=30) and control (n=30) groups. In the 4th month of pregnancy, melatonin was implanted subcutaneously behing the ear of each animal in the melatonin group, and 1 ml of saline was applied subcutaneously to the control group. Colostrum samples from all the sheep and blood samples from both sheep and lambs were taken within the first hour after birth, before the lambs were suckled. Colostrum IgG levels were seen to be higher in the melatonin group compared to the control group ($p<0.001$); higher in the group with no female offspring compared to the group with female offspring ($p<0.001$) and higher in twins compared to singletons ($p<0.001$). The colostrum IgG levels were higher in ewes with singleton birth in both the melatonin and control groups compared to those with no female offspring at birth ($p<0.001$). Serum total antioxidant capacity (TAC) levels in sheep was higher in the melatonin group than in the control group ($p<0.001$). Serum total oxidant capacity (TOC) and oxidative stress index (OSI) levels were higher in the control group than in the melatonin group ($p<0.001$). The serum TAC level in lambs was higher in the melatonin group than in the control group ($p<0.001$), and was higher in singleton lambs than in twins ($p<0.001$). The serum TOC and OSI levels were higher in the control group than in the melatonin group ($p<0.001$) and higher in singleton lambs than in twins ($p<0.001$). In conclusion, the use of prepartum melatonin may be considered in sheep breeding enterprises to improve the antioxidant defence system of the body and improve the quality of colostrum for newborn lambs to be able to gain sufficient immunity.

Keywords: Melatonin; Colostrum; IgG; Oxidative Stress; Sheep.

INTRODUCTION

Melatonin is an indolamine, which is synthesized and secreted by the pineal gland in the brain. This molecule, which is abundant in almost all living organisms in nature is a chemical compound with a molecular weight of 232 g/mol with both hydrophilic and hydrophobic properties (1). Oxidative stress can be defined as an imbalance between the amount of free radicals and reactive products or oxidants and the amount of antioxidants that protect the body from these metabolites. This situation affects the organism as a whole and causes significant damage to the molecules and cellular structures in the body (2). Melatonin removes reactive oxygen species (ROS) and reactive nitrogen species (RNS) from the body and has some important functions halting the formation of free radicals. By decreasing the effect of oxidative stress, it functions as an antioxidant, increasing antioxidant defences,

and thereby protecting cells and tissues from damage. It has both hydrophilic and hydrophobic properties and cleanses hydroxyl radicals (OH) and reduces oxidative stress in both water and lipid parts of the body (3).

Colostrum is a liquid that is synthesized in the last stages of pregnancy. It accumulates in the mammary gland and ceases to be produced immediately after birth. It differs from normal milk in terms of content, taste, color and consistency, as it contains immune complements, growth factors and various nutrients necessary for the defence systems, nutrition and development of newborns after birth (4).

Colostrum is a source of antibodies in passive immunity in newborns as it contains 80 times more antibodies than normal milk (5). There is a significant concentration of immunoglobulin (Ig) (6), and the most important factor affecting the quality of colostrum is the amount of gamaglobulin (IgG) in immunoglobulins. The IgG contained in colostrum accounts for 75% of immunoglobulins. The most commonly used method for calculating the quality of colostrum is the determination of the IgG level (7).

In ruminants, including sheep, the placenta does not allow the transmission of immune substances to the offspring through the maternal bloodstream during pregnancy and therefore lambs are born as agammaglobulinemic. However, the transfer of immune substances in the colostrum provides immunity until the offspring acquire the ability to produce their own antibodies, thus temporary passive immunity is obtained and they are protected from diseases (8).

As the only source for the initiation of postpartum immunity, colostrum is important for the supply of immunoglobulin G, leukocytes, cytokines, growth factors and various nutrients necessary for the immune system in the neonatal period (4).

Lambs and kids need to have sufficient passive immunity to be protected against environmental pathogens in the postpartum period. There is a positive relationship between vitality and passive immunity level in newborns. The neonatal mortality rate has been reported to be high in lambs and kids with passive transfer failure (4, 9).

The use of antioxidants in the prenatal period has been shown to improve the quality of colostrum (10), and it has been reported that antioxidants play a role in the synthesis of IgG and its migration through mammary-specific receptors (11). It has been stated that the dry period serum antioxidant level affects the quality of colostrum (12), while antioxidants in the colostrum content protect immunoglobolina in the colostrum against oxidative stress damage (13).

The aim of this study was to investigate the effect of subcutaneous implantation of melatonin in the 4th month of pregnancy in Awassi sheep on oxidative stress markers (blood serum TAC and TOC levels) and colostrum quality (colostrum IgG levels) by considering the sex of offspring. The data obtained were examined in terms of the feasibility of using this method in veterinary breeding practice.

MATERIALS AND METHODS

Animal Material

This study was conducted between August 2021 and February 2022 at the Faculty of Veterinary Practice Farm of Harran University, located in the Eyyubiye district of Şanlıurfa Province, southeast Türkiye, at an altitude of 517 m, 37°07'18.1" N latitude and 38°49'13.0" E longitude. The animal sample comprised 60 Awassi sheep, aged 2-4 years, each weighing mean 53.96±1.09 kg, with a body condition score ranging from 2-3 (1=Extremely weak, 5=Obese) (2.63±0.05), which had previously given birth at least once, and had no genital system conditions. During the study period, the sheep grazed freely on pasture, and were taken into a closed pen for the process of oestrus synchronization, and the collection of blood and colostrum samples. The sheep were fed a mixture of hay (5.2%), clover (32.9%) and milk feed (61.9%) and were provided with fresh water *ad libitum.*

Oestrus Synchronization and Oestrus Tracking

In order to provide a sample during pregnancy and to induce lambing within a certain period of time, progesterone-based oestrus synchronization was applied in the breeding season. Progesterone impregnated vaginal sponges (Medroxyprogesterone acetate, Esponjavet®, Hipra Animal Health, Türkiye) were placed in the vagina to remain in the vagina for 12 days. On the 11th day, 2 ml of PGF2a (Dinoprost tromethamine, Dinolytic®, Zoetis, Türkiye) was administered intramuscularly. On the 12th day after insertion, the vaginal sponges were removed and 500 IU PMSG (PMSG, Oviser®, Hipra Animal Health, and Türkiye) was injected intramuscularly. After the injection of PMSG, oestrus was followed up using teaser rams for 30 minutes at 8 hour-intervals for 3 days, and the sheep showing oestrus

were hand-mated with rams of the same breed, with predetermined fertility.

Ultrasonographic Examinations and Melatonin Implantation

Ultrasonographic pregnancy examinations of the sheep at 35-45 days following the mating and during the 4 months of melatonin application were performed transrectally and transabdominally using a real-time B-mode ultrasound device (Hasvet 838 ultrasound device, Antalya, Türkiye) with a linear probe at a frequency of 5 MHz.

The ultrasound examinations were all performed by the same researcher, with a record kept of the screen settings of depth, gain, focus, and brightness for all the images. The sheep determined to be pregnant were separated into 2 groups as the melatonin (n=30) and control (n=30) groups. The melatonin group was treated with a subcutaneous implant behind the ear (Melatonin, Regulin®, CEVA, Türkiye), and the control group received1 ml saline solution subcutaneously.

Collecting Milk Colostrum and Blood Samples

Colostrum samples of 15 mL in total from a single mammary lobe after the teat ducts were emptied were withdrawn into centrifuge tubes (15mL, Isolab®, Germany) following the rules of asepsis and antisepsis before the lambs were suckled within the first hour after birth. The samples were transported to the laboratory on ice, and then centrifuged at 3000 rpm for 10 minutes. The colostrum serum obtained from each sample was transferred to a 2 ml microcentrifuge tube and stored at -80˚C until analysis.

In order to determine oxidative stress (TOC and TAC) from the sheep and lambs in the study group, blood samples from the Vena jugularis were withdrawn into tubes containing 5 ml of coagulation activator in accordance with the procedures of asepsis and antisepsis within 1 hour after delivery (before the lambs suckled). The blood samples taken were delivered to the laboratory under cold chain conditions and centrifuged for 10 minutes at 3000 rpm. The obtained serum samples were transferred to 2 ml micro centrifuge tubes and stored at -80˚C until the relevant analyses. The sex and birth weight of each lamb born from all the sheep were recorded, with weight recorded according to whether a singleton or twin birth.

Biochemical Analysis

The serum TAS levels of the sheep and lambs in the study groups were determined spectrophotometrically at 660 ηm using a commercial kit (Total Antioxidant Status, NN21117A, Rel Assay Diagnostics®, Mega Medicine, Gaziantep, Türkiye). The serum TOS levels were determined spectrophotometrically (Molecular Device SpectraMax M5 Plate Reader, Pleasanton, California, United States) at 530 ηm using a commercial kit (Total Oxidant Status, NN211290, Rel Assay Diagnostics®, Mega Medicine, Gaziantep, Türkiye). The oxidative stress index (OSI) was calculated as OSI=([TOC {mmol/L}]/[TAC {mmol} Trolox equivalent]/1×100) (14). IgG levels of colostrum serum were evaluated using the ELISA method with a commercial kit (Sheep Immunoglobulin G, IgG, Cat.No. E0019Sh, BT LAB®, Zhejiang, China).

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences software (SPSS for Windows; version 26.0). A 2×2×2 factorial design was established in which group (control and melatonin), number of offspring and sex of offspring were fixed to determine the level of parameters examined in the treatment of mothers with melatonin. Differences between groups were changed to the General Linear Model (GLM) procedure by adding additional coding to the syntax menu. The data in the tables, graphs, and results section were expressed as mean±standard error (SEM) values. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Pregnancy and Number of Offspring: There was no pathological conditions that might have affected the general health status of the sheep during the pregnancies. It was observed that the sheep stopped ruminating a few days before the parturition and remained separated from the herd. These sheep were placed in separate compartments (2x2m) and their births were followed up. Birth occurred on 150±2 days of pregnancy, all were by the vaginal route, single (151.10±0.11) or twin (150.82±0.16) without requiring help and no maternal problems were encountered in the postpartum period. As a result of the births, 18 male and 24 female offspring were born from 18 single and 12 twin births in the melatonin

Table 1. A 2×2×2 ANOVA summary table in which the group, number of offspring and sex of offspring are presented the sheep.

Factors			IgG level	Serum TAC level	Serum TOC level	OSI level	Gestation period (days)
Group	**Sex of Offspring**	**Single and Twin Births**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
Control	No female offspring	Single	43.60±0.89	2.07±0.02	14.45±0.04	6.97±0.06	151.22±0.24
		Twin	54.11±1.54	2.08±0.04	14.36±0.8	6.89±0.11	150.33±0.41
	Female offspring	Single	24.39±0.80	2.08±0.02	14.50±0.04	6.95±0.05	150.90±0.21
		Twin	53.58±1.01	2.08±0.02	14.47±0.05	6.93±0.07	150.571±0.27
Melatonin	No female offspring	Single	69.77±0.94	2.31±0.02	12.47±0.04	5.35±0.06	151.375±0.25
		Twin	64.37±1.33	2.34±0.03	12.45±0.06	5.32±0.09	151.25±0.36
	Female offspring	Single	43.12±0.84	2.34±0.02	12.51±0.04	5.34±0.06	150.90±0.22
		Twin	64.11±0.94	2.33±0.02	12.48±0.04	5.34±0.06	151.12±0.25
Main effects and interactions			***P value**	***P value**	***P value**	***P value**	***P value**
Group			**<0.001**	**<0.001**	**<0.001**	**<0.001**	>0.05
Sex of offspring			**<0.001**	>0.05	>0.05	>0.05	>0.05
Single and Twin Birth			**<0.001**	>0.05	>0.05	>0.05	>0.05
Group x Sex of Offspring			**<0.05**	>0.05	>0.05	>0.05	>0.05
Group x Single and Twin Birth			**<0.001**	>0.05	>0.05	>0.05	>0.05
Group x Sex of Offspring x Single and Twin Birth			**<0.05**	>0.05	>0.05	>0.05	>0.05

Total antioxidant capacity (TAC), total oxidant capacity (TOC), Oxidative Stress Index (OSI), Standard error of the mean (SEM)

group, and 18 male and 22 female offspring were born from 20 single and 10 twin births in the control group.

A 2×2×2 ANOVA summary table in which the group, number of offspring and sex of offspring are fixed in the sheep and lambs is presented in Table 1 and Table 2.

Colostrum Immunoglobulin G Levels: The colostrum IgG levels were higher in the melatonin group than in the control group ($p<0.001$). According to the sex of offspring born, the colostrum IgG level was higher in the sheep with male offspring at birth than in the group with female offspring at birth ($p<0.001$). According to single and twin births, colostrum IgG levels were higher in sheep giving birth to twins than in sheep giving birth to singletons ($p<0.001$). Colostrum IgG levels were higher in the ewes with singleton birth compared to ewes not giving birth to females in both the melatonin and control groups ($p<0.001$). In the melatonin and control groups, there was no differences in ewes that gave birth to twins according to whether or not there were males or female offspring ($p>0.05$).

Total Antioxidant, Total Oxidant Capacity and Oxidative Stress Index of Blood Serum in Sheep: The blood serum TAC level was higher in the melatonin group than in the control group ($p<0.001$). The blood serum TOC and OSI levels were higher in the control group than in the melatonin group ($p<0.001$). There was no difference in blood serum TAC, TOC and OSI levels between the sexes of lambs born and single and twin births ($p>0.05$). There was no differences in interaction between the group*lamb sex, group*single and twin births and group*lamb sex*single and twin births ($p>0.05$).

Total Antioxidant, Total Oxidant Capacity and Oxidative Stress Index of Blood Serum in Lambs: The blood serum TAC level was higher in the melatonin group than in the control group ($p<0.001$). The blood serum TAC level was higher in singleton lambs than in twin lambs ($p<0.001$). However, there was no significant difference between the sexes of lambs born ($p>0.05$). There was no difference between the group*lamb sex and the group*lamb sex*single and twin birth interactions ($p>0.05$). The blood serum TOC and OSI levels were higher in the control group than in the melatonin group ($p<0.001$). The blood serum TOC and OSI levels were higher in singleton lambs born

Table 2. A 2×2×2 ANOVA summary table in which the group, number of offspring and sex of offspring are fixed in the lambs.

Factors			Serum TAC level	Serum TOC level	OSI level	Birth weight (gr)
Group	**Sex of Offspring**	**Single and Twin Birth**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
Control	Male	Single	1.78±0.01	15.52±0.03	8.70±0.05	4.14±0.71
		Twin	0.94±0.01	7.38±0.03	7.84±0.05	3.76±0.71
	Female	Single	1.77±0.00	15.56±0.03	8.77±0.04	3.95±0.64
		Twin	0.94±0.00	7.39±0.03	7.86±0.04	3.50±0.64
Melatonin	Male	Single	1.99±0.01	12.72±0.03	6.37±0.05	5.73±0.64
		Twin	1.08±0.01	6.16±0.03	5.69±0.05	3.71±0.71
	Female	Single	2.01±0.00	12.74±0.03	6.33±0.04	4.03±0.71
		Twin	1.07±0.00	6.17±0.03	5.71±0.04	3.47±0.64
Main effects and interactions			**P value**	**P value**	**P value**	**P value**
Group			**<0.001**	**<0.001**	**<0.001**	>0.05
Sex of offspring			>0.05	>0.05	>0.05	>0.05
Single and Twin Birth			**<0.001**	**<0.001**	**<0.001**	>0.05
Group x Sex of Offspring			>0.05	>0.05	>0.05	>0.05
Group x Single and Twin Birth			**<0.001**	**<0.001**	**<0.001**	>0.05
Group x Sex of Offspring x Single and Twin Birth			>0.05	>0.05	>0.05	>0.05

Total antioxidant capacity (TAC), total oxidant capacity (TOC), Oxidative Stress Index (OSI), Standard error of the mean (SEM)

than in twin lambs ($p<0.001$), with no significant difference between the sexes of lambs born ($p>0.05$). No difference was determined between the group*lamb sex and the group* lamb sex*single and twin birth interactions ($p>0.05$).

Parameters Related to Gestation Period in Sheep and Birth Weight in Lambs: In the parameters of gestation period in sheep and birth weight in lambs, there was no significant difference between the groups in terms of the sexes of lambs born compared to single and twin births ($p>0.05$). No difference was determined between the group*lamb sex, group*single and twin births and group*lamb sex* single and twin births interactions ($p>0.05$).

DISCUSSION

The melatonin hormone, the effect of which was investigated in this study, is synthesized from the pineal gland as a result of the transmission of photoperiodic signals to the reproductive neuroendocrine axis by the effect created by the day-night change. It is an immunomodulatory (helping to support immune function) factor and is produced by immunocompetent (lymphocytic tissue cells involved in immune reactions) cells. It also works as an antioxidant by removing free radicals and activating enzymes (15). It is known that melatonin plays a role in the season-related regulation of the immune system, and its effect on IgG production has been shown (16, 17). In a study of mice it was observed that melatonin administered in the evening increased the primary antibody response *in vivo* in erythrocytes after 5 days (16). It has also been reported that increased melatonin levels by artificial darkening in elderly rats may prevent immunosuppression by increasing IgG and IgM levels (17). In the literature reviews of sheep, which constitute the material of the current study, there is limited information on the use of exogenous treatments to improve the quality of colostrum.

It has been stated that since immunoglobulins cannot cross the placental barrier in sheep with epitheliocorial placenta structure, it is important to provide these components from the sheep colostrum to enhance the viability of newborn lambs (18). In a previous study examining the colostrum IgG level in Awassi sheep, the IgG level of the colostrum sample taken after birth was reported to be 6.09 g/dl (19). The IgG level has been reported as 49.50±4.36g/L (20) in colostrum samples of Rasa Aragonesa sheep and as 30.89±0.87g/L in Karagül sheep (21). Other studies have shown IgG levels

in colostrum to be 50-70mg/mL in different sheep breeds (Rambouille, Targhe, Columbia, Finnish hybrids, Lacaun, East Frisian, Suffolk, Lori bakhtyari, Shaul) (22-25). In studies conducted on goats, it has been reported that the postpartum level of colostrum IgG was 50-72mg/mL (26-28).

In the current study, the colostrum IgG levels in Awassi sheep were determined to be 43.92±0.55mg/mL in the control group, which was similar to the findings of previous studies (22-25). Although studies of other species were not directly related to the current study, they provide an idea in terms of colostrum IgG level monitoring. It is thought that the reason for the difference in the reference range in colostrum IgG levels may be related to factors such as breed, diet, season and number of offspring.

It has been previously reported that IgG levels were higher in the melatonin group in the colostrum sample taken after birth when melatonin was administered as a subcutaneous implant in the 4th month of pregnancy (20). In the current study, as a result of melatonin application in the 4th month of pregnancy, it was found that the IgG level of colostrum was significantly higher in the melatonin group than in the control group, and this was in accordance with the only literature on exogenous melatonin in sheep. In the current study, the increase in the amount of IgG in colostrum after melatonin implantation was probably due to melatonin activating immune system cells either directly through melatonin receptors or indirectly due to changes in steroid hormones (29). Abecia *et al.* (20) reported that colostrum IgG levels were low when the fetus was female in single and multiple offspring ewes in all study groups with and without melatonin. While a significant difference was reported in ewes giving birth to singletons, no significant difference was detected in ewes giving birth to twins. In the present study, a low level of colostrum IgG was measured in the female lambs. In addition, the colostrum IgG level was higher in twins compared to singletons in this study. While there was no difference in colostrum IgG level in twin pregnancies in this study, the low IgG level in single pregnancies in the presence of a female fetus is compatible with the literature. In the current study, according to the sex of the offspring at birth, while there was no difference in twin pregnancies, the incidence of miscarriage of a female fetus in single pregnancies was seen to be consistent with the data in the literature. The possible cause of this condition was thought to be the concentration dilution effect. It has been reported that female offspring production has a positive effect on milk yield in Florida goats (30) and Churra and Lacaune sheep (31). In the current study, colostrum production was not measured, presumably ewes bearing females will produce the most colostrum, so that even with no differences in IgG production, any molecule diluted in colostrum will have a lower concentration (20). A similar situation has been observed in dairy cows, and those with female calves were reported to produce higher colostrum and milk during lactation than those with male calves, and the total immunoglobulin concentration was higher in males (32).

In the present study, the fact that sheep with female lambs have low IgG levels was found to be similar to the literature references. Milk production can be affected by the sex of offspring through sex-specific fetal hormones, which have been determined to have the potential to affect placental and mammary gland tissue (33). In the current study, sex-specific hormones released by the fetus may have affected colostrum IgG secretion. However, no association between the sex of the offspring and colostrum IgG levels has been reported in other studies (34, 35). Melatonin applications in Assaf and Lacaune sheep did not affect milk production and quality of milk due to colostrum melatonin treatment (36). In another similar study, it was shown that the application of melatonin during pregnancy improved the oxidative stress status of sheep under heat stress, increasing milk production during multiple pregnancies (37).

During pregnancy, both sheep and fetuses are exposed to oxidative stress caused by an increased amount of reactive oxygen species (ROS) (38). It has been stated that measuring antioxidants separately does not fully reflect the antioxidant capacity of the body, and therefore the colorometric value of TAC, which reflects the total of all antioxidants in the biological system, should be measured for this purpose. In studies conducted in cows, it has been reported that the TAC and TOC levels will vary according to the measurement methods (39) and nutritional differences (40). In the present study, all sheep were under the same care and feeding conditions and an attempt was made to provide a sample using the same analytical method in TAC-TOC measurements.

It is known that application of melatonin implants four times on days 0-40-80 and 120 during pregnancy in sheep increases serum TAC levels and improves redox status, in-

creases the average number of lambs born per sheep, the body weight of the lambs, and milk production (37). It has been reported that TAC values are high in single pregnancies with different antioxidant applications in pregnant sheep (41). In the present study, serum TAC levels were higher in sheep and lambs in the melatonin group ($p<0.001$), and serum TOC and OSI levels were lower in the melatonin group ($p<0.001$). This effect, which occurs as a result of the application of melatonin, an antioxidant, is consistent with the literature references. Melatonin increases the TAC level and decreases the TOC levels by stimulating the synthesis of antioxidants such as GPx and reducing by-products of oxidants such as lipid peroxidase (42, 43). In the present study, it was also determined that twin lambs have significantly lower serum TAC levels than single lambs showing compatibility with the limited literature data (41). In addition, the serum TOS and OSI levels in the current study were observed to be significantly lower in twin lambs than in singleton lambs, but it was not possible to evaluate this fully due to the absence of a similar study. Similar studies in the future may help in this regard.

In a study conducted on Holstein cows, it was reported that TOS levels were higher in the group that did not receive prenatal antioxidant applications (44). It has been reported that antioxidant applications in the prenatal period increase serum TAC levels and decrease TOC levels during the postpartum period (45). In another study conducted on cows, the effect of different prenatal antioxidants on TAC was investigated and it was reported that serum TAC levels were significantly higher in the treatment group compared to the control group during the postpartum period (46). In studies conducted on other species, the increase in serum TAC levels and decrease in TOC levels with the use of antioxidants during pregnancy support the results of the current study.

In pregnant ewes in a number of studies, the administration of melatonin has been shown to have variable effects on the live birth range of lambs when given orally (12 mg per day, from day 90 of pregnancy to birth) or implanted (18 or 36 mg, from day 100 to birth) (47-50). In the present study, melatonin implantation in the last period of pregnancy did not affect the birth weight of lambs born in the control and melatonin groups ($p>0.05$). In a study conducted on Merino sheep, prenatal melatonin was applied, but there was reported to be no difference between the birth weights of single and twin lambs (50). This suggests that melatonin, which was administered only in the last month of pregnancy, was insufficient to create the expected effect on the weight of the lambs born. It is thought that melatonin-induced growth development potential may have been minimized because the sheep in the current study were fed optimally with appropriate rations throughout pregnancy.

The administration of melatonin to sheep during pregnancy is known to prolong the gestation period by 1-2 days (50). In the present study, no difference was observed between the groups. It can be deduced that this condition, similar to birth weight, is due to the fact that melatonin was given in a more limited time period and was unable to stimulate the desired effects.

CONCLUSIONS

In conclusion, the results of this study demonstrated that the prepartum application of melatonin, an antioxidant, increased the antioxidant defence system by decreasing the TOS levels and increasing the TAS levels in sheep and lambs.

It was determined that melatonin applications as subcutaneous implants in the prepartum period significantly increased the level of colostrum IgG in the melatonin group compared to the control group. It was observed that the colostrum IgG level was higher in the ewes that did not have female offspring at birth in the control group compared to those that received melatonin as subcutaneous implants in the prepartum period. In addition, it was observed that melatonin applications as subcutaneous implants in the prepartum period did not differ in twin birth groups, but differed in single birth groups.

It was concluded that the application of melatonin applied in the last part of the prepartum period had no effect on the duration of labor and birth weight.

In sheep breeding enterprises it is recommended that prepartum antioxidant applications be used to reduce reactive oxygen products and improve the body's antioxidant defence system, to increase the quality of colostrum and to provide adequate immunity to the newborn lambs.

Due to the limited number of studies on melatonin applications in sheep, there is a need for further studies on the subject taking into account factors such as season, breed, dosage, and duration of implementation.

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CONFLICT OF INTEREST STATEMENT

The authors of this article have no conflict of interests to declare.

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Investigation of Changes in Biochemical Parameters in Some Diseases Occurring During the Transition Period in Simmental Cows

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ABSTRACT

The transition period is important in cows. This study was conducted to evaluate the effects of retained fetal membranes (RFM), clinical mastitis, and metritis on biochemical and selected mineral levels in Simmental cows. Cows were divided into five groups; cows with RFM (n=17), clinical mastitis (n=25), metritis (n=21) and postpartum healthy cows (n=21) within 21 days postpartum, and also prepartum healthy cows (n=20) in the 15±5 days before the expected parturition date. The activity of alkaline phosphatase (ALP) (85.18±15.83 U/L), aspartate transaminase (AST) (123.02±19.15 U/L), and gamma-glutamyl transferase (GGT) (28.18±2.66 U/L) in the metritis group increased compared to the prepartum healthy cows. Moreover, GGT (41.83±14.61 U/L) and a myocardial band of creatine kinase (CK-MB) (155.25±27.85 U/L) activities were highest in the RFM group, while creatine kinase – N-acetyl-cysteine activity (CK-NAC) (540.45±157.67 U/L) and creatinine concentration (2.29±0.88 mg/dL) were observed in the metritis group. Total protein (6.39±0.38 g/dL) concentration was highest in the case of mastitis. Urea, on the other hand, was highest in the metritis group with a concentration of 61.40±17.38 mg/dL. Our results showed changes in the biochemical profile of cows with RFM, clinical mastitis, and metritis. Biomarker profiles were determined using receiver-operator characteristic (ROC) curves. It was determined that activities of ≥ 89 (U/L) AST, ≥ 24 (U/L) GGT, and ≥ 106 (U/L) CK-MB for metritis, ≥ 21(U/L) GGT for RFM, and ≥ 105 (U/L) CK-MB for mastitis can be used in the preliminary diagnosis. Also, further studies with a larger cow cohorts are recommended.

Key words: Cows; Mastitis; Metritis; Receiver Operating Characteristics; Transition Period.

INTRODUCTION

The transition period, defined as the period between three weeks before calving and three weeks after calving, is a demanding period for cows (1). Infectious disorders occur in the first weeks postpartum (2). Approximately 90% of cows are exposed to bacterial contamination during the two weeks postpartum. Bacteria are eliminated over time; nevertheless, in some cases, these bacteria remain in the lumen of the uterus in cows. This contributes to the occurrence and higher prevalence of postpartum diseases (3, 4).

Puerperal metritis is one of the most important diseases in the postpartum period in cattle (3). This disease is characterized by fetid watery red-brown uterine discharge and an abnormally enlarged uterus, as well as high fever (≥39.5˚C) and systemic disease symptoms, within 21 days after calving. Clinical metritis within 21 days postpartum is limited to

a uterus that has not completed involution. Purulent and foul-smelling symptoms from the vagina without signs of systemic disease are recognized (3). One of the diseases seen in this period is acute puerperal mastitis. Mastitis is a very common disease in dairy cows and one of the most costly diseases for the dairy industry (5).

Biochemical tests used in the early diagnosis and prevention of diseases that cause abundant postpartum economic losses in cows could be beneficial for the dairy industry. It may contribute to the development of protocols for the treatment and prevention of metritis, retained fetal membranes (RFM), and mastitis in the postpartum period. Ruprechter *et al.* (6) and Paiano *et al.* (7) demonstrated that the evaluation of some biochemical parameters in the prepartum period in Holstein cows could be used to predict the health of dairy cows in the postpartum period.

To the best of our knowledge, even though there are biochemical parameters and literature about peripartum diseases of Holstein cows, there is a dearth of information about the changes in the biochemical profile of mastitis, RFM, and metritis diseases in Simmental cows. Therefore, the purpose of this study was to characterize the relationships between changes in the biochemical profiles of peripartum Simmental cows and to screen biomarkers of disease status in Simmental cows with metritis, mastitis, and RFM.

MATERIALS AND METHODS

Animals and sampling

The study used 4-6 year old multiparous (2nd and 3rd lactation) Simmental cows (n=104) weighing 450-500 kg and was conducted on a livestock farm in Kastamonu (Türkiye). This study was conducted following approval by the Kastamonu University Local Ethics Committee of Animal Experimentation. The cows were diagnosed with metritis according to the criteria of Sheldon *et al.* (3), RFM according to the criteria of Beagley *et al.* (8), and mastitis according to the criteria of Cobirka *et al.* (9). Cows were excluded from the study when there was more than one disease evident. In the study, blood samples were collected from cows with RFM (n=17), clinical mastitis (n=25), metritis (n=21), and postpartum healthy cows (n=21) within 21 days postpartum, and also prepartum healthy cows (n=20) in the 15±5 days before the expected parturition date. The blood samples were collected from the jugular vein into tubes without anticoagulants. The blood samples were centrifuged at 6,000 rpm for 8 minutes, and sera were transferred to the eppendorf tubes. The sera were stored at -20°C until biochemical analysis.

Biochemical analysis

Serum albumin, direct bilirubin, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), aspartate transaminase (AST), the myocardial band of creatine kinase (CK-MB), creatine kinase – N-acetyl-cysteine (CK-NAC), unsaturated iron-binding capacity (UIBC), total protein, urea, creatinine, magnesium, phosphorus, calcium, and glucose concentrations were measured with an automatic biochemistry analyzer (Gesan Chem 200, Italy), in accordance with the manufacturer's guidelines.

Statistical methods

Statistical analyses were performed using SPSS Version 22.0 (Chicago, Illinois, USA). The conformity of the data of the parameters examined in the study to the normal distribution was examined visually (probability graphs and histogram) and using the *Shapiro-Wilk* test. It was determined whether the data showed normal distribution or not. According to the evaluation, it was determined that all the data did not meet the parametric test assumptions, that is, they did not show a normal distribution. Therefore, the Kruskal-Wallis test, which is a nonparametric test, was used for intergroup comparisons for all parameters. Mann-Whitney-U test with Bonferroni correction was also used in the *post-hoc* pairwise analysis ($P<0.05$).

Biomarker profiles were determined using receiver-operator characteristic (ROC) curves. The area under the curve (AUC)>0.90 was considered highly accurate, AUC between 0.70 and 0.90 was considered moderate accuracy, AUC between 0.5 and 0.7 was considered low accuracy, and AUC≤0.5 was considered a chance result. In the evaluation of the area under the curve, it was accepted that the diagnostic value was not statistically significant for parameters with $P>0.05$ (10).

RESULTS

Table 1 and Table 2 show the results of the biochemical profiles. The highest ALP activity was found in the metritis

Table 1: Concentration of biochemical parameters of groups I of Simmental cows

Groups (n)	ALT (U/L)	ALP (U/L)	AST (U/L)	GGT (U/L)	Albumin (g/dL)	Direct bilirubin (mg/dL)	Total bilirubin (mg/dL)
Prepartum healthy cows (20)	23.50±10.36^{b}	63.75±10.36^{abc}	23.30±1.43^{a}	23.30±1.43^{a}	3.06±0.11	0.07±0.01^{a}	0.14±0.02^{a}
Clinical mastitis (25)	19.42±3.11^{ab}	53.46±7.59^{ab}	90.96±7.49^{ab}	28.86±2.92^{ab}	3.25±0.18	0.21±0.06^{b}	0.27±0.04^{ab}
RFM (17)	12.00±0.01^{a}	73.25±15.08^{bc}	129.25±36.64^{b}	41.83±14.61^{b}	2.88±0.05	0.14±0.02^{ab}	0.40±0.08^{a}
Clinical metritis (21)	18.22±2.07^{ab}	85.18±15.83^{c}	123.02±19.15^{b}	28.18±2.66^{ab}	2.94±0.21	0.16±0.02^{ab}	0.43±0.05^{b}
Postpartum healthy cows (21)	27.23±4.68^{b}	41.66±3.25^{a}	133.92±41.16^{b}	22.90±0.77^{a}	2.87±0.05	0.11±0.02^{ab}	0.30±0.07^{ab}
P	0.001	0.025	0.047	0.009	0.089	0.000	0.000

$^{a, b, c}$: The difference between groups with different superscripts in the same column is statistically significant, (P<0.05).

Table 2: Concentration of biochemical parameters of groups II of Simmental cows

Groups (n)	Creatinine (mg/dL)	CK-MB (U/L)	CK-NAC (U/L)	Total protein (g/dL)	UIBC (µg/dL)	Urea (mg/dL)
Prepartum healthy cows (20)	1.38±0.10^{a}	100.15±11.34^{a}	198.10±15.27^{a}	6.39±0.38^{a}	185.35±9.78	37.25±0.91^{a}
Clinical mastitis (25)	1.17±0.05^{a}	112.50±3.17^{a}	148.71±16.01^{a}	7.55±0.17^{b}	183.00±19.21	28.85±3.83a
RFM (17)	1.28±0.048^{a}	155.25±27.85^{b}	457.08±80.91^{b}	6.55±0.11^{a}	214.00±16.48	27.00±1.23^{a}
Clinical metritis (21)	2.29±0.88^{b}	134.54±13.11^{ab}	540.45±157.67^{b}	7.22±0.29^{ab}	151.70±14.03	61.40±17.38^{b}
Postpartum healthy cows (21)	1.24±0.08^{a}	107.14±9.27^{a}	327.80±83.85^{ab}	6.81±0.18^{ab}	180.00±9.64	36.52±1.95^{a}
P	0.047	0.036	0.009	0.004	0.052	0.002

$^{a, b}$: The difference between groups with different superscripts in the same column is statistically significant, (P<0.05).

group (85.18±15.83 U/L) compared to postpartum healthy cows (41.66±3.2 U/L) (P<0.05). Compared to the prepartum healthy cows, mastitis, metritis, and RFM groups had higher AST and GGT activities (P<0.05). Alanine aminotransferase activity was the lowest in the RFM group (12.00±0.01 U/L) (P=0.001). The highest direct bilirubin concentration was found in the mastitis group (0.21±0.06 mg/dL) (P=0.000), while total bilirubin was found in the metritis group (0.43±0.05 mg/dL) (P=0.000). Creatine concentration (2.29±0.88 mg/dL) and CK-NAC (540.45±157.6 U/L) activity were found to be higher in the metritis group, while CK-MB (155.25±27.85 U/L) activity was the highest in the RFM group (P<0.05). While the total protein concentration is at its lowest level in the prepartum period (6.39±0.38 g/dL), it increased when mastitis (7.55±0.17 g/dL) occurred (P<0.05). In terms of the urea concentration, it seemed that the metritis group (61.40±17.38 mg/dL) had increased when compared with the other groups (P<0.05). There was no difference between the groups with regard to albumin (P=0.089) and UIBC (P=0.052) concentrations.

Table 3 shows blood mineral constituents and glucose concentrations. Blood calcium concentrations were higher in prepartum (9.83±0.10 mg/dL) and postpartum healthy cows (10.33±0.10 mg/dL), yet mastitis (9.61±0.24 mg/dL), metritis (9.00±0.32 mg/dL), and RFM (9.75±0.21 mg/dL) groups were lower (P<0.05 for all conditions mentioned above). Magnesium (1.86±0.11 mg/dL) and phosphorus (3.81±0.4 mg/dL) concentrations were the lowest in the RFM group, while glucose concentration was the lowest in the postpartum healthy cows (38.95±5.17 mg/dL) (P<0.05).

Receiver-operator characteristic curve analysis results for AST, GGT, and CK-MB activities for metritis, GGT activity for RFM, and CK-MB activity for mastitis are given in Table 4. Receiver-operator characteristic curve analysis values were determined for AST, GGT, and CK-MB activities with P<0.05.

Table 3: Blood mineral substance and glucose concentrations in groups of Simmental cows

Groups (n)	Phosphorus (mg/dL)	Calcium (mg/dL)	Magnesium (mg/dL)	Glucose (mg/dL)
Prepartum healthy cows (20)	5.36±0.21^{c}	9.83±0.10^{bc}	3.08±0.37^{ab}	57.70±5.33^{b}
Clinical mastitis (25)	5.10±0.31^{bc}	9.61±0.24^{b}	2.03±0.29^{a}	64.73±5.29^{b}
RFM (17)	3.81±0.43^{a}	9.75±0.21^{b}	1.86±0.11^{a}	59.16±7.24^{b}
Clinical metritis (21)	5.06±0.55^{bc}	9.00±0.32^{a}	2.85±0.44^{ab}	72.81.± 7.21^{b}
Postpartum healthy cows (21)	4.22±0.20^{ab}	10.33±0.10^{c}	3.95±0.54^{b}	38.95±5.17^{a}
P	0.005	0.001	0.031	0.000

$^{a, b, c}$: The difference between groups with different superscripts in the same column is statistically significant, (P<0.05).

Table 4: ROC analysis results of AST, GGT, and CK-MB activities in groups of Simmental cows for metritis, mastitis, and RFM

	Item	Optimized Cutoff	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P
Metritis	AST (U/L)	≥ 89	82	75	0.786	0.009
	GGT (U/L)	≥ 24	73	70	0.736	0.032
	CK-MB (U/L)	≥ 106	100	75	0.786	0.009
RFM	GGT (U/L)	≥ 21	91	55	0.750	0.023
Mastitis	CK-MB (U/L)	≥ 105	93,3	75	0.740	0.016

AUC: Area under the curve

DISCUSSION

Intense septicemia caused by intrauterine infection may damage the liver tissue, causing an increase in the activity of liver enzymes and thus deterioration of liver function (11). Risk factors for uterine infections and mastitis include hypocalcemia, hypomagnesemia, and hepatocellular damage (i.e. increased GGT, ALT, ALP, and AST) (12, 13). Based on the liver function results, it was possible to detect an increase in GGT and AST activities in the metritis group, indicating damage to the liver tissue in the group before the clinical diagnosis of metritis (7). In this study, it was determined that ALP, AST, and GGT activities increased when the disease developed compared to prepartum healthy cows, but ALT activity was lowest in the metritis and RFM groups. Dervishi *et al.* (14), found the activities of GGT and AST to be higher in the metritis group than in the healthy cows. Furthermore, it was stated that ALP, AST, and ALT activities increased in the mastitis group compared to healthy cows. In the same study, it was determined that total protein concentration increased in mastitis cases (15). When mastitis and metritis groups were compared with postpartum healthy cows, it was also found in our study that total protein increased. In another study, it was stated that there was no statistical difference in total protein concentration between the prepartum and during lactation. Since the disease did not develop during late pregnancy, early lactation, and mid-lactation, it was thought that there was no change in total protein concentration (16).

Serum AST concentration showed a significant difference between the groups and was higher in cows with RFM at day 10±4 and 3±1 day relative to calving (P<0.05), but there was no statistical difference at 10±4 and 30±4 days postpartum. Also, it was noted that there were no differences in ALP and ALT activities, glucose, urea, albumin, and phosphorus concentrations between cows with and without RFM (17). Furthermore, in another study, there was no difference in GGT activity and total protein concentration in pre/postpartum blood samples for cows with the RFM (18). Although there is no difference between groups in albumin concentration according to our study, studies have shown that it may be at a lower level in cases where RFM and metritis are present (19, 20). Contrary to our study, it was stated that albumin concentration increased in cows with mastitis (21). The prepartum detection of this increase brought to mind that mastitis can be used for pre-detection (6).

Our study showed that urea and creatine concentrations

increased in the metritis group, but decreased in cows with RFM and mastitis. Paiano *et al.* (7) and Senosy *et al.* (22) found that urea and creatine concentrations decreased in cows with metritis in their studies. On the other hand, when the GGT activity, total protein, creatine, and urea concentrations in the prepartum period are compared with the postpartum period, it is stated that there was no difference (18). It has been found that cows with vaginal discharge in the postpartum period had lower urea and albümin concentrations, while they showed higher serum GGT activity compared to the control group (23).

As for serum calcium concentrations, the findings of the present study showed lower calcium concentrations than postpartum healthy cows when RFM, metritis, or mastitis were present in cows, as determined in previous studies (24, 25). Akar and Yildiz (26) found the calcium level to be low in cows with RFM in their study, but there was no difference in magnesium levels compared to the control group. On the other hand, it was also stated that there was no difference in calcium levels between cows that displayed RFM and those which did not (27). In the present study, phosphorus, calcium, and magnesium levels were found to be the lowest in the RFM group. In addition, a decrease in the level of these minerals was observed in cows with mastitis. In another study conducted on cows with mastitis, it was determined that the above-mentioned minerals decreased (28).

Patbandha *et al.* (29) demonstrated higher sensitivity (75%), specificity (66.67%), and accuracy than Ospina *et al.* (30) for metritis diagnosis using a prepartum blood non-esterified fatty acids threshold value. They reported 37% sensitivity and 80% specificity, respectively. Milk lactose could detect the distinction between infected and non-infected udder quarters in cows with an 81% accuracy (31) and in buffaloes with 83.76% accuracy (32). Also, Pyorala (33) stated that milk lactose could be used to identify the distinction between mastitis and healthy quarters with an accuracy of 73.9 to 77.1%. This study, it is considered that activities of ≥ 89 (U/L) AST, ≥ 24 (U/L) GGT, and ≥ 106 (U/L) CK-MB for metritis, ≥ 21 (U/L) GGT for RFM, and ≥ 105 (U/L) CK-MB for mastitis can be used in the preliminary diagnosis of these diseases in Simmental cows. The overall test performance of these findings is moderate and their practical use for diagnosing these diseases is uncertain. Considering the association of elevated CK-MB of Simmental cows with metritis, CK-MB remains an important tool to test research hypotheses, especially when a more objective parameter for the metritis is needed.

CONCLUSIONS

In conclusion, our results show that there are biochemical changes in Simmental cows with metritis, mastitis, and RFM in the transition period. Regular monitoring of the specified parameters in the postpartum period in dairy cows may be useful for veterinarians and dairy farm owners to predict uterine health and mastitis. To better understand the role of the aforementioned parameters in the pathogenesis of RFM, mastitis, and metritis, further studies with a larger cohort of cows should be conducted with periodic blood samples taken before the onset of these disease symptoms and at different times in Simmental cows.

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Bartonella bovis in Cattle in Nigeria: Molecular Detection and the Analysis of Risk Factors

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ABSTRACT

Cattle are the most important source of animal protein to humans in Nigeria. They are predominately raised under the extensive system of production. Although, low cost, this management system exposes the animals to several ectoparasites and vector-borne infections, with veterinary and public health consequences. Bartonellosis is an emerging vector-borne infection with veterinary and zoonotic implications. This study examined 462 blood samples from cattle in Nigeria for the presence of *Bartonella* DNA using PCR and sequencing approach. DNA fragments of the citrate synthase gene (*gltA*) and RNA polymerase beta subunit gene (*rpoB*) of *Bartonella bovis* were detected in 43 (9.3%) and 6 (1.3%), respectively, of the samples examined. The *gltA* and *rpoB* sequences from this study had high identities of 97.6% to 99.8% with GenBank deposited sequences of *B. bovis.* Phylogenetic analysis recovered the *gltA* and *rpoB* nucleotide sequences from this study in a monophyletic clade with *B. bovis* sequences from diverse mammals from other countries. Prevalence of *B. bovis* was associated ($p<0.05$) with animals older than two years of age and samples collected from abattoirs. This is the first report of *B. bovis* in cattle in Nigeria. More studies are required to determine the potential public health implications of these findings considering the high rate of detection in animals slaughtered for human consumption and the difficulties in enforcing meat inspection laws.

Keywords: Bartonellosis; Cattle; PCR; *gltA*; *rpoB*; Nigeria.

INTRODUCTION

Cattle are the most common type of large domesticated ungulate found throughout much of the world. They comprise hundreds of breeds that are recognized worldwide (1). In Nigeria, cattle are the single most important livestock species in terms of animal protein supply, value and biomass (2, 3). They are not only the main source of meat, milk, skin, bone, blood and horn products, but they are also used for draught power, transportation of people and loads, as well as to lift water from deep wells (3, 4, 5). About 75 percent of ruminant livestock population in Nigeria are found in the Sahel agro-ecological zone and are managed under the pastoral system (3, 6). Although, this production system is low cost, it exposes the animals to diseases, consequently, affecting productivity and endangering public health.

Bartonellae are considered as emerging pathogens, being increasingly associated with a number of diseases in both humans and animals (7, 8). They are fastidious Gram-negative bacteria that infect and persist in mammalian erythrocytes and endothelial cells and are found in a wide range of wild and domesticated mammals (5). Several species of *Bartonella* have been isolated from blood of ruminants in Africa, Asia,

Europe, and North and South America (4, 9-15). Among cattle, *Bartonella bovis* has been implicated in causing bovine endocarditis (16, 17). Other species such as, *Bartonella chomelii, Bartonella rochalimae, Bartonella schoenbuchensis, Bartonella vinsonii* subsp. *arupensis,* '*Candidatus* Bartonella davousti' have also been isolated from ruminants (13, 15, 18-20). The latter list is likely to increase with more studies and improvements in the detection methods. *Bartonella* spp. are usually transmitted to animals and humans through blood-feeding arthropod vectors such as fleas, lice, ticks and sandflies (12, 21).

The reported prevalence of *B. bovis* in cattle varies widely across studies from different geographic regions, i.e., 6.8% in Poland (22), 11.8% in Greece (15), 24% in Italy (23) and 70% in French Guyana (11). On the African continent, the first report of *B. bovis* infection in cattle was in Cote d'Ivoire, West Africa (4). Subsequently, there were reported prevalence of 27.8% in Senegal (13) and 15.3% in cattle in Algeria (14). *Bartonella* spp. of zoonotic importance have been reported in rodents and bats and their ectoparasites in Nigeria (24, 25). However, to date there is no report on *Bartonella* species infection in cattle in Nigeria. Therefore, this study was aimed at using molecular approach to detect and characterize *Bartonella* spp. in cattle in Nigeria and to determine the risk factors for infection.

MATERIALS AND METHODS

Ethical Approval

Approval for this study was granted by the Institutional Animal Care and Use Committee (IACUC), National Veterinary Research Institute (NVRI), Vom, Nigeria, approval number: AEC/03/108/21. Informed consent was obtained from management of abattoirs and cattle owners before the animals were sampled.

Study Area and Sample Collection

The study was conducted on blood samples collected from cattle from the three agro-ecological zones (AEZs) of Nigeria between July to December 2021. Samples were collected from two, five and three states in the Sahel, Savanna and Guinea AEZs, respectively (Fig.1). Blood samples were collected from 50 cattle slaughtered in each abattoir located in Jalingo, Maiduguri and Katsina, 35 in Lafiya abattoir and 54 in Jos abattoir. The remainder of the tested samples were obtained from sedentary herds in Ekiti (n=4), Kaduna (n=8), Nasarawa (n=15), Jos (n=26), Akwa Ibom (n=29), Ogun (n=41) and Kwara (n=100).

Before sampling, visual examination was conducted to assess the body condition of each cattle. Data on the age, gender and breed of the animals were recorded for each study site. Trained personnel properly restrained animals and 5 mL of blood was drawn from the jugular vein into ethylene diamine tetra-acetic acid (EDTA) tubes. Samples were kept in cold boxes packed with ice and transported to the Molecular Biology laboratory, Parasitology Division, NVRI, Vom, where they were kept at -20°C until analysis.

DNA extraction

DNA was extracted from anticoagulated blood using the Quick-DNA™ Miniprep Plus kit (Zymo Research, USA) with slight modification of the manufacturer's protocol. Briefly, 20 µL of proteinase K was added to 200 µL of anticoagulated blood and equal volumes of BioFluid in a 1.5 mL micro centrifuge tube. The mixture was vortexed for 15 seconds and incubated at 55°C for three hours. 420 uL of DNA binding buffer was added to the lysate, vortexed briefly and centrifuged at 16,000 xg for 3 minutes. The mixture was then transferred to a Spin column and the procedure was continued according to the manufacturer's instructions. The DNA was eluted in 80 µL of elution buffer and stored at -20°C until analysis.

Amplification of *Bartonella* spp. DNA from cattle blood by conventional PCR

All the DNA samples extracted from cattle blood in this study were initially screened for the presence of *Bartonella* spp. 350 bp citrate synthase (*gltA*) gene using the primers CSH1F (5' GCG AAT GAA GCG TGC CTA AA-3') and BhCS1137 (5'-AAT GCA AAA AGA ACA GTA AAC A-3') (26). Positive samples in the *gltA* amplification were tested for the amplification of the 850 bp RNA polymerase beta subunit gene (*rpoB*) using the primers 1400F (5'-CGC ATT GGC TTA CTT CGT ATG-3') and 2300R (5'-GTA GAC TGA TTA GAA CGC TG-3') (27).

The PCR mix consisted of 16 µL of 2X Master Mix with standard buffer (New England Biolabs Inc.), 0.6 µL of each primer (10 mM), 5 µL of template DNA and 8.8 µL of DNA/RNA-free water (BioConcept, Switzerland) in a final volume of 31 µL. Amplification for the *gltA* gene

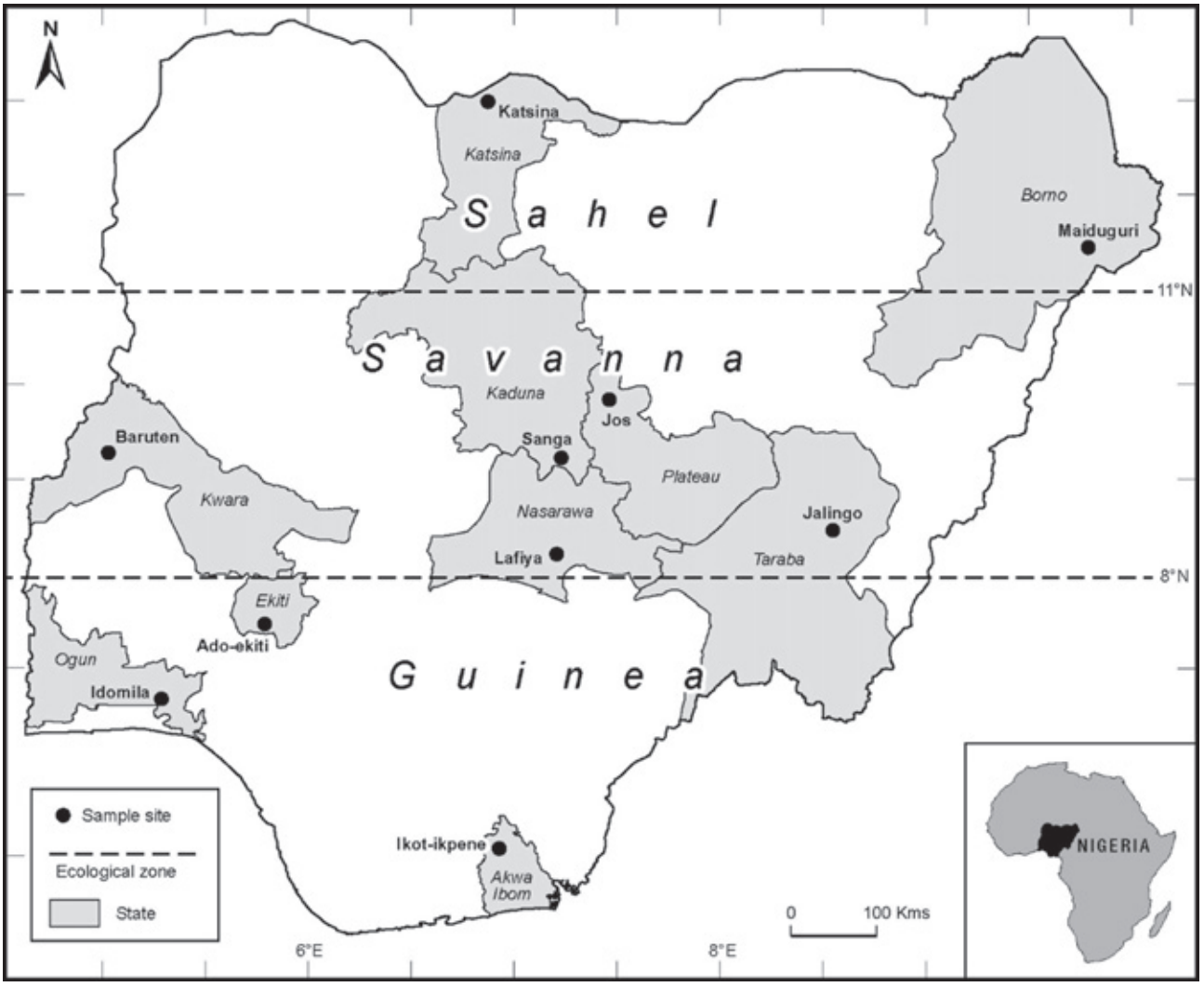

Fig.1. Map of Nigeria, West Africa showing the study sites.

involved 30 seconds at 94°C initial denaturation followed by 35 cycles of 30 seconds at 94°C, 1 minute at 51°C (annealing), and 1 minute at 68°C followed by a final elongation at 68°C for 8 minute. The amplification of the *rpoB* gene was similar except annealing which was done at 53°C for 30 seconds. The conventional PCR was conducted on a GenAMP 7400 (Applied Biosystems, Foster City, CA), in the Molecular Biology Laboratory, Parasitology Division, NVRI Vom, Nigeria. The DNA of *Bartonella elizabethae* obtained from a commensal rodent in Nigeria was used as a positive control. A non-template control (NTC) containing all the reaction mix except DNA was included in each PCR run. The PCR products were electrophoresed in a 1.2% agarose gel stained with SafeView™ Classic (Applied Biological Materials, Canada) and were visualized under a Blue light Transilluminator (Cleaver Scientific, UK) for the size of amplified fragments by comparison to a 100-bp DNA molecular weight marker. Positive amplicons were sequenced at the Center for Genomic Technologies, Hebrew University of Jerusalem, Israel using the PCR primers.

Nucleotide and phylogenetic analysis

Sequences were edited manually in the software Geneious Prime 2022.0.1 (https://www.geneious.com) and were compared to reference sequences available in the GenBank using the BLASTn algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Nucleotide sequences were quality checked and ambiguous base calls or missing data were coded with N´s or the corresponding ambiguity code. Double nucleotide peaks in otherwise high-quality sequence parts

Table 1. Prevalence of *Bartonella bovis*-DNA in cattle in Nigeria

Study location	No. positive/no. tested (%)	
	gltA	*rpoB*
Katsina	5/50 (10.0)	1/50 (2.0)
Jalingo	16/50 (32.0)	4/50 (8.0)
Borno	2/50 (4.0)	0 (0)
Ekiti	0/4 (0)	0 (0)
Kwara	0/100 (0)	0 (0)
Kaduna	0/8 (0)	0 (0)
Akwa Ibom	0/29 (0)	0 (0)
Ogun	6/41 (14.6)	0 (0)
Plateau	7/80 (8.8)	1/80 (1.3)
Nasarawa	7/50 (14.0)	0 (0)
Total (%)	43/462 (9.3)	6/462 (1.3)

were scored as mixed haplotype infection and haplotype diversity was assessed in Geneious Prime. Sequences were aligned using the MAFFT algorithm (28, 29). Reference sequences were retrieved from GenBank, mainly based on the dataset of Goncalves *et al.* (30) and added to alignments of the sequences of the study (all accession numbers are listed in the phylogenetic trees, see Figs 2 & 3). The program ModelTest-NG was used to test different DNA substitution models (31). The phylogenetic analyses of the partial *rpoB* gene consisting of a total of 792 nucleotides (nt) comprising 38 sequences including two new sequences of this study and the substitution model TIM3+G+I was used. The phylogenetic analyses of the partial *gltA* gene of a total of 378 nt comprised 37 sequences including five new sequences of this study and the substitution model TIM3+G was used. For Maximum likelihood (ML) analysis, we used raxmlGUI version 2.0.6 (32). Nodal support was evaluated using 1000 thorough bootstrap and consensus. Phylogenetic trees were displayed in FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Sequences obtained in this study were deposited in the GenBank under the following accession numbers: *gltA* (OM317751– OM317755); *rpoB* (OM317756 & OM317757).

Statistical analysis

The prevalence of *B. bovis* DNA was calculated as percentage of the total samples examined. The association between the detection of *B. bovis* DNA based on the *gltA* gene and the variables: age (≤ 2 years old versus > 2 years old), body condition score (good, medium or poor), source of sample (abattoir or herds), agro-ecological zones (Sahel, Savanna or Guinea), sex and breed of animals were analyzed. Univariate analysis was performed for each risk factor using the Chi-square test. The analysis was performed using the R Statistical Software (33). The level of significance was set at $p \leq 0.05$.

RESULTS

Blood samples were collected from 462 cattle from ten states located in the three agro-ecological zones of Nigeria (Table 1, Fig. 1). From this number, 239 of the samples (51.7%) were obtained from cattle slaughtered in abattoirs in four cities, while 223 (48.3%) where from cattle in herds located in different parts of Nigeria. The majority of the cattle were females; (278/462, 60.2%) and 72.3% (334/462) were older than two years of age. Eight breeds of cattle were sampled, although the bulk of them, 360 (77.9%) were of the White Fulani breed (Table 2). 288 of the 462 (62.3%) of the cattle sampled were from the Savanna agro-ecological zone, followed by 100 and 74 from the Sahel and the Guinea zones, respectively (Table 2, Fig. 1). Overall, 43 (9.3%) and 6 (1.3%) out of the 462 of the cattle examined were positive for *B. bovis* DNA based on the *gltA* and *rpoB* genes, respectively (Table 1). The highest prevalence (32.0%) of *Bartonella bovis* DNA was detected in cattle from an abattoir in Jalingo, followed by 14.6% in cattle from a sedentary herd in Ogun State. Although the prevalence

Fig. 2. Maximum likelihood phylogenetic analysis of 387 bp *gltA* sequences of *Bartonella* species. Bootstrap values are indicated. The sequences of this study are highlighted in bold and accession numbers are given in parentheses. The geographic origin (country) and the vertebrate host of each sequence are provided. The taxon *Brucella melitensis* was used as an outgroup.

of *B. bovis* DNA varied among the different categories of cattle examined, significant association ($p<0.05$) was found with older cattle (>2 years) and those slaughtered in the abattoirs, but not with the gender, breed, body condition or ecological zones (Table 2).

Table. 2: Prevalence and relative risks of *Bartonella bovis* in cattle in Nigeria

Variables	Number of animals tested			Prevalence (%)	95% CI	χ2	P
	Positive	Negative	Total				
Source of sample							
Abattoir	36	203	239	15.1	0.108-0.202	18.04	0.00002*
Herds/Farms	7	216	223	3.1	0.013-0.064		
Gender							
Male	14	170	184	7.6	0.042-0.124	0.73	0.39
Female	29	249	278	10.4	0.071-0.146		
Breed							
White Fulani	34	326	360	9.4	0.066-0.130	2.32	0.94
Bokoloji	2	15	17	11.8	0.015-0.364		
Sokoto Gudali	1	23	24	4.2	0.001-0.211		
Keteku	0	1	1	0.0	0.000-0.975		
N'dama	0	1	1	0.0	0.000-0.975		
Red Fulani	3	27	30	10.0	0.021-0.265		
Ambala	1	3	4	25.0	0.006-0.806		
Wadara	2	23	25	8.0	0.010-0.260		
Age							
Adult (>2years)	40	294	334	12.0	0.087-0.160	11.47	0.0007*
Young (≤2years)	3	125	128	2.3	0.005-0.067		
Body condition							
Good	20	153	173	11.6	0.072-0.173	1.70	0.42
Medium	17	191	208	8.2	0.048-0.128		
Poor	6	75	81	7.4	0.028-0.154		
Ecological zone							
Sahel	7	93	100	7.0	0.029-0.139	1.18	0.55
Savanna	30	258	288	10.4	0.071-0.145		
Guinea	6	68	74	8.1	0.030-0.168		

χ2 = Chi-square, P= significant value

Nucleotide and phylogenetic analysis

Partial sequences of the *gltA* gene were amplified and sequenced for five samples. Sequences of four samples matched *B. bovis,* which share sequence identities of 97.6– 99.8%. One sample (JA53) did not feature any ambiguity bases and therefore represents a single haplotype, whereas the other three sequences (OG11, OG40, T33) were mixed haplotype infections, featuring several double nucleotide peaks in the electropherograms. The phylogenetic analysis grouped all four sequences with other reference sequences of *B. bovis* in one monophyletic clade with high support (bootstrap value of 94). The fifth sequence (sample T1, mixed haplotype infection) was closer related to the species *Bartonella grahamii* according to the phylogenetic analysis with low bootstrap value of 36 (Fig. 2). The reference sequence with highest sequence identity of 97.38% was an uncultured *Bartonella* sp. sample from a spleen of a small mammal in Equatorial Africa (Fig. 2).

The two partial *rpoB* gene sequences of the Nigerian *Bartonella* samples of the study shared a sequence identity of 98.8%. Both sequences contained ambiguous base calls which indicate mixed haplotype infections. The phylogenetic

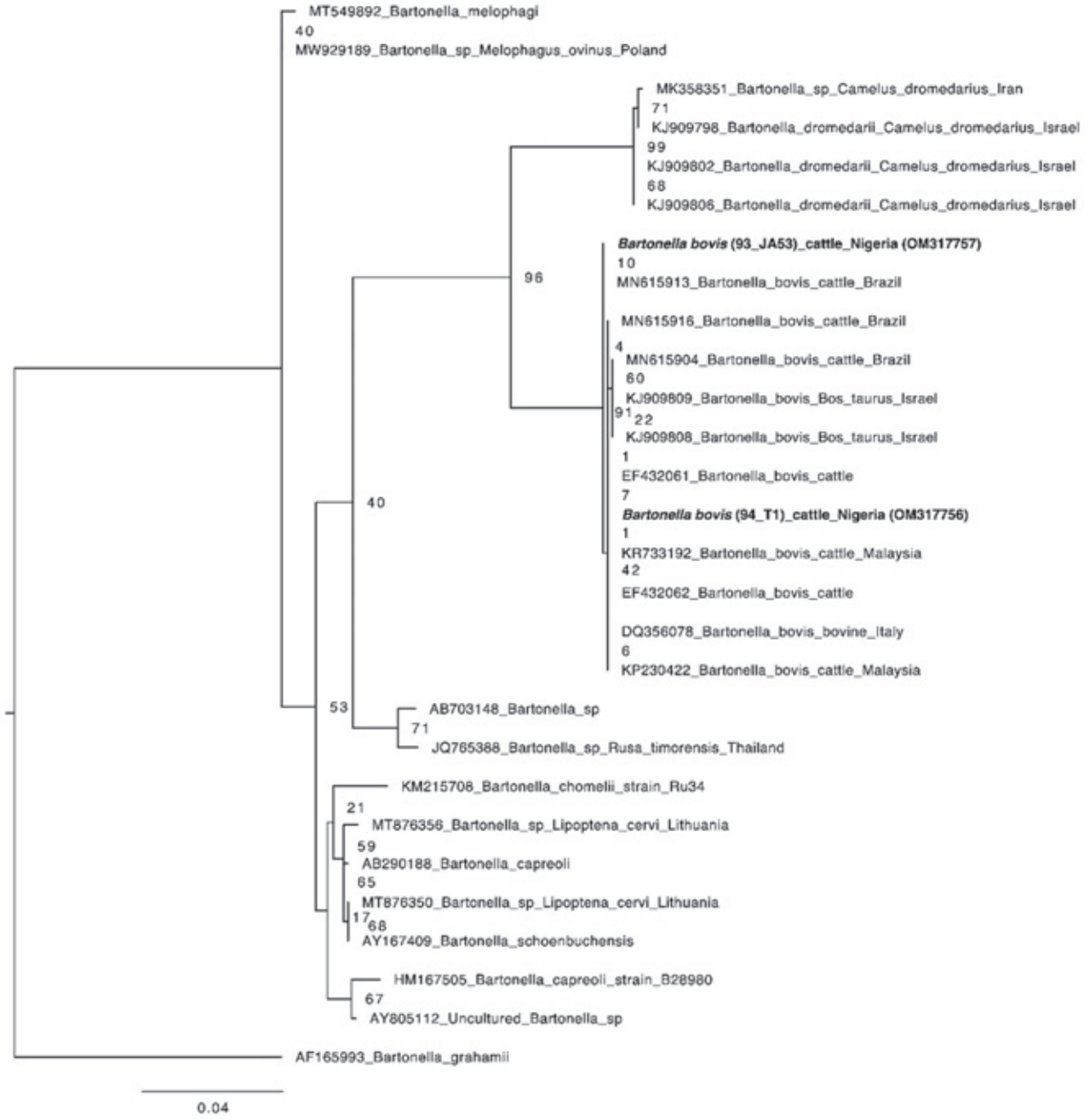

Fig. 3. Maximum likelihood phylogenetic analysis of 792 bp *rpoB* sequences of *Bartonella* species. Bootstrap values are indicated. The sequences of this study are highlighted in bold and accession numbers are given in parentheses. The geographic origin (country) and the vertebrate host of each sequence are provided. The taxon *Bartonella grahamii* was used as and outgroup. The phylogenetic analysis of *rpoB* recovered both Nigerian sequences within the monophyletic group of *Bartonella bovis* sequences from cattle from e.g., Brazil, Israel, Malaysia, and Italy with high support (bootstrap value of 91) as sister clade to the species *Candidatus* Bartonella dromedarii (named *Bartonella dromedarii* in the tree) (bootstrap value of=96).

analysis of the *rpoB* sequences placed both Nigerian sequences within the monophyletic group of *B. bovis* sequences from cattle from Brazil, Israel, Malaysia, and Italy with high support (bootstrap value of 91) as sister clade to the species *Candidatus* Bartonella dromedarii (bootstrap value of = 96). The nucleotide sequence of the sample T1 that clustered with sequences *B. grahamii* in the *gltA* dendrogram was clustered with *B. bovis* in the *rpoB* phylogenetic tree with high bootstrap (Fig 3).

DISCUSSION

Molecular detection and nucleotide sequence analysis of *Bartonella* spp. in cattle in Nigeria, confirmed that all the sequences obtained in this study belong to *B. bovis*. The prevalence of 9.3% (*gltA*) and 1.3% (*rpoB*) of *B. bovis* DNA in cattle in this study was lower than the previous reports in cattle of 27.8% in Senegal (13) and 15.3% in Algeria (14). However, a zero prevalence of *Bartonella* spp. has been reported in cattle from Kenya (5). The difference may be due to the study design, the genes targeted and the sensitivities of the assays used in each of the studies. Only the DNA of *B. bovis* was detected in cattle in this study, unlike the studies from Algeria and Senegal, where *B. chomelii* and a novel spp. '*Candidatus* Bartonella davousti' were reported in addition to *B. bovis* (13, 14). Our findings were similar to the first report of *Bartonella* spp. in cattle on the African continent where only *B. bovis* was recovered by both culture and PCR amplification (4). In this study, the prevalence of *B. bovis* was associated with older cattle (>2 years) similar to the report from Spain (20) but, in contrast to the report from France and Algeria (14, 16). Generally, high prevalence of vector-borne diseases have been associated with older animals, especially those raised under the extensive management due to continuous challenge by hematophagous arthropods (34, 35). Furthermore, there was an association between samples obtained from cattle slaughtered in the abattoirs with detection of *B. bovis* DNA.

Likewise, a study in Senegal reported the isolation from cattle blood of a *Bartonella* spp. with unique genetic features, which are different from other species of the *Bartonella* genus. This isolate is being proposed as a potentially novel *Bartonella* spp., highlighting the probable role of cattle as a potential reservoir of *Bartonella* spp. (13).

Beef is the main source of animal protein for humans in most of Nigeria. Each year large numbers of cattle and other livestock are slaughtered at designated abattoirs, slaughterhouses or slaughter slabs to produce meat for public consumption. However, sometimes slaughter may also take place at home or at an outdoor slaughter facility designated for religious and cultural practices (3). Although, veterinary public health officials are mandated to inspect and certify meat targeted for human consumption, enforcement of certain decisions have been hampered due to obsolete or lack of legislation, to back such actions. Worst still, it has been observed that in defiance to directives from veterinary public health officials to trim some infected parts from a carcass before passing the meat for public consumption, butchers cut some of the affected parts and eat it raw to justify the fitness of the meat for human consumption. Hence, butchers and meat processors usually operate without proper public health supervision thereby endangering the health of consumers. A relatively high proportion of the Nigerian populace are immunosuppressed either due to malnutrition or infections with HIV or malaria, thereby predisposing them to several emerging infectious diseases (36-39). Therefore, the role of *Bartonella* spp. in causing disease in immunosuppressed individual deserves attention. For example, a study conducted in South Africa reported that 10% of outpatients attending HIV clinics in an urban center were bacteremic with *B. henselae* (40). As for 2015, Nigeria was rated as having the world's second highest burden of people living with HIV/AIDS (41). Added to this, the predominant cattle production system in Nigeria brings humans into close contact with domestic animals and ectoparasites, which may facilitate the transmission of zoonotic pathogens or even non-pathogenic species to cause disease in immunocompromised people. *Bartonella bovis* may be a potential agent of zoonosis in humans similar to what was demonstrated with *B. rochalimae* that was first detected in a Pulex flea and subsequently isolated from a patient with fever and splenomegaly (42).

In conclusion, the detection of *B. bovis* DNA in cattle in Nigeria, most especially in cattle slaughtered for human consumption in the face of poor sanitation, poor health condition and the inability to strictly enforce meat inspection in most abattoirs is a source of public health concern. More studies in line with the One Health concept should be conducted to elucidate the epidemiology and public health implication of *Bartonella* spp. in Nigeria. Efforts should be intensified to improve the sanitary condition in the abattoirs and to effectively enforce meat inspection policy across the country.

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CONFLICT OF INTEREST / COMPETING INTERESTS

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Molecular Characterization of Methicillin- and Multidrug-Resistant *Staphylococcus pseudintermedius* Strain Isolated from a Case of Feline Otitis Externa

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ABSTRACT

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an emerging zoonotic pathogen in veterinary medicine. Whole-genome sequencing (WGS) is highly preferred today as a valid method for molecular typing of bacterial pathogens due to its high discriminatory power and characterization efficiency. This study describes the WGS–based characterization of an MRSP strain named HMKU-VET-MRSP-2020 from a case of cat otitis externa in Türkiye. The strain was classified as MLST sequence type 71 and SCC*mec* type IIIA. WGS analysis indicated the presence of several antimicrobial resistance genes and mutations consistent with the resistance phenotype. Phylogenetic analysis showed that the strain was clusterized with the isolates from dog clinical cases previously reported from different countries of Europe and one human isolate from the USA. The study is the first report on the isolation and molecular characterization of MRSP from a cat in Türkiye and provides insights into the zoonotic potential of this microorganism.

Keywords: Cat; Methicillin Resistance; *Staphylococcus pseudintermedius*; Whole-Genome Sequencing.

INTRODUCTION

Staphylococcus pseudintermedius is an opportunistic pathogen that colonizes companion animals' skin and mucous membranes asymptomatically (1). *S. pseudintermedius* is the most frequent cause of skin and soft tissue infections in dogs than in cats (2). The lower colonization rates observed in cats are attributed to less adhesion of *S. pseudintermedius* to feline corneocytes than to canine corneocytes (3). However, in cats, *S. pseudintermedius* can be isolated from various infections related mostly to the skin, including dermatitis, otitis, and wound infections (1).

In 2006, methicillin-resistant *S. pseudintermedius* (MRSP) emerged and reached worldwide high prevalence rates (4). The frequent reports of multi-drug resistance (MDR) in MRSP isolates greatly complicate veterinary clinicians' therapeutic interventions (5, 6). MRSP has an epidemic clonal population structure (4), and certain dominant MDR MRSP lineages were frequently reported in particular continents, namely ST71 and ST258 in Europe, ST68 in North America, and ST45 and ST112 in Asia (4). *S. pseudintermedius*-related human infections have also been reported due to transmission from pet animals to humans (2, 7, 8).

This study was, therefore, conducted to perform a Whole-genome sequencing (WGS)-based characterization of an MDR *S. pseudintermedius* strain isolated from a case of feline otitis externa in Türkiye.

MATERIALS AND METHODS

Isolation and identification

The MRSP strain was isolated from a 3.5-year-old female Tekir cat's left ear with otitis externa in the Microbiology Laboratory, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University. The strain was first identified using phenotypic tests (colony morphology, Gram staining, and catalase test) for genus level. The species identification was performed by the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer using the Biotyper 3.1 software (Bruker Daltonics, Germany) and the Vitek2® automated system (bioMérieux, Marcy l'Etoile, France) in parallel.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the strain was determined using the standard disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (9). The following commercial disks were used: penicillin (1 U, P), oxacillin (1 µg, OXA), erythromycin (15 µg, E), gentamicin (10 µg, CN), clindamycin (2 µg, DA), trimethoprim-sulphamethoxazole (1.25/23.75 µg, SXT), ciprofloxacin (5 µg, CIP), tetracycline (30 µg, TE), and fusidic acid (10 µg, FA). *S. aureus* ATCC 25213 was used as a control strain.

DNA extraction, library preparation, and sequencing

The genomic DNA was extracted from the strain using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quantity and quality of the extracted DNA were measured using a Qubit 3.0 (Thermo Fisher Scientific, Waltham, MA, USA). The sequencing libraries of genomic DNA were prepared with the Illumina TruSeq DNA Nano Library Prep Kit (Illumina, San Diego, CA, USA) and paired-end (2×150 bp) sequencing was performed on the NovaSeq platform (Illumina, San Diego, CA, USA). After trimming low-quality reads and removing adapter sequences using Trimmomatic v 0.36 (10), the quality of both raw reads and trimmed reads was assessed using FastQC v 0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/; accessed on 26 March 2022). The *de novo* genome assembly was conducted using the SPAdes algorithm (v 3.14.1) by applying the default parameters (11). The quality of assembly was evaluated using QUAST 4.5 (12), and contigs longer than >200 bp were included in further analysis. The genome assembly data were deposited at NCBI under accession no. JAJICA000000000.

WGS-based characterization of MRSP strain

The MLST type, SCC*mec* type, and the presence of acquired antimicrobial resistance genes of the strain were searched using the bioinformatic tools available at the Center for Genomic Epidemiology (CGE) platform (http://www.genomicepidemiology.org/). Mutations in the topoisomerase II (*gyr*A) and topoisomerase IV (*grl*A) genes that mediate fluoroquinolone resistance in the strain were analyzed using BLASTn. To this end, reference *gyr*A and *grl*A sequences were downloaded from strain CCUG 49543^T (NCBI accession numbers AM262968 and AM262971, respectively) and queried against a custom BLAST database. Gene predictions and annotations of the *de novo* assembled genomes were annotated using NCBI Prokaryotic Genome Annotation Pipeline for annotation (13). For the phylogenetic comparison of the strain, WGS of 46 *S. pseudintermedius* isolates of animal and human origin from different countries were retrieved from PATRIC and NCBI databases. The tree constructed using the presence and absence of accessory genes were provided in Roary outputs (accessory_binary_genes.fa.newick). The phylogenetic tree was visualized using an interactive web tool iTOL (14).

RESULTS

The strain was found to be resistant to penicillin, oxacillin, tetracycline, ciprofloxacin, erythromycin, clindamycin, gentamicin and trimethoprim-sulphamethoxazole, and was named HMKU-VET-MRSP-2020.

The sequenced reads were assembled into a draft genome consisting of 141 contigs, with a total length of 2 726 834 bp and N_{50} of 14816 bp. The average G+C content was 37.34%. *In silico* analysis of the assembled genome revealed eight antimicrobial resistance (AMR) genes conferring resistance to: (*i*) beta-lactams (*bla*Z and *mec*A); (*ii*) aminoglycosides (*aac(6')-aph(2")*, *aph(3')-III* and *ant(6)-Ia*); (*iii*) macrolide, lincosamide and streptogramin (MLS) group (*erm*B); (*iv*) tetracyclines (*tet*K); and (*v*) trimethoprim (*dfrG*). Based on the MLST, the strain belonged to sequence type (ST) 71 and harbored SCC*mec* type III (3A).

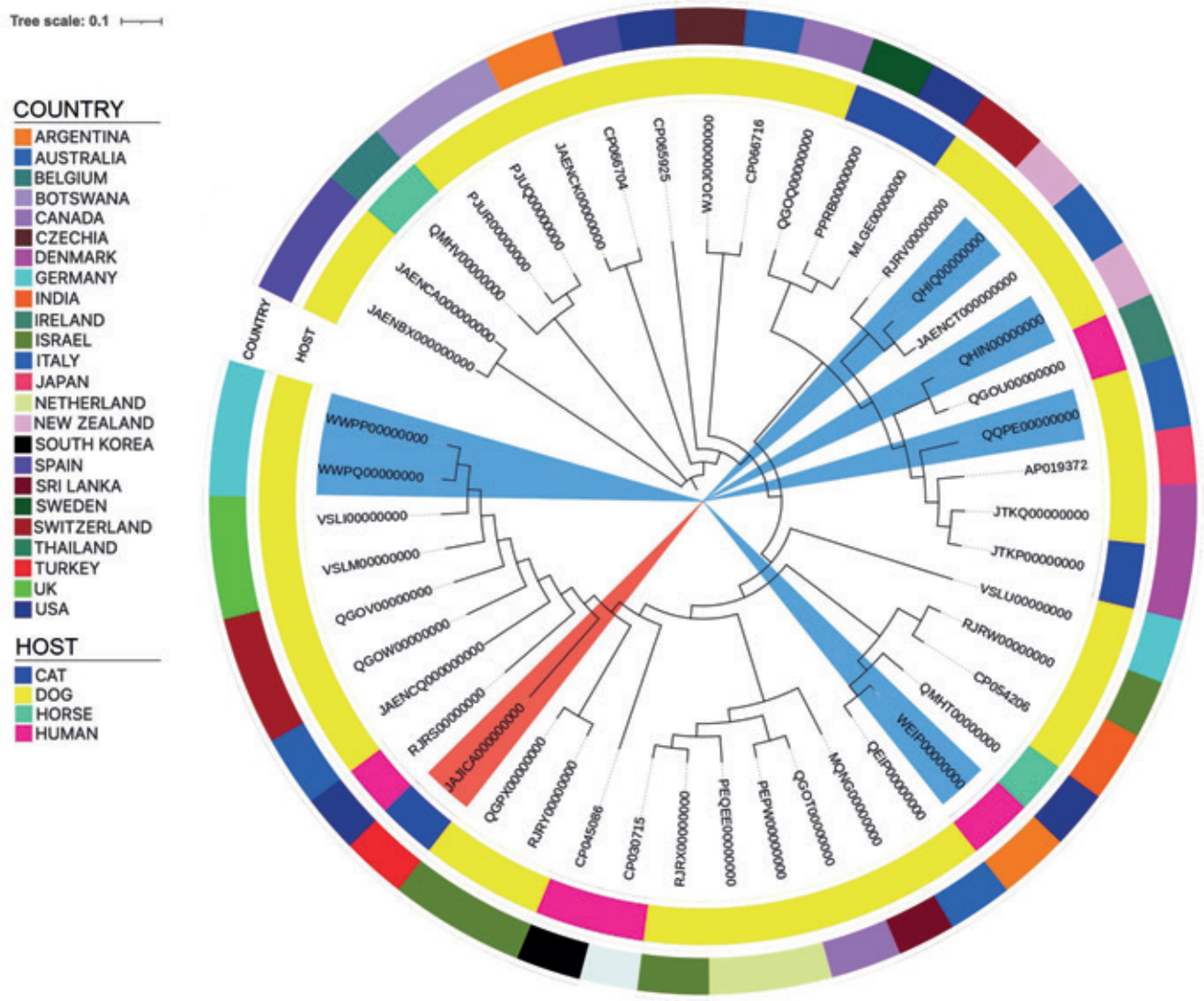

Figure 1. The phylogenetic tree is based on the core genome of *S. pseudintermedius* from different regions of the world. The strain from this study is highlighted with a red color shade. MRSP isolates were highlighted with a blue color shade.

The phylogenetic analysis based on genomes of 46 *S. pseudintermedius* of human and animal origin revealed three main clades. The strain is closely related to dog clinical isolates from Italy, Switzerland, the UK, and Germany and one human isolate from the USA (Figure).

DISCUSSION

This study shows the WGS-based molecular characterization of the MRSP strain isolated from otitis externa of a cat in Türkiye. The WGS revealed that the strain belonged to ST71, which is also the most frequently reported MDR clone in Europe for dogs, cats, and humans including both clinical patients and healthy carriers (1, 4, 15) and is increasingly reported worldwide (16-18). It has been reported that the presence of ST71 in humans is often connected with previous contact with dogs (19, 20). Therefore, it could be speculated that owners of cats are also at risk of being infected with MRSP. More importantly, Latronico *et al.* (21) showed that MRSP ST71 strains of human origin adhered equally well to canine and human corneocytes. Thus, it could be said that MRSP ST71 strains have a high adhesion ability to human skin.

Thus far, both methicillin-susceptible and resistant *S.*

pseudintermedius have not been reported to be isolated from cats in Türkiye. The cat was admitted to our faculty clinics with the complaint of otitis externa that did not respond to treatment. Although it is not possible to determine the exact source of the infection, previously reported risk factors by Lehner *et al*. (22), such as previous hospitalization, frequent visits to veterinary settings, close contact with dogs, along with the administration of glucocorticosteroids and antibacterial chemotherapeutics might have played a role as the source of infection.

As previously reported in Europe, the MRSP strains belonging to ST71 have been significantly associated with resistance to beta-lactams, gentamicin, erythromycin, clindamycin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole (15, 17). In concordance with these studies, similar genetic determinants responsible for resistance were also present in the strain. This makes increasingly selecting of antimicrobial therapy very limited. Due to the zoonotic potential of MRSP and the horizontal transfer of resistance genes, continuous monitoring of phenotypic and genotypic antimicrobial profiles is important.

In conclusion, this study provides important data on phenotypic and genotypic features of MDR MRSP from a clinical case of a cat. According to our knowledge, this study is the first report on WGS analysis of *S. pseudintermedius* from the cat in Türkiye. The findings of the study also indicate an urgent need for national surveillance of MRSP in companion animals, veterinary clinics, and veterinary personnel to reduce the burden of this important veterinary pathogen, with particular emphasis on the detection of emerging MDR MRSP clones such as ST71.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Prevalence of Shiga Toxin-Producing O157 and Non-O157 *Escherichia coli* in Anatolian Buffaloes (*Bubalus bubalis*)

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ABSTRACT

Shiga toxin producing *Escherichia coli* (STEC) serotypes are recognized as potentially important food-borne pathogens for humans. Ingestion of *E. coli* contaminated food is largely known to originate from livestock. Cattle and sheep herds hold the majority of agricultural revenue in Türkiye but Anatolian water buffaloes have often been underestimated for foodborne pathogens. The aim of this study is to determine virulence genes harboring *E. coli* O157 and six major non-O157 STEC (O26, O45, O103, O111, O121, and O145) serotypes in feces of healthy Anatolian water buffaloes by using multiplex PCR (mPCR) method. Of the collected 458 fecal samples from healthy live animals, we have performed virulence and serotype targeting mPCR following direct DNA extraction from collected samples. Results indicate that there is 0.9% of O157 prevalence while six major non-O157 *E. coli* have not been identified. The characterization results of the virulence genes also showed that *eae* is most prevalent (5.7%) followed by *ehxA* (3.9%) and stx_1 (3.1%). In this study, we have shown Anatolian buffaloes might have a relationship with other O-type *E. coli* strains. Non-O157 STECs, which are often disregarded in both animals and humans, should be investigated. As a consequence, gaining regional or national data collection will allow to implement better effective diagnosis and treatment options.

Keywords: *E. coli* O157; non-O157; STEC; Anatolian Buffaloes.

INTRODUCTION

Escherichia coli (*E. coli*) consists of a diverse and large group of bacterial organisms residing within the gut of humans and animals (1). Although most *E. coli* variants are harmless or result in asymptomatic colonization, some pathogenic variants can cause gastroenteritis, including hemorrhagic colitis (HC) or extra-intestinal infections such as urinary tract infections, hemolytic uremic syndrome (HUS) or other severe systematic infections including pneumonia (2).

Diarrhea-causing pathogenic *E. coli* variants are identified under several groups according to their O-type serogroup classification and pathogenesis with clinical implications: entero-aggregative *E. coli*, entero-pathogenic *E. coli* (EPEC), diffuse-adherent *E. coli*, entero-invasive *E. coli,* and entero-haemorrhagic *E. coli* (3). Shiga toxin-producing *E. coli* (STEC) agents are mostly associated with the entero-haemorrhagic group of variants but several types of diarrheagenic *E. coli* pathovariants other than O157 can also produce shiga toxin (4). In a public outbreak that emerged in Germany in 2011, it was found that non-O157 shiga toxin-producing *E. coli* O104 was responsible for mass public infections. Among the infected patients (3842 cases) in Germany, tragedy 22.1% of people developed HUS, and the outbreak resulted in

1.4% mortality. A detailed investigation confirmed that the consumption of contaminated vegetable sprouts led to the infection (5). Many large sporadic outbreaks have emerged in the past between 1984 and 2009 but non-O157 STEC cases rarely have been reported due to the absence of identification and characterization of non-O157 STEC methods (6). In a different outbreak led by foodborne infection, it has been reported that 23 children from South Australia (1995) and 10 children from Norway (2006) developed HUS due to non-O157 STEC; O111 and O103 respectively (7, 8). As a result, we are now much more aware of the importance of both O157 and non-O157 STEC for public health issues due to unexpected sources of infection and outbreak.

E. coli strains may acquire virulence factors such as shiga toxin 1 and 2 (*stx₁, stx₂*) intimin (*eae*), and enterohaemolysin (*ehxA*). It was suspected that there was a close relationship between virulence factors and clinical manifestations. Shiga toxins are cytotoxins that inhibit protein synthesis and cause host cell death. Intimin is shared by STEC and EPEC implements which is tightly attach to epithelial cells. Therefore, causes attaching and effacing (A/E) lesions in the intestinal mucosa. Especially HC and HUS cases with severe diarrhea were determined to be closely related to STEC types carrying the *eae* gene (9). Enterohaemolysin is thought to provide an iron source to stimulate *E. coli* growth by lysis of erythrocytes and release of heme from hemoglobin (10). Hence, virulence genes incorporating *E. coli* strains or their combination with various virulence genes maybe closely associated with clinical symptoms of intestinal or extra-intestinal infections.

Foodborne STEC infections are caused mainly by contaminated water sources via close contact with fecal materials of farm animals, primarily cattle followed by goats, sheep, and pigs (11). Although most of the daily dietary needs of humans are primarily supplied from cattle, buffaloes are also considered as a source of meat and dairy products.

Türkiye continues breeding Anatolian buffaloes, descended from Mediterranean water buffalo, for its economic value and special aromas of dairy products. Previously, epidemiological investigation of O157 and non-O157 serogroup STEC isolation studies were carried out in farm animals including cattle and sheep in Türkiye, however there is less known about Anatolian water buffaloes as a potential source of STEC. Moreover, prevalence studies of STEC in Anatolian water buffaloes are limited. A few studies have been conducted in Anatolian water buffaloes showing the prevalence of O157:H7 STEC, but the presence of non-O157 STEC has not yet been elucidated (12-16). Besides, the importance of virulence genes harboring *E. coli* serotypes in relation to public health should also need to be highlighted.

The purpose of this study was to determine *E. coli* O157 and six major non-O157 (O26, O45, O103, O111, O121, O145) *E. coli* serotypes in feces of healthy Anatolian water buffaloes by characterization of encoded virulence genes: shiga toxin 1 (*stx₁*), shiga toxin 2 (*stx₂*), intimin (*eae*) and enterohaemolysin (*ehxA*) using a mPCR assay.

MATERIALS AND METHODS

Sampling

Sample collection was carried out at 83 different buffalo farms (animal numbers varies between 5 and 25) within Sivas city, Central Anatolia, Türkiye. A total of 458 fecal samples were directly taken from the rectum of healthy Anatolian buffaloes of 4 years and older using a disposable sterile glove per animal to eliminate cross contamination. Fecal samples were placed in sterile disposable plastic containers. Samples were kept in a car cooler fridge that maintained 4˚C inner temperature and then transferred to the laboratory.

Bacterial DNA isolation

DNA isolation was performed on samples immediately upon arrival to laboratory as described by the manufacturer of QIAamp DNA Stool Mini Kit (QIAGEN, Germany). A spectrophotometry absorbance-based method at A260/280 ratio was applied using NanoDrop (DeNovix, USA) to verify and quantify the amount (µg) of isolated DNAs. To verify the purity of DNA, the ratio of A260/280 around 1.8 was accepted as pure, otherwise samples were considered contaminated and extraction was repeated. The following controls were used in this study: O157 retrieved from American Typing Cell Culture (ATCC, 43894) and six non-O157 serotype reference strains retrieved from Staten's Serum Institute (Copenhagen, Denmark, SSI-95211 for O26; SSI-87256 for O45; SSI-82170 for O103; SSI-82118 for O111; SSI-82130 for O121; SSI-82280 for O145).ATCC 43895 strain also used as positive control for the *stx₁, stx₂, eae, ehxA* genes. DNA samples were labeled and stored at -20˚C until *E. coli* characterization.

Table 1. The list of primer pairs used in mPCR to identify O-type and virulence genes.

Target Gene	Primer ID	Nucleotide sequence (3'-5')	Product size (bp)	Tm(°C)	Ref
wzxO45	O45-F	GGGCTGTCCAGACAGTTCAT	890	65.5	(17)
	O45-R	TGTACTGCACCAATGCACCT		66	
wzxO103	O103F2	GCAGAAAATCAAGGTGATTACG	740	61.7	(17)
	O103R2	GGTTAAAGCCATGCTCAACG		63.3	
stx1	stx1-F	TGTCGCATAGTGGAACCTCA	655	64.8	(18)
	stx1-R	TGCGCACTGAGAAGAAGAGA		64.9	
wbqO121	O121-F2	TCAGCAGAGTGGAACTAATTTTGT	587	64.4	(17)
	O121-R2	TGAGCACTAGATGAAAAGTATGGCT		65.6	
wzxO145	O145F5	TCAAGTGTTGGATTAAGAGGGATT	523	63.9	(17)
	O145R5	CACTCGCGGACACAGTACC		65.6	
stx2	stx2-F	CCATGACAACGGACAGCAGTT	477	66.5	(18)
	stx2-R	TGTCGCCAGTTATCTGACATTC		64	
wzxO26	O26F4	AGGGTGCGAATGCCATATT	417	63.8	(17)
	O26R4	GACATAATGACATACCACGAGCA		64	
eae	eae-F	CATTATGGAACGGCAGAGGT	375	63.7	(18)
	eae-R	ACGGATATCGAAGCCATTTG		62.1	
rfbEO157	rfbE-F	CAGGTGAAGGTGGAATGGTTGTC	296	66.5	(19)
	rfbE-R	TTAGAATTGAGACCATCCAATAAG		60.6	
wzxO111	O111F2	TGCATCTTCATTATCACACCAC	230	62.6	(17)
	O111R2	ACCGCAAATGCGATAATAACA		62.9	
ehxA	ehxA-F	GCGAGCTAAGCAGCTTGAAT	199	64.7	(18)
	ehxA-R	CTGGAGGCTGCACTAACTCC		65.5	

E. coli serotype screening and STEC detection by mPCR

For the *E. coli* serotype screening, we performed multiplex PCR (mPCR) method consisting of both O-type and virulence gene targeting primer pairs listed as in Table 1. The sample screening for O-type and virulence genes was performed in two separated steps in all extracted DNA samples (n=458). In the first mPCR panel step, a mixture of O-type specific primer pairs was employed to determine O26, O45, O103, O111, O121, O145 and O157 types. In the second mPCR panel, a mixture of virulence genes targeting primer pairs was applied to identify *stx1, stx2*, *eae* and *ehxA* virulence traits in collected sample mixtures. The mPCR was accomplished in a total 50 µl mixture reaction volume that was constituted of the following molecules and reagents; 1XPCR buffer [75mM Tris-HCl, 20mM $(NH_4)_2SO_4$, 0.1% Tween–20; pH 8.8], 2.5 mM $MgCl_2$, 250 µM of each dNTP, 1.5 U Taq DNA polymerase (MBI, Therma Scientific Fermentas, Lithuania), 5 ρmol of each primer and 5 µl of isolated DNA (25 ηg). Reference strain DNA samples were also included to study for each run along with distilled water as a negative control. Thermal cycle (T100, BIORAD) conditions were set to single cycle at 94°C for 5 minutes; 35 cycles at 94°C for 30 seconds, 67°C for 80 seconds, then cooled down to 4°C. PCR products were run in 1.5% agarose by constant 100V pulsing voltage. The gel was soaked in 10mg/ml ethidium bromide for 30 minutes, and then visualized using UV transilluminator (Vilber Lourmat Quantum ST4, France). Low amplified or nonspecific bands showing samples were repeated to verify the results.

Statistical analysis

The chi-square test was used to reveal the statistical significance of the differences between PCR results of the shiga toxin positive *E. coli* O157 and non-O157 shiga

Table 2. Distribution of positive *E. coli* O serotypes and virulence genes in fecal samples of Anatolian buffaloes.

Sample no*	Farm**	O serotype***	*stx1*	*stx2*	*ehxA*	*eae*
5	Hayran	ND	–	–	+	–
6		ND	–	–	–	+
7		ND	–	–	+	+
8		ND	+	–	–	–
9	Pazar	ND	–	–	–	+
18	Catal	ND	+	–	–	–
21		ND	–	–	–	+
24		ND	–	–	–	+
25		ND	–	–	+	–
45	Guney	O157	+	–	+	–
46		O157	+	–	–	–
66	Alaca	ND	–	–	–	+
67		ND	–	–	+	–
70		ND	–	–	–	+
87	Mentes	ND	+	–	–	–
91		ND	–	–	–	+
96		ND	–	–	+	–
97		ND	–	–	–	+
118	Eken	O157	+	–	–	–
125		ND	–	–	–	+
144	Konak	ND	–	–	+	–
204	Hasdemir	ND	+	–	–	+
209		ND	–	–	–	+
219	Polat	ND	+	–	+	+
221		ND	–	–	+	+
228		ND	–	–	–	+
251	Akca	ND	+	–	+	+
252		ND	–	–	+	+
253		ND	–	–	+	–
256		**ND**	**–**	**–**	**–**	**+**
274	Sarica	O157	+	–	–	+
305	Bostan	ND	+	–	+	+
307		ND	–	–	+	+
308		ND	–	–	+	–
311		ND	–	–	–	+
345	Kara	ND	+	–	–	+
386	Pinar	ND	+	–	–	+
417	Bey	ND	+	–	+	+
440	Yayla	ND	–	–	–	+
443		ND	–	–	+	–
457	Soran	ND	–	–	+	–
41	**18**	**–**	**14**	**0**	**18**	**26**

*: Of the 458 fecal samples 41 were found PCR positive result for the investigated genes in this study. **: These 41 samples were fell within 18 of sampled 83 farms. ***: *E. coli* O26, O45, O103, O111, O121, O145 and O157 serotypes were examined and only four samples were found positive O157 but none of the samples were positive for other serotypes subjected in this study. *stx1*: shiga toxin 1, 14 samples were found positive for *stx1*. *stx2*: shiga toxin 2, none of the samples were found positive for stx2. *ehxA*: enterohaemolysin, 18 samples were found positive for *ehxA*. *eae*: intimin, 26 samples were found positive for *eae*. ND: Not determined. +: Gene present. –: Gene absent.

toxin positive samples and their distribution at the farm level. Values with p≤0.05 were considered statistically significant.

RESULTS

STEC prevalence

From 458 collected fecal samples from healthy Anatolian buffaloes, only 4 (0.87%) tested positive for O157 but none for non-O157 *E. coli* serotypes (O26, O45, O103, O111, O121, and O145). The *stx*$_1$ gene was detected in all O157 positive samples; however the *stx*$_2$ gene was not. Of the 458 samples, 10 individual samples were carrying *stx*$_1$ and none of them was found positive for seven major O serotypes being examined (Figure 1). The prevalence of O157 STEC and non-O157 STEC were estimated to be 0.87% and 2.18%, respectively, with a total STEC prevalence of 3.06% (Figure 2a). The difference between O157 and non-O157 STEC positivity rates was not significant (p>0.05). When the results were considered at the farm level, STECs were determined in 13.25% (11/83) of the herds. Within these 11 herds, the proportion of STEC positive samples found in range between 5% and 8%, with an overall proportion of 3.06% (14/458). The positivity of STEC O157 is 3.62% (3/83) while non-O157 STEC positivity is 9.64 % (8/83) at farm level (Figure 2b). The difference between these results were not significant (p>0.05).

Characterization of STEC

The O157 positive samples (n=4) all possessed *stx*$_1$ (100%) gene, while none of them were found positive for *stx*$_2$. It was also found that 10 out of 458 of the fecal samples were also positive for *stx*$_1$ but not for the seven major O serotypes examined. These samples were considered as non-O157 and not major six STEC serotypes which were out of subject of this study. These results were listed in Table 1. The prevalence of *eae* and *ehxA* were found around 5.7% and 3.9% of the fecal samples of Anatolian buffaloes, respectively. When non-STEC samples (n=27) were analyzed, results revealed that only 9 samples harbored the *ehxA* virulence gene alone while only 14 samples showed positivity for *eae*. Out of 27 non-STEC samples, only four samples possessed the combination of both *eae* and *ehxA* genes. Although we identified the presence of *ehxA* and *eae* virulence genes in non-STEC samples, we also detected those genes other than shiga toxins in STEC identified samples. Results indicated that, of the 14 STEC samples, only one sample was identified positive for *ehxA* (*stx*$_1$+*ehxA*) while *eae* determined alone in four samples (*stx*$_1$+*eae*). Among the shiga toxin-positive samples, we also determined the combination of virulence *eae* and *ehxA* genes in four samples (*stx*$_1$+*eae*+*ehxA*). Both virulence genes (*eae* and *ehxA*) were not found in the remaining five STECs.

DISCUSSION

Among the members of the global market, Türkiye was holding 4th place in terms of milk production from buffaloes in the early 2000's however production was reduced significantly due to modernization in agricultural revenue and substitution of Anatolian buffaloes with Holstein heifers (20). Although the majority of dairy products are supplied from cattle and sheep herds in Türkiye, Anatolian water buffaloes still have a significant economic impact due to the nutritional values of buffalo milk and their dairy products particularly mozzarella cheese. STEC isolation and identification in meat and dairy products of ruminants has been demonstrated however, there is less information known about STEC presence in buffaloes (21, 22). Although the presence of STEC O157:H7 in Anatolian buffaloes has been studied, non-O157 STEC and its characterization remains unqualified.

This study provides the first results of seven major STECs including the presence of virulence genes (*stx*$_1$, *stx*$_2$, *eae* and *ehxA*) from fecal samples of Anatolian water buffaloes reared in Central Anatolia, Türkiye. Several studies have shown the success of PCR techniques to characterize *E. coli* and STEC with 100% specificity and 98% sensitivity (17, 23); therefore, mPCR assay was employed for the detection of both virulence genes and *E. coli* O serotypes in the present study.

There is no known human STEC infection caused by meat or dairy products of Anatolian buffaloes in Türkiye but the presence of *E. coli* O157:H7 in Anatolian buffaloes in different regions was investigated (13-15). A study conducted in western Türkiye evidenced *E. coli* O157:H7 presence for the first time in raw milk and fecal samples of Anatolian water buffaloes, which showed 3.7%, and 1.4% positivity in fecal and milk samples, respectively (14). In a different study, 3.8% *E. coli* O157:H7 positivity was reported in Buffaloes residing in Northern Anatolia of Türkiye (15). In the current investigation, of the collected samples, we have identified

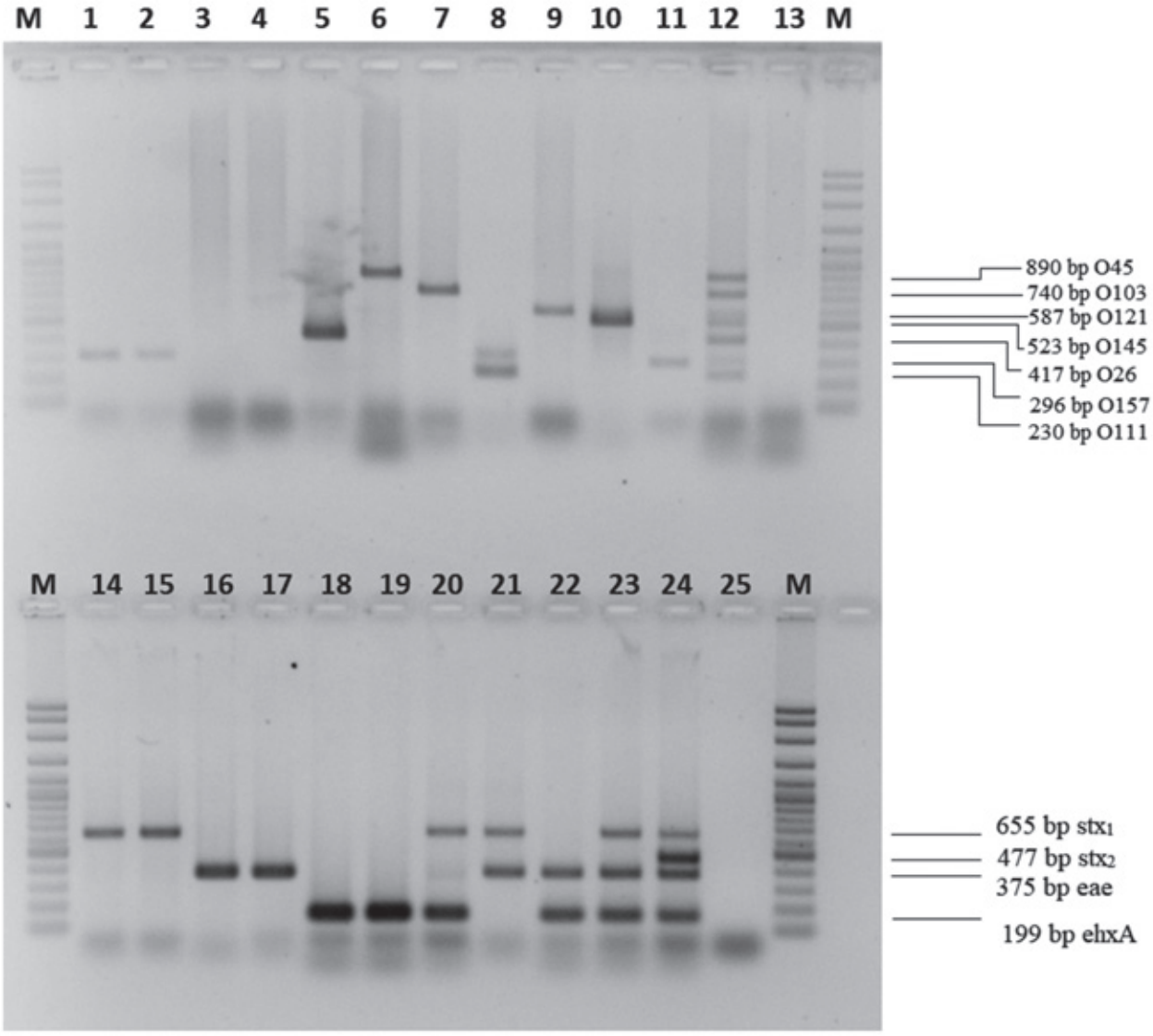

Figure 1: The multiplex PCR results of of DNA samples obtained from fecal samples of Anatolian buffaloes. The image representing the DNA amplicons in EthBr stained agarose gel electrophoresis.

Top line: Multiplex PCR results of *E. coli* O serotype. M: 100 bp DNA ladder, Lane 1-2: O157 positive field samples, Lane 3-4: Negative field samples. Lane 5: O26 positive control (SSI-95211), Lane 6: O45 positive control (SSI-87256), Lane 7: O103 positive control (SSI-82170), Lane 8: O111 positive control (SSI-82118), Lane 9: O121 positive control (SSI-82130), Lane 10: O145 positive control (SSI-82280), Lane 11: O157 positive control (ATCC 43895), Lane 12: Mixed DNA sample of all reference positive controls, Lane 13: Negative control (distilled water).

Bottom line: Multiplex PCR results of virulence genes. M: 100 bp DNA ladder, Lane 14-15: *stx1* positive field samples, Lane 16-17: *eae* positive field samples, Lane 18-19: *ehxA* positive field samples, Lane 20: *stx1+ehxA* positive field sample, Lane 21: *stx1+eae* positive field sample, Lane 22: *eae+ehxA* positive field sample, Lane 23: *stx1+eae+ehxA* positive field sample, Lane 24: *stx1+stx2+eae+ehxA* positive control (ATCC 43895), Lane 25: Negative control (distilled water).

0.87% prevalence for O157 but flagella antigen (H) was not studied. Besides, a study conducted on carcasses and rectal swab samples of buffalos to investigate the presence of *E. coli* O157:H7, none of the collected samples reacted in O157 and H7 agglutination assays, indicating no positivity (13). Such differences might be related to the chosen methods, bacterial load in animal (samples) or different geographical areas being investigated.

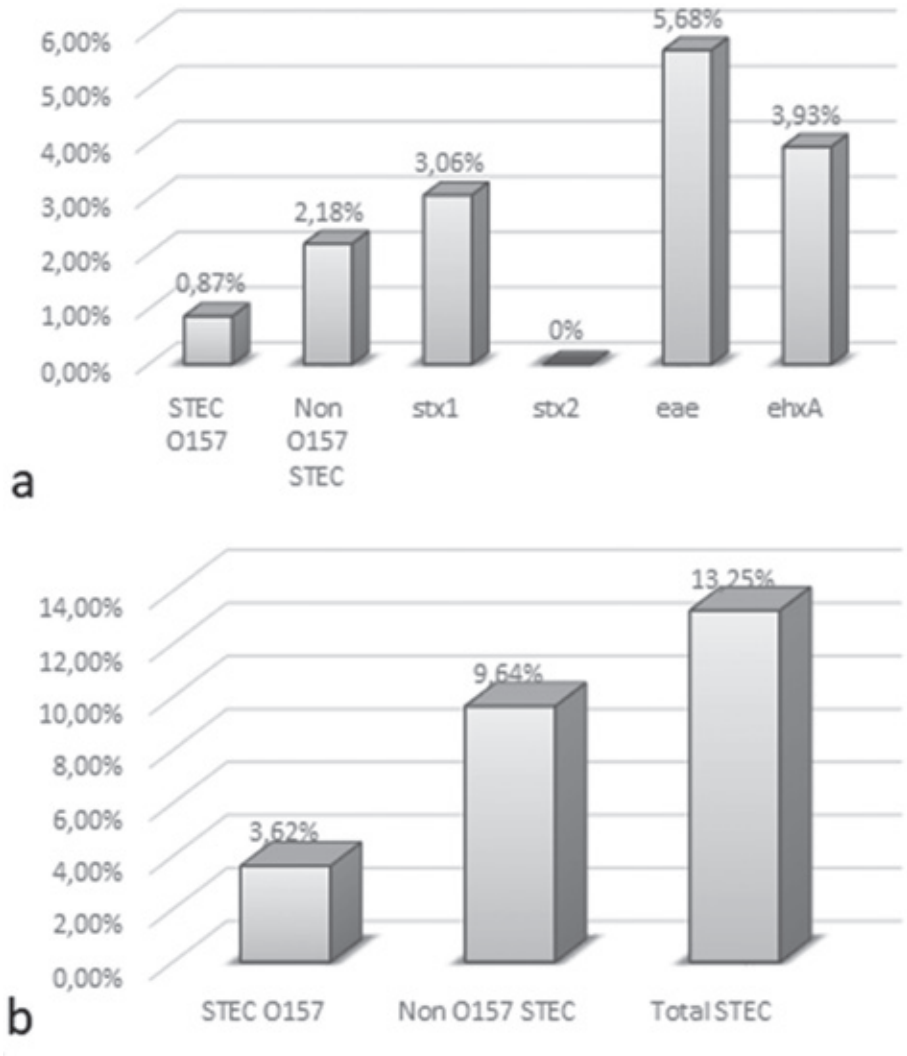

Figure 2: The prevalence of STECs and virulence genes identified in feces samples of field collected Buffaloes.

The bar graph (a) showing the prevalence of STECs and virulence genes in total collected samples (n=458) while second bar graph (b) representing the distribution of STECs in farms (n=83). *E. coli* O26, O45, O103, O111, O121, O145 serotypes were not detected and omitted in graph. There was no statistical difference between O157 and non O157 STEC prevalence of collected samples ($p>0.05$). The difference between O157 and non-O157 STEC positivity rates of farms was also not significant ($p>0.05$).

Our virulence gene screening results revealed that 14 samples out of 458 examined samples tested positive for *stx₁* (3.1%) virulence trait. None of the samples assayed were positive for *stx₂* whereas 5.7% and 3.94% of samples were carrying *eae* and *ehxA* respectively. We also showed the presence of *ehxA* or *eae* genes in non-STEC samples indicating that some *E. coli* strains carry at least one of the virulence genes other than shiga toxins. Therefore, those *eae* and *ehxA* carrying non-STEC strains can be considered as EPEC. It could be assumed that Anatolian water buffaloes or their dairy and meat products have a lower potential risk for public health when compared to other ruminants bred in Türkiye; nonetheless, they can play a role in foodborne infections when other non-O type STECs and EPECs are considered.

Water buffaloes are also considered important livestock; however, their products in several countries and economic values can vary. A study conducted in fecal samples of buffaloes in Italian rearing farms showed a 14.5% of STEC O157 prevalence, suggesting the water buffaloes should be

taken into account as a potential reservoir of STEC (24). In 2013, a STEC outbreak emerged and resulted in 22 cases of HUS in Italy due to shiga toxigenic O26 contamination, suspected from raw milk or vegetables, and this increased the awareness concerning non-O157 STECs (25). A recent study, carried out in different animal products has indicated that raw buffalo milk retained 35.71% pathogenic *E. coli* agents, which harbored *stx$_1$, stx$_2$, eae,* and *ehxA*. It was also shown in the same study that prevalence of O157, O26, and O111 was about 50%, 40%, and 10% respectively, while O45, O111, and O121 serotypes were not determined (26). A study from Bangladesh screened fecal samples of buffaloes and portion of STEC was about 11%, among which 7% and 5% of them possessed the *stx$_1$* and s*tx$_2$* respectively, suggesting buffaloes could pose an added public risk in rural areas in Bangladesh. However, O-type *E. coli* was not investigated in that study (27). In terms of O-type serogroup characterization, a study conducted in Southeastern Brazil showed a 37% prevalence of non-O157 STEC in fecal swab samples taken from buffaloes (28). Among the STEC positive samples, 38.5% and 22% harbored the *stx$_1$* and *stx$_2$* gene respectively, while none harbored the *eae* gene. Curiously, none of the samples tested positive for common O serogroups (O26, O103, O111, O145, O157) (28). Similarly, we also could not identify six major O-type *E. coli* (O26, O45, O103, O111, O121, and O145) in fecal samples of Anatolian water buffaloes. This finding could be explained by the prevalence of common O-type serogroups that can show a distinct and diverse relationship among animals at the species level.

Another study revealed that there was a strong relationship between *eae* gene with some certain O-type serogroups (O5, O26, O69, O84, O103, O111, O145, and O157 (29). As stx$_2$ causes more severe human infection than *stx$_1$*, the presence of other virulence genes could enhance clinical signs (30). Even though, virulence genes harboring STEC and non-STEC serogroups other than major O-types were not investigated in the present study, we have provided detailed investigation of major serotypes along with common virulence genes in terms of STEC prevalence in Anatolian water buffaloes in Türkiye. Future studies should be directed to characterize STECs and EPECs prevalence in a broad range spectrum of O-type antigens and virulence genes.

Although dairy products are plausibly contaminated during milking and production, animal carcasses are most commonly contaminated with pathogenic bacteria during slaughtering and dressing. In the current study, *E. coli* O157 and six non-O157 major serogroups (O26, O45, O103, O111,121, O145) were investigated by PCR in Anatolian water buffaloes for the first time and the presence of six major *E. coli* other than O157 which were not identified. Our findings revealed that the most prevalent virulence genes identified in healthy Anatolian water buffaloes were *eae* followed by *stx$_1$*. Even though most of the *E. coli* strains are known as harmless and sometimes referred to as commensal bacteria for mammals, acquirement of virulence traits through evolutionary processes can change their clinical manifestations. It has been shown that the presence of virulence genes alone or in combination with other virulence genes may enhance the pathogenicity of *E. coli*. Those acquirements include the attachment of *E. coli* to the intestinal wall by *eae* and increase in cytopathogenic abilities by shiga toxins (stx$_1$, stx$_2$). Furthermore, some animals infected with *E. coli* strains possessing *ehxA* showed clinical signs of hemolysis (10). It has been anticipated and discussed in detail that there is a close relationship between clinical outcomes and virulence genes. Some studies also showed virulence genes can contribute resistance to some antibiotics (31, 32).

To minimize foodborne O157 and non-O157 STEC human infections, it is highly crucial to determine which serotypes and virulence genes are most prevalent and what are the possible transmission routes to humans such as the consumption of meat, milk or vegetables. In this study, we observed that Anatolian buffaloes might have a relationship with other non-O157 STECs but they could not be determined due to current facilities. The epidemiology of STECs especially non-O157 STECs in Türkiye could be clarified by more comprehensive studies including other animal species as well. Therefore, it can lead to developing new control measures for the prevention of possible human outbreaks and to reduce the economic losses of food producers. Investigation of non-O157 STECs, which are mostly ignored in both animals and humans, should also be considered. Thus, obtaining regional or national data will provide more effective diagnosis and treatment opportunities.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

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INVITED LECTURES

Habituated Wild Boar in the City of Haifa: Management Pattern to Cope with

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Establishment of wild boar populations in Haifa city, which are based on anthropogenic food sources, caused continues conflict while losing their fear from people. In former years, intensive culling of few hundreds individuals per year, was the only management action that was done. Feeding of domestic cats was very familiar in large parts of the city, which attract wild boars. The amount of culling from 5 years increased to greater than 3 times from 150 to 430 individuals per year while the conflict endured. A joint project of Haifa municipality and Israel Nature and Park Authority (INPA) in order to reduce the wild boar population, was carried out according to the following principles principles: 1. Decrease food and water source by efficient sanitation. 2. Fencing and blocking wild boar paths using robust fence (1.2 meter height) from open areas to the external neighborhoods. 3. Capturing and culling of intractable individuals that habituated and threatened the residents. Monitoring the activities effect was estimated by municipality weekly reports, GPS collared wild boars and camera traps. Intensive and tight sanitation of garbage cans in streets and parks contribute to > 8 times decrease of tipping down of garbage bins events in the last year. Extensive enforcement of few hundred cases in the last year, was executed to deal with feeding wild boar by residents. There was sharp decrease of wild boars entering fenced and sanitized neighborhoods documented by GPS and tagged wild boars carried out after few months. A few dozen of intractable individuals from some neighborhoods that were culled, contributed to a large decrease in wild boar appearance. Tight sanitation alongside blocking the entrance paths to neighborhoods presented an effective tool to decrease of conflict with wild boar in cities and settlements. Culling individuals should be only a complementary activity to deal with habituated individuals.

A Quest for Alternative Management of Wild Boars

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The wild boar (*Sus scrofa*) is a medium size ungulate, native to Europe, Asia and North Africa, and an introduced species to North America and Australia. Wild boars have expanded their populations and geographic distribution over the past few decades, leading to increased conflicts with humans and often leading to habitat modifications. The most common management tool to minimize their conflict with humans has been culling. Yet, studies, show that culling wild boars might have an reverse effect, leading to higher reproductive potential and to elevated population size. Under elevated hunting pressure the yearling males, which naturally form their own small packs, stayed with their natal groups. Furthermore, we found that under high hunting pressure females' hair progesterone levels were significantly higher compared to that of females roaming under low hunting pressure. Our study may suggest that the increased access of yearling males to the females in their groups, due to hunting pressure, might contribute to the high reproduction levels observed under culling regimes. We believe that wildlife managers should develop alternative methods for culling to deal with the increased worldwide conflict with humans. For example, we found that both sanitary measurements and the addition of mud puddles in an urban forest significantly decreased the conflict between humans and wild boars.

Insights from a 20 Years' Research on Free-Roaming Cats

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The domestic cat had been distributed around the globe mainly as a pet. Over the years, it formed non-domiciliary populations known as free-roaming cats. Free-roaming cats often suffer from impaired welfare and were shown to cause adverse environmental effects, such as ecological damage, nuisances and public health hazards. The management of these populations is implemented mainly by the trap-neuter-return (TNR) method, as it is considered as a humane control method. Despite the extensive use of this method, there is disagreement among researchers, regulators, and animal organizations, in regard to the effectiveness of this method in reducing free-roaming cat numbers, improving their welfare, and reducing the environmental adverse effects that they might cause. In our research, we examined few aspects of TNR effectiveness, along with the performance of a uniquely designed controlled field experiment, over a 12-y period and spanning a 20-km^2 urban area. We found positive correlation between neutering and cat health and survival. High intensity-TNR reversed population growth, reaching an annual approximately 7% reduction, only when it was applied in geographic contiguity. This population reduction was limited by a rebound increase in cat reproduction and longevity. We conclude that cat population management by TNR should be performed with high intensity, uninterruptedly, and in geographic contiguity to enable population reduction. To enhance management effectiveness and mitigate compensatory effects, we recommend further evaluating an integrated strategy that combines TNR with complementary methods (e.g., vital resource regulation, ill cat euthanasia, and adoption).

SCIENTIFIC ABSTRACTS

Clinical Utility of Serum Fructosamine in Long-Term Monitoring of Diabetes Mellitus in Dogs

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Serum fructosamine (sFA) is used to assess glycemic control in diabetic dogs. Nevertheless, its interpretation is hindered by several limitations. This study evaluated the long-term diagnostic performance of sFA, along with clinical signs, in assessing glycemic control in diabetic dogs. This retrospective study included insulin-treated (for ≥1 month) diabetic dogs. sFA, body weight, appetite, polyuria/polydipsia and clinical scores (CS; well-controlled DM, CS-0; uncontrolled diabetes mellitus, CS-1) were recorded during follow-up visits. The study included 50 dogs (302 visits; median, 6 visits/dog; range, 2-20), of which 33 (66%) achieved CS-0. sFA was higher (P<0.001) on visits with CS-1 (mean, 563 µmol/L; 95% confidence interval [CI], 533-592) compared to visits with CS-0 (mean, 495 µmol/L; 95%CI, 467-523). Increase in sFA was associated (P<0.001) with increased OR of CS-1 (OR, 1.26; 95%CI, 1.15-1.39). sFA was moderately predictive of CS (area under the ROC curve, 0.72; 95%CI, 0.67-0.77; P<0.0001). sFA cutoff of 486 µmol/L, had 75% sensitivity and 59% specificity in predicting the CS. sFA was lower (P=0.04) when hypoglycemia was suspected or reported (mean, 501 µmol/L; 95%CI, 437-565) than in other visits (mean, 561 µmol/L; 95%CI, 528-593). Acute comorbidity contributed (P=0.009) to discordant sFA and CS. sFA was moderately accurate in classifying the CS in diabetic dogs. Incorporating sFA in monitoring potentially improved the CS. Decreases of sFA over follow-ups were indicative of improved CS, but might be suggestive of hypoglycemic episodes. Acute comorbidities decreased the diagnostic accuracy of sFA. Additional monitoring tools are advised when sFA and the CS are conflicting.

Effects of Dietary Anti-Oxidants on Production and Welfare Markers in Heat Stressed Dairy Cows

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Heat stress negatively affects production and welfare of dairy cows, and increases oxidative stress. The objectives were to examine the effects of a plant extracts supplement (AXT; Axion ThermoPlus, CCPA, France) as anti-oxidants during heat load on production and indices of stress and welfare in dairy cows. Forty-two multiparous mid-lactation cows during peak summer (THI=77) were fed for 2 weeks either a standard milking cows' diet

(CTL, n=14), or supplemented with 100 g/d of AXT (100AXT, n=14), or 150 g/d of AXT (150AXT, n=14). The cows were cooled 5 times a day; then, half of the cows from each treatment were cooled or not cooled for 2 weeks, after which cows were switched for additional 2 weeks. Data were analyzed for effect of treatment, cooling and their interaction with PROC MIXED of SAS. Milk yields and dry matter intake were higher in 100ATX than in CTL, but not different in AXT150 compared to controls. The percentage of hours that VT was >39˚C was lower in AXT100 and in AXT150 than in CTL. Welfare indices: rumination time and laying down times were higher in 150AXT than in CTL, with intermediate values in 100AXT (not statistically significant compared to control). Supplementation of 100AXT during heat load increased feed intake, and production. Both doses lowered VT compared to controls. AXT150 improved welfare indices in dairy cows. This work indicated that plant antioxidants may be beneficial to heat stressed cows, and improve performance and welfare indices.

The Seroprevalence of *Neospora* spp. in the Israeli Equine Population and its Association with Pregnancy and Abortion

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Neospora protozoan parasites are endemic worldwide, infect livestock and companion animals and are a leading cause of abortions in cattle. In horses, *N. caninum* and *N. hughesi* have been associated with fetal loss, and neurological disorders, respectively. This study aimed to evaluate the exposure to *Neospora* in the Israeli equine population, in pregnant mares and in aborting mares. Serum samples from horses and thoracic fluid from aborted fetuses were tested for *Neospora* exposure by the indirect fluorescent antibody test (IFAT). Tissue samples from aborted fetuses were tested by polymerase chain reaction (PCR). The seroprevalence in 334 apparently healthy horses sampled at 30 farms throughout Israel was 24%, with older age (p=0.026) and housing management (p=0.033) significantly associated with seropositivity in univariable, but not in multivariable, analysis. *Neospora* seroprevalence in 152 pregnant mares from 36 farms was 66.4%, with older age (p=0.006) and Arabian breed (p=0.005) significantly associated with seropositivity in univariable, but not multivariable analysis. The seroprevalence in 107 of these mares that were re-sampled after parturition decreased to 48.6%. The seroprevalence found in 31 aborting mares was 70.9% and the molecular prevalence in their aborted fetuses was 41.9%. Sequence analyses of the PCR results identified all parasites as *N. caninum.* This study revealed high exposure of equines to *Neospora* parasites, with increased seropositivity in pregnant and aborting mares. Increased seropositivity during pregnancy may reflect increased parasite replication due to immunosuppression, which led to increased chance of fetal infection. These finding suggest that *N. caninum* could be a significant cause of abortion in horses in Israel.

Evaluation of External Physical Features for Estimation of Endotracheal Tube Diameter in Dogs

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Endotracheal intubation, using the largest possible endotracheal tube (ETT), is essential for airway management in canine anesthesia. Despite studies correlating tracheal diameter to inter-nare distance, weight or other external measures, evidence-based guidelines for ETT selection are lacking. We prospectively recruited 36 dogs, 10 brachycephalic, 26 non-brachycephalic, weighing 2.2 - 75 kg, scheduled for head and neck computerized tomography (CT). Tracheal diameter at the level of the second cervical vertebra was measured on the CT scan. We recorded weight, body condition score, and external measurements, using a tape measure, including length of the humerus, and the minimal distance between nares, the eye and nare, eye and canine tooth, and both eyes. Correlations were evaluated using Spearman or Pearson correlation. Parameters were compared between groups using t-test, Mann-Whitney U test and Fischer Z transformation. Mean (± SD) Tracheal diameter was significantly smaller in brachycephalic dogs (13.50±4.16 vs 17.61±4.93). Weight had a significantly stronger correlation, and inter-nare distance a significantly weaker correlation, to tracheal diameter in non-brachycephalic dogs (r=0.91 and 0.47 respectively) vs. brachycephalic dogs (r=0.67 and 0.88). Inter-nare distance was smaller than the inserted ETT in 86.1% of cases. After excluding factors with multicollinearity, an equation comprised of head conformation and weighted combination of inter-nare-, inter-eye-, and eye to nare- distances as well as weight, predicted tracheal diameter, describing 76% of tracheal diameter variation. This study describes a novel method for estimating ETT size using external markers, which is superior to inter-nare distance or weight.

Determination of Isometric Points in the Stifle of a Dog using a 3D Model

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Isometry is the term used to describe points on bones contributing to a joint that remain equidistant from one another as the joint flexes and extends. In cases of cranial cruciate ligament (CCL) rupture isometric points define the ideal location to place the material used in the repair. Isometric points have never been confirmed experimentally in three dimensions (3D) in the dog's stifle, which is the aim of this study. A static 3D model of the stifle was generated from a computer tomography scan of one dog, and a kinetic model was generated, from data collected from sensors attached to the tibia, when flexing the stifle through 80°. Kinetic data was superimposed on the static model by aligning specific points which were defined for both models. This allowed the tibia to rotate and translate relative to the femur based on the kinetic data. The contour of the distal femur

and proximal tibia were converted into point clouds and the distance between each point on the femur and all the points on the tibia was measured at 15 different positions. A total of 3681 isometric points were identified, with all points located in 2 pairs of isometric areas. One pair of isometric areas was at the insertions of the cranial cruciate ligament on the femur and tibia. A second pair was on the lateral aspect of the stifle. A better understanding of the location of isometric areas may lead to refinements in intra- and extra-capsular techniques used to treat cases of ruptured CCL.

Retrospective Analysis of Factors Affecting Clinical Outcome in Dogs with Primary Nodal Diffuse Large B-Cell Lymphoma Treated with CHOP-Based Chemotherapy

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Numerous factors are known to affect the prognosis of dogs with chemotherapy-treated lymphomas. This is the first retrospective study on dogs with lymphoma in Israel. The objective of this study was to identify prognostic factors for dogs receiving CHOP-based chemotherapy for primary large B-cell lymphoma (BCL). Medical records of dogs treated for BCL at the Koret School of Veterinary Medicine from 2017 to 2022 were reviewed. Factors potentially related to prognosis were statistically analyzed. Forty-six dogs were included in the study. The complete remission rate was 87% (40 dogs). Median progression-free survival (PFS) for the entire population was 159 days (range 31-664). Median overall survival (OS) was 279 days (range 13-1196). Factors significantly associated with OS in the lowest quartile (≤279 days, n=22) only included presence of anemia at diagnosis (OR 8.87; 95% CI 0.98-80.18; P=.04). Factors significantly associated with OS in the upper quartile (>279 days, n=20) included higher number of grade 1 neutropenia events during treatment with vincristine (mean 1.23 (range 0-4) vs. 2.38 (range 0-6) P=0.02) and cyclophosphamide (mean 0.4 (range 0-2) vs. 1.44 (range 0-3) P <0.01) and higher number of dose delays (mean 2 (range 0-5) vs. mean 3.9 (range 0-10) P=0.04). The results of this study suggest anemia at diagnosis may be associated with a poor outcome in dogs receiving CHOP-based chemotherapy for BCL, whereas grade 1 neutropenia during treatment and, as a result, dose delays, may be associated with a better outcome.

Acute Kidney Injury in Dogs: Etiology, Clinical and Clinicopathologic Findings, Prognostic Markers and Outcome

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Acute kidney injury (AKI) is a common condition in dogs, leading to severe uremia and associated with high morbidity and mortality. The current study objectives were to characterize the etiology, clinical and clinicopathologic findings and outcome in a large cohort of dogs diagnosed with AKI. This retrospective study included 249 client-owned dogs hospitalized at a veterinary teaching hospital and diagnosed with AKI. One hundred and sixty-four dogs (66%) survived. The most common clinical signs at presentation were lethargy (90%), anorexia (83%), and vomiting (68%). Putative etiologies included ischemic/inflammatory (58%), infectious (8%), nephrotoxicity (6%), or other (5%). Hospital acquired AKI was documented in 9% of the dogs. Median serum creatinine (sCr) at presentation and maximal sCr during hospitalization were 4 mg/dL (range, 1.1-37.9) and 4.6 mg/dL (range, 1.1-43.1), respectively. Maximal documented sCr was used for classifying dogs to IRIS AKI grades as follows: Grade I, 6 (2%), Grade II, 38 (15%), Grade III, 89 (36%), Grade IV, 77 (31%), and Grade V, 39 (16%). The overall mortality significantly increased with IRIS AKI grade (P=0.009). Anuria was significantly more common among non-survivors compared with survivors (P=0.002). Forty-seven (18.8%) dogs were treated with hemodialysis, of which 60% survived. Survival was substantially higher than previously documented. Non-infectious etiologies, higher AKI IRIS grade, acidemia, thrombocytopenia and hypoalbuminemia were associated with a negative prognosis.

דחיית טיפולים (ממוצע 2 (טווח 5-0) לעומת 3.9 (טווח 10-0); P=0.04). תוצאות המחקר מעידות כי אנמיה בעת האבחון יכולה להיקשר עם תוצאה גרועה בכלבים עם לימפומה של תאי B המקבלים כימותרפיה מבוססת CHOP בעוד נויטרופניות מדרגה 1 במהלך הטיפול וכתוצאה מכך, דחיית טיפולים, יכולות להיקשר לתוצאה טובה יותר.

פגיעה כלייתית אקוטית בכלבים: אטיולוגיה, ממצאים קליניים וקליניקופתולוגיים, מרקרים פרוגנוסטיים, ותוצאה

ד' רימר, ה' חן, מ' בר־נתן ו־ג' שגב

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פגיעה כלייתית אקוטית הינה שכיחה בכלבים, מובילה לאורמיה חמורה ומזוהה עם תחלואה ותמותה גבוהות. מטרת המחקר הנוכחי היא לתאר את האטיולוגיה, הממצאים הקליניים והקלינוקופתולוגיים ותוצאות הפגיעה בכלבים שאובחנו עם פגיעה כלייתית אקוטית. המחקר הינו רטרוספקטיבי, הכולל 249 כלבים שאושפזו בבית חולים וטרינרי אוניברסיטאי ואובחנו עם פגיעה כלייתית אקוטית. מאה ששים־וארבעה כלבים (66%) שרדו. הסימנים הקליניים הנפוצים בהגעה היו לתרגיה (90%), אנורקסיה (83%), והקאות (68%). אטיולוגיות משוערות כללו איסכמיה/דלקת (58%), זיהום (8%), נפרוטוקסיות (6%), או אחר (5%). פגיעה כלייתית שאירעה בעת אשפוז תועדה ב־9% מהכלבים. חציון ריכוז קריאטינין בסרום בהגעה ומרבי בעת האשפוז היו 4 מ"ג/ד"ל (טווח, 1.1-37.9) ו־4.6 מ"ג/ד"ל (טווח, 1.1-43.1), בהתאמה. הכלבים סווגו לדרגת פגיעה כלייתית על פי IRIS, ועל סמך ריכוז מרבי של קריאטינין בסרום בעת האשפוז: דרגה I, 6 (2%), דרגה II, 38 (15%), דרגה III, 89 (36%), דרגה IV, 77 (31%), ודרגה V, 39 (16%). התמותה עלתה עם העלייה בדרגת הפגיעה הכלייתית באופן מובהק (*P*=0.009). אנאוריה היתה שכיחה יותר בקרב כלבים שלא שרדו (*P*=0.002). ארבעים־ושבעה (18.8%) כלבים טופלו בהמודיאליזה, מתוכם 60% שרדו. בהשוואה למחקרים קודמים, השרידות במחקר זה גבוהה באופן יחסי. נמצא קשר בין אטיולוגיה שאינה זיהומית, חומרת הפגיעה הכלייתית, חמצת, ספירת טסיות נמוכה או אלבומין נמוך, עם פרוגנוזה שלילית למחלה.

הטובוס שהוכנס ב־86.1% מהמקרים. לאחר ניתוח של מולטיקולינאריות נמצאו ארבע מדדים: משקל, מרווח בין נחיריים, מרווח בין עיניים ומרווח בין העין לנחיר, אשר יחד עם מבנה הגולגולת יצרו משוואה אשר מנבאת בצורה הטובה ביותר את קוטר הקנה ומתארת 76% מהשונות בקוטר הקנה. מחקר זה מדגים שיטה חדשה לנבא את קוטר הקנה בכלבים באמצעות שימוש במדדים חיצוניים, אשר הינו אמין יותר מאשר שימוש במרווח בין נחיריים או משקל בלבד.

קביעת נקודות איזומטריות בברך של כלב באמצעות מודל תלת ממד

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נקודות איזומטריות הנן שתי נקודות הממוקמות בשני צידיו של מפרק ושומרות על מרחק קבוע במהלך תנועתו. במקרים של קרע ברצועה צולבת קדמית, נקודות איזומטריות מגדירות את המיקום האידיאלי להנחת המשתל המשמש לתיקון. מטרת מחקר זה היא לאתר נקודות איזומטריות בברך הכלב, לראשונה בשימוש מודל תלת ממדי ניסויי. מודל תלת־ממדי סטטי נוצר מסריקת CT של ברך של כלב, ומודל קינטי נוצר מנתונים שנאספו במהלך כיפוף של ה־Tibia לאורך 80°, מחיישנים אשר הונחו עליה. הנתונים הקינטיים הועברו למודל הסטטי בעזרת נקודות מתאר מוגדרות וקבועות אשר סומנו עבור שני המודלים ולמעשה אפשר ל Tibia לנוע ולנטות ביחס לעצם ה Femur בהתבסס על הנתונים הקינטיים. מתאר עצם ה Femur הדיסטלית וה Tibia הפרוקסימלית הוסבו לצבר נקודות (point clouds) והמרחק בין כל נקודה על עצם ה־Femur לכל הנקודות על עצם ה Tibia נמדד בכל אחד מ־15 המנחים שנבדקו. בסך הכל, אותרו 3681 נקודות איזומטריות כאשר כל הנקודות ממוקמות ב־2 זוגות של אזורים איזומטריים. זוג אחד ממוקם באספקט הלטרלי של הברך. הבנה טובה יותר של מיקומם של האזורים האיזומטריים עשויה להוביל לשיפור טכניקות intra ו־extra capsular המשמשות לטיפול במקרי קרע רצועה צולבת קדמית.

מחקר רטרוספקטיבי של הגורמים המשפיעים על התוצאה הקלינית של כלבים עם לימפומה של תאי B שטופלו עם פרוטוקול כימותרפי CHOP

ע' אינהורן, ג' דנק, א' חנאל ו־ע' יס

ביה"ס לרפואה וטרינרית ע"ש קורט, האוניברסיטה העברית בירושלים, רחובות, ישראל

ידוע כי מספר פקטורים משפיעים על הפרוגנוזה של כלבים עם לימפומה המטופלים בכימותרפיה. זהו המחקר הרטרוספקטיבי הראשון על כלבים עם לימפומה בישראל. מטרת המחקר הייתה לזהות פקטורים פרוגנוסטיים עבור כלבים שקיבלו כימותרפיה בפרוטוקול CHOP עבור הטיפול בלימפומה של תאי B. נבחנו התיקים הרפואיים של כלבים שטופלו ללימפומה של תאי B בבית החולים הווטרינרי האוניברסיטאי בין 2017 ל־2022. פקטורים העשויים להיות קשורים לפרוגנוזה נבחנו באמצעות שיטות סטטיסטיות שונות. ארבעים ושישה כלבים נכללו במחקר. רמיסיה מלאה הושגה ב־87% מהכלבים (40 כלבים). חציון שרידות ללא התקדמות המחלה (PFS) עבור כל אוכלוסיית המחקר היה 159 ימים (טווח 664-31). חציון השרידות הכולל (OS) היה 279 ימים (טווח 1196-13). פקטורים שנקשרו באופן מובהק סטטיסטית עם זמן שרידות נמוך מהחציון (≤279 ימים) כללו רק אנמיה בעת האבחון (OR 8.87; 95% CI 0.98-80.18; P=.04). פקטורים שנקשרו באופן מובהק סטטיסטית עם זמן שרידות גבוה מהחציון (>279 ימים) כללו מספר גבוה יותר של אירועי נויטרופניה מדרגה 1 במהלך הטיפול בוינקריסטין (ממוצע 1.23 (טווח 4-0) לעומת 2.38 (טווח 6-0); P=0.02) וציקלופוספמיד (ממוצע 0.4 (טווח 2-0) לעומת 1.44 (טווח 3-0); P<0.01) ומספר גבוה יותר של

חום. תוצאות המחקר מעודדות ומצביעות לראשונה על תוסף תזונתי שיכול לשפר מדדי רווחה התנהגותיים, להועיל בהורדת טמפ' הגוף בפרות בתנאי עומס חום סביבתי, וזאת יחד עם עלייה בתנובת החלב ובצריכת המזון.

הימצאות סרולוגית של טפילי ניאוספורה בסוסים בישראל, והקשר להריון ולהפלות

ל' מימון,[1] א' שטיינמן,[2] י' קלצ'קו[2], ש' תירוש־לוי,[1,2] ג' שוורץ,[2] י' בלינדר,[1] ג' בנעט,[2] מ' לשקוביץ־מזוז[1]

[1]החטיבה לפרזיטולוגיה, המכון הוטרינרי ע"ש קמרון, בית דגן, ישראל
[2]בית הספר לוטרינריה ע"ש קורט, האוניברסיטה העברית בירושלים, רחובות, ישראל

טפילי *ניאוספרה* הינם טפילים פרוטוזואלים עם תפוצה עולמית אשר מדביקים חיות משק ומחמד ומהווים גורם משמעותי להפלות בבקר. בסוסים, המין *ניאוספורה קאנינום* דווח כגורם הפלות ו*ניאוספורה יוגסי* כגורם לבעיות נוירולוגיות. מטרת עבודה זו היתה להעריך מה ההמצאות הסרולוגית של *ניאוספורה* באוכלוסיית הסוסים בישראל, בסוסות בהריון ובסוסות מפילות. החשיפה הסרולוגית לניאוספורה הוערכה באמצעות בדיקת IFAT לדגימות סרום שנאספו מסוסים ונוזל חזה שנאסף מנפלים. המצאות מולקולארית של ניאוספורה נבדקה בדגימות מאיברים של נפלים באמצעות PCR. ההמצאות הסרולוגית של ניאוספורה בקרב 334 סוסים בריאים מ־30 חוות מכל הארץ היתה 24%. גיל מבוגר (p=0.026) וממשק אחזקת הסוסים (P=0.033) נמצאו כקשורים לחשיפה בניתוח חד גורמי, אך לא בניתוח רב גורמי. ההמצאות הסרולוגית של ניאוספורה בקרב 152 סוסות הרות שנדגמו ב־36 חוות היתה 66.4%. גיל מבוגר (p=0.006) וגזע ערבי (p=0.005) נמצאו כקשורים לחשיפה בניתוח חד גורמי, אך לא בניתוח רב גורמי. ההמצאות הסרולוגית בקרב 107 מהסוסות שנדגמו שוב לאחר המלטה היתה 48.6%. ההמצאות הסרולוגית בקרב 31 סוסות מפילות היתה 70.9% וההמצאות המולקולארית אצל הנפלים של אותן סוסות היתה 41.9%. ריצוף דוגמאות ה־PCR החיוביות אפיין את כל הטפילים כ*ניאוספורה קאנינום*. תוצאות מחקר זה הראו חשיפה גבוהה לטפילי *ניאוספורה* באוכלוסיית הסוסים בישראל (ועליה בחשיפה מאז 2002) וגבוהה עוד יותר בסוסות בהריון. העליה בחיוביות סרולוגית בזמן הריון עשויה לשקף התרבות מוגברת של הטפילים בשל הדיכוי החיסוני בהריון וסיכון מוגבר להדבקת העובר. תוצאות אלו מורות על כך ש*ניאוספורה* עשוי להיות גורם משמעותי להפלות בסוסות בישראל.

הערכת שימוש במדדים חיצוניים לניבוי קוטר קנה הנשימה בכלבים

ס' סימנובסקי,[1] מ' יפה,[1] א' אפשטיין,[1] י' ברוכים,[1,2] ד' פארי[1] ו־י' קושניר[1]

[1]בית הספר לרפואה וטרינרית ע"ש קורט, האוניברסיטה העברית בירושלים, רחובות, ישראל
[2]טיפול נמרץ בן שמן, מרכז מומחים וטרינרי, בן שמן, ישראל

אינטובציה, באמצעות הצינור התוך־קני (טובוס) הגדול ביותר, חיונית לאבטחת דרכי אוויר בכלבים מורדמים. מספר מחקרים מראים מתאם בין קוטר הקנה למרווח בין הנחריים, משקל, או מדדים אחרים בכלבים, אך אין כיום קווים מנחים מבוססי מחקר לבחירת טובוס בכלבים. למחקר זה גויסו 36 כלבים 10 ברכיצפלים ו־26 שאינם ברכיצפלים, במשקל 2.2-75 ק"ג, שהיו צפויים לעבור הדמיית סיטי של הראש והצוואר. קוטר הקנה נמדד בגובה החולייה הצווארית השנייה מתוצאות הסריקה. לכל כלב נמדד משקל, מצב גופני, וכן באמצעות סרגל: אורך הזרוע, המרווח המינימלי בין הנחריים, בין העין לנחיר, בין העין לניב, ובין שתי העיניים. מתאמים בין המדדים החיצוניים לקוטר הקנה הוערכו באמצעות מתאם ספירמן או פירסון. השוואה בין הקבוצות נעשתה באמצעות מבחן t, מבחן מאן וויטני־U, ומבחן המרה פישר־Z. קוטר הקנה הממוצע (±סטיית תקן) היה קטן יותר בכלבים ברכיצפלים (4.16±13.50 לעומת 4.93±17.61) למשקל היה מתאם חזק יותר ולרווח בין הנחריים מתאם חלש יותר לקוטר הקנה בכלבים לא ברכיצפלים (r=0.91 ו־0.47 בהתאמה) לעומת כלבים ברכיצפלים (r=0.67 ו־0.88 בהתאמה). המרווח בין הנחריים היה קטן מהקוטר החיצוני של

השימושיות הקלינית של פרוקטוזאמין בסרום במעקב ארוך־טווח אחר סוכרת בכלבים

א' ארוך, מ' מזעקי־טובי, ו' אבו אחמד, י' עובדיה ו־ש' קוזי

ביה"ס לרפואה וטרינרית ע"ש קורט, האוניברסיטה העברית בירושלים, רחובות, ישראל

פרוקטוזאמין משמש להערכת השליטה הגליקמית בכלבים סוכרתיים; האינטרפרטציה שלו מסובכת עקב מספר מגבלות. מחקר זה העריך את הביצוע האבחוני ארוך הטווח של פרוקטוזאמין, יחד עם הסימנים הקליניים, להערכת השליטה הגליקמית בכלבים סוכרתיים. מחקר רטרוספקטיבי זה כלל כלבים סוכרתיים שטופלו (לפחות חודש) באינסולין. ריכוז פרוקטוזאמין בסרום, משקל גוף, תיאבון, נוכחות שתייה/השתנה מוגברות וניקוד קליני (CS; שליטה טובה, 0-CS; סוכרת לא נשלטת, 1-CS) נרשמו במשך ביקורות המעקב. המחקר כלל 50 כלבים (302 ביקורות; חציון 6/כלב; טווח, 2-20), מהם 33 (66%) הגיעו ל־0-CS. פרוקטוזאמין היה גבוה יותר ($P<0.001$) בביקורים בהם היה 1-CS (ממוצע, 563; מיקרומול/ל'; רווח בר־סמך 95%, 533-592) לעומת ביקורים בהם היה 0-CS (ממוצע, 495; רווח בר־סמך 95%, 467-523).

עלייה בפרוקטוזאמין נקשרה ($P<0.001$) עם עלייה בסיכוי (OR) ל־1-CS (OR, 1.26; רווח בר־סמך 95%, 1.15-1.39). פרוקטוזאמין חזה את ה־CS במידה בינונית (שטח מתחת לעקומת receiver operator characteristics, 0.72; רווח בר־סמך 95% 0.67-0.77; $P<0.0001$). נקודת חתך ריכוז פרוקטוזאמין של 486 מיקרומול/ל' הראתה רגישות 75% וסגוליות 59% בחיזוי ה־CS. פרוקטוזאמין היה נמוך יותר ($P=0.04$) כשהיפוגליקמיה דווחה או נחשדה (ממוצע, 501 מיקרומול/ל'; רווח בר־סמך 95%, 437-565) לעומת ביקורות אחרות (ממוצע, 561 מיקרומול/ל'; רווח בר־סמך 95%, 528-593). מחלות נלוות חדות תרמו ($P=0.009$) לחוסר התאמה בין פרוקטוזאמין לבין ה־CS. פרוקטוזאמין מדויק במידה בינונית בסיווג ה־CS בכלבים סוכרתיים. שילוב פרוקטוזאמין במעקבים פוטנציאלית משפר את הניקוד הקליני. ירידה בפרוקטוזאמין לאורך הביקורות מכוונת לקיום שיפור בניקוד הקליני, אך עשויה להצביע על אפיזודות היפוגליקמיה. נוכחות מחלות נלוות חדות מפחיתה את הדיוק האבחוני של פרוקטוזאמין. מומלץ לשלב אמצעי ניטור נוספים כשהתוצאות של פרוקטוזאמין והניקוד הקליני סותרות.

השפעת תוסף נוגדי חמצון צמחיים על ייצור ומדדי רווחה בפרות חלב בתנאי עומס חום

ג' דאדאם,[1] ד' דניאל,[2] ע' פלך,[3] ג' קרא,[1,2] ה' קמר,[1] י' לבון,[4] ע' מועלם[1] ו־מ' זכות[1]

[1]המחלקה לחקר בקר וצאן, המכון לחקר בע"ח, מכון וולקני, ראשון לציון, ישראל

[2]המחלקה למדעי בעלי החיים, הפקולטה לחקלאות, מזון וסביבה ע"ש רוברט ה. סמית, האוניברסיטה העברית בירושלים, רחובות, ישראל

[3]שה"מ, משרד החקלאות, ראשון לציון, ישראל

[4]התאחדות יצרני החלב, קיסריה, ישראל

עומס חום פוגע בביצועים וברווחה של פרות חלב וגורם לעלייה בעקה החימצונית. מטרת המחקר לבחון את השפעת מתן תוסף המכיל תמציות צמחים כנוגדי חמצון (Axion Thermorplus, CCPA, France) על ייצור החלב, מדדי רווחה ועקה בפרות חלב בתנאי עומס חום. 42 פרות בוגרות באמצע התחלובה ברפת ההזנה הפרטנית בוולקני חולקו ל־3 טיפולי הזנה בשיא הקיץ (THI=77): 1. ביקורת – מנת העדר (n=14). 2. AXT100 – תוסף של 100 ג' ליום (n=14). 3. AXT150 – תוסף של 150 ג' ליום (n=14). לאחר שבועיים של הזנה בהם הפרות צוננו 5 פעמים ביום, מחצית מהפרות בכל טיפול תזונתי צוננו 5 פעמים ביום או לא צוננו כלל למשך שבועיים. לאחר מכן, הפרות שלא צוננו כעת צוננו ולהיפך למשך שבועיים נוספים. המודל הסטטיסטי בחן את השפעת ההזנה, הצינון והאינטראקציה ביניהם באמצעות PROC MIXED של SAS. תנובת החלב וצריכת המזון היו גבוהים יותר ב־100AXT לעומת הביקורת, אך לא היו שונים בין AXT150 לבין הביקורת. אחוז השעות שטמפ' הגוף הייתה מעל 39 מעלות היה נמוך יותר ב־100AXT וב־150AXT מאשר בביקורת. מדדי הרווחה: משך זמן העלאת הגרה וזמן הרביצה היו גבוהים יותר ב־150AXT לעומת הביקורת, עם ערכי ביניים בקבוצת ה־100AXT (לא מובהק לעומת הביקורת). מתן תוסף נוגדי חמצון בכמות 100AXT שיפר תנובת חלב, צריכת מזון, ושני המינונים הורידו טמפ' וגינלית לעומת הביקורת. מתן 150AXT שיפר מדדי רווחה בפרות חלב בתנאי עומס

האתגר בניהול אוכלוסיות של חזירי בר

א' שיינס

ביולוגיה וסביבה, אוניברסיטת חיפה־אורנים, טבעון, ישראל

חזיר הבר (*Sus scrofa*) הוא פרסתן בגודל בינוני, המצוי ביבשות אירופה, אסיה וצפון אפריקה, ואף הוכנס לצפון אמריקה ואוסטרליה. בעשורים האחרונים חזירי הבר הרחיבו את אוכלוסייתם ואת התפוצה הגיאוגרפית שלהם, מה שהוביל לעימותים מוגברים עם בני האדם ולעתים קרובות לשינויים בבתי הגידול. כלי הוויסות הנפוץ ביותר לצורך מיזעור הקונפליקט עם בני אדם היה חיסול, לרוב ע"י ציד. עם זאת, מחקרים, כולל שלנו, מראים שלציד חזירי בר עשויה להיות השפעה הפוכה, דבר המוביל לפוטנציאל רבייה גבוה יותר ולגידול באוכלוסייה. תחת לחץ ציד מוגבר, הזכרים הצעירים, היוצרים באופן טבעי להקות קטנות משלהם, נשארו עם קבוצות הלידה שלהם. יתר על כן, מצאנו כי בלחץ ציד גבוה רמות הפרוגסטרון בשיער של הנקבות היו גבוהות משמעותית בהשוואה לזו של נקבות המשוטטות בלחץ ציד נמוך. המחקר שלנו עשוי להצביע על כך שעקב לחץ ציד, הגישה המוגברת של זכרים צעירים לנקבות בקבוצות שלהם עשויה לתרום לרמות הרבייה הגבוהות שנצפו באזורי ציד גבוה. אנו מאמינים שאנשים המתמודדים עם ניהול אוכלוסיות של חיות בר צריכים לפתח שיטות חלופיות לחיסול על מנת להתמודד עם הסכסוך המוגבר שלהם עם בני האדם. לדוגמה, מצאנו שהגברת שמירה על תנאים סניטרים בערים ותוספת של שלוליות בוץ ביער העירוני הפחיתו משמעותית את הקונפליקט בין בני האדם לחזירי הבר.

תובנות מעשרים שנות מחקר על חתולים משוטטים

ע' גינטר

מרצה אורחת, האוניברסיטה העברית בירושלים (תוכנית MVPH וקורסים בריאות ציבור ורפואה וטרינרית רשותית), ישראל
יועצת, השירותים הווטרינרים, משרד החקלאות ופיתוח הכפר, ראשון לציון, ישראל

חתול הבית הופץ ברחבי העולם בעיקר כחיית מחמד, אך במהלך השנים, נוצרו אוכלוסיות שאינן ביתיות הקרויות חתולים משוטטים. חתולים משוטטים עלולים לסבול מפגיעה ברווחתם, בד בבד עם גרימת נזקים סביבתיים כגון: פגיעה אקולוגית, מטרדים לחברה האנושית וסיכון לבריאות הציבור. ויסות אוכלוסיות החתולים המשוטטים נעשה בעיקר באמצעות שימוש בשיטת הלכידה־עיקור־החזרה, מאחר וזו נחשבת כשיטת ויסות הומנית. למרות השימוש הנרחב בשיטה זו, קיים חוסר הסכמה בקרב חוקרים, רגולטורים ועמותות למען בעלי־חיים לגבי יעילות השיטה בהפחתת גודל אוכלוסיית החתולים המשוטטים, צמצום פגיעתם הסביבתית ושיפור רווחתם. במחקר הנוכחי, בחנו את יעילותה של שיטת הלכידה־עיקור־החזרה במספר היבטים, לצד העמדת מחקר שדה מבוקר בעל מבנה ייחודי, שנמשך על פני 12 שנים וכולל שטח עירוני של 20 קמ"ר. במחקר זה, מצאנו קורלציה חיובית בין עיקורים לבריאות והישרדות החתולים. ביצוע עיקורים בעצימות גבוהה הביא להיפוך מגמת הגידול במספר החתולים, עם ירידה שנתית ממוצעת של 7% בגודל האוכלוסיה, רק כאשר העיקורים בוצעו ברציפות גיאוגרפית. ההפחתה בגודל האוכלוסיה הוגבלה על ידי מנגנוני פיצוי שכללו עליה ברביה ובתוחלת החיים. ניתן להסיק כי כדי להשיג הפחתה בגודל אוכלוסיית החתולים, מוטב לבצע את הלכידה־עיקור־החזרה בעצימות גבוהה, באופן מתמשך על פני שנים וברציפות גיאוגרפית. על מנת לשפר את יעילות השיטה בהתמודדות עם תהליכי פיצוי האוכלוסייה, מומלץ ליישם גישה משולבת הכוללת שיטות משלימות כגון: שליטה במקורות מזון, המתת חסד לחתולים חולים ומסירה לאימוץ.

הסימפוזיון ה־44 לרפואה וטרינרית: רווחת בעלי־חיים

ביה"ס לרפואה וטרינרית ע"ש קורט, האוניברסיטה העברית בירושלים, ישראל

הסימפוזיון היה בחסות **לין ופיל הימלשטיין, ארה"ב**

פרס התקציר הטוב ביותר היה בחסות **רויאל קנין, ישראל**

הרצאות מוזמנות:

חזירי בר בחיפה: דרכי התמודדות עם קונפליקט

ע' דולב,[1] י' אולק[2] ו־ע' גל[1]

[1]רשות הטבע והגנים, מחוז צפון, ישראל
[2]היחידה לתכנון, סביבה וקיימות – עיריית חיפה, חיפה, ישראל

התבססות אוכלוסיות חזירי בר בחיפה גרמו להתפתחות קונפליקט מתמשך. בשנים עברו בוצע דילול בהיקף של מאות פרטים בשנה, תוך שהיקף הדילול גדל משנה לשנה, ללא ירידה בהיקף הקונפליקט. פרויקט משותף של עיריית חיפה ורט"ג לצמצום אוכלוסיית חזירי הבר בחיפה, החל בסוף שנת 2021 והתבסס על העקרונות הבאים לפי סדר קדימויות הבא: 1. צמצום מקורות מזון ומים – ביצוע סניטציה ומניעת מזון זמין, עצירת האכלה מכוונת, קיבוע פחים, ריקון פחים בשעות ערב והסברה. הכל במטרה לצמצם את האינטרס של החזירים להיכנס לשכונות להשגת מזון. 2. גידור וחסימה של נתיבי הכניסה נועד למנוע כניסת חזירים לשכונות בחיפושם אחר מזון. מבנה השכונות יוצר נתיבי חדירה מוגדרים של חזירים. 3. טיפול והוצאה של פרטים סוררים – פרטים מקרב החזירים שהורגלו למצוא מזונם בשכונות ואיבדו החשש מבני אדם עלולים להיות מסוכנים, ונדרש טיפול להוצאתם מהמערכת. הפעולות לצמצום מקורות מזון, כפי שפורטו למעלה, הביאו לירידה של עד פי 8 בכמות אירועי הפיכת הפחים. במקביל, מופו עשרות מוקדי האכלת בע"ח וניתנו מאות דוחות למאכילים. נתיבי כניסה של חזירי בר לשכונות נחסמו בעזרת גידור קשיח בשלוש שכונות הגובלות בפארק הכרמל. מעקב אחר מספר חזירים בעזרת תגי אוזן וקולרי GPS הראה שינוי חד בשיעור הכניסה לשכונות שטופלו. כתוצאה מהרגלת פרטים רבים להאכלת יד, איבדו רבים מהם את החשש מבני אדם והפכו מסוכנים. פעילות סניטציה ואכיפה לצד חסימת נתיבי כניסה לשכונות, מראה יעילות משמעותית בהפחתת פעילות החזירים בשכונות וצמצום הקונפליקט. עם זאת, חזירים שנותרו פעילים בשכונות והתרגלו לקבל מזון בני אדם, צפויים להיות אגרסיביים בניסיונותיהם להשיג מזון ועלולים לדרוש מזון בצורה כוחנית, ועל כן נאלצנו להוציא פרטים אלה מהמערכת ולהמיתם.

אפיון מולקולרי של זן חיידק *Staphylococcus pseudintermedius* עמידים למתיצילין ובעל multi-drug resistance מבודד ממקרה של דלקת אוזניים בחתול

א' אסלנטאס[1] א' אולגן,[1] מ' ביאילי[2] ו־ב' בויאויקלטאי[3]

[1] המחלקה למיקרוביולוגיה, הפקולטה לרפואה וטרינרית, אוניסרסיטת הטאי מוסטפה קמאל, הטאי, טורקייה.
[2] אוניברסיטת הטאי מוסטפה קמאל, בית ספר למדעי הבריאות הטאי, טורקייה.
[3] המכון ביופרמטיקה אוניברסיטת טכני למזרח התיכון, אנקרה, טורקייה.

חיידקי *Staphylococcus pseudintermedius* עמידים למתיצילין (MRSP) מהווים פתוגנים זואונוטיים מגיחים ברפואה וטרינרית. ריצוף גנומי (whole-genome sequencing) הינה כיום השיטה המועדפת לסיווג מולקולרי של פתוגנים בקטריאליים בזכות רזולוציה גבוהה והיכולת לבצע אפיון מקיף של חיידקים. בעבודה הנוכחית אופיין הגנום של תבדיד MRSP זן HMKU-VET-MRSP-2020 אשר בודד ממקרה של דלקת אוזניים בחתול בתורכיה. התבדיד שויך ל־MLST ST71 ו־SCC*mec* סוג IIIA. רצף הגנום הראה נוכחות של מגוון גנים ומוטציות אשר עשויים להקנות לחיידק עמידות לאנטיביוטיקה. ניתוח פילוגנטי הראה כי זן זה שייך לצבר של זנים אשר בודדו מזיהומים קליניים בכלבים במדינות שונות באירופה וזן נוסף אשר בודד מבן אדם בארה"ב. זהו התיאור הראשון של בידוד ואפיון מולקולרי של MRSP מחתולים בתורכיה. עבודה זו שופכת אור על הפוטנציאל הזואונוטי של חיידק זה.

הימצאות חיידקי *Escherichia coli* Shiga toxin-producing זן O157 וזני non-O157 בבופלו אנטולי (*Bubalus bubalis*)

ר' קלין,[1] מ"נ מוגלקו,[1] מ' קרהן,[1] ט' טורן,[2] ה' עיזידן[2] ו־א' ברבר[3]

[1] המחלקה למיקרוביולוגיה, הפקולטה לרפואה וטרינרית, אוניברסיטת סיווס קומהוריית, סיווס, טורקייה.
[2] המחלקה לוירולוגיה, הפקולטה לרפואה וטרינרית, אוניברסיטת סיווס קומהוריית, סיווס, טורקייה.
[3] המחלקה למדעים ביו־רפואיים ואבחוניים, הקולג' לרפואה וטרינרית, אוניברסיטת טנסי, נוקסוויל, טנסי, ארה"ב.

חיידקי Shiga Toxin-producing Escherichia coli (STEC) מסרוטיפים שונים הינם פתוגנים הומניים חשובים הנישאים במזון. מקור הזיהום בדרך הוא כלל במזון מן החי. עדרי בקר וצאן מהווים את ענפי החי העיקריים בטורקייה, וממעיטים בחשיבות הבופלו אנטולי (Anatolian buffalo) כמקור לפתוגנים הנישאים במזון. מטרת עבודה זו הייתה לחקור את ההימצאות של גנים לאלימות בחיידקי ***E. coli*** סרוטיפ O157 וכן של ששת הסרוטיפים החשובים הנוספים (O26, O45, O103, O111, O121, O145) בדגימות צואה של בופלו בריאים באמצעות multiplex PCR (mPCR). סך הכול 458 דגימות צואה נאספו. חיידקי O157 זוהו ב־0.9% של הדגימות. שאר הסרוטיפים לא זוהו כלל. מבין הגנים שנחקרו, הגן *eae* היה הכי נפוץ (5.7%), אחריו הגן *ehxA* (3.9%) ו־*stx1* (3.1%). איסוף נתונים אודות ההימצאות של חיידקי STEC ברמה האזורית והלאומית חשובה לקביעת דרכי אבחון וטיפול.

חקירת שינויים במדדים ביוכימיים החלים בשל מצבי מחלה שונים בתקופת המעבר בפרות סימנטל.

ט׳ ספק,[1] א׳ ילמז[2] ו־ריסונלי[4,3]

[1] אוניברסיטת קסטמונו, הפקולטה לרפואה וטרינרית, מחלקה למיילדות וגינקולוגיה, קסטמונו, טורקייה.
[2] אוניברסיטת סירית, הפקולטה לרפואה וטרינרית, מחלקה למיילדות וגינקולוגיה, סירית, טורקייה.
[3] אוניברסיטת טורקית קרגיז־מנאס, הפקולטה לרפואה וטרינרית, מחלקה למיילדות וגינקולוגיה, ביסקק, קירגיזסטן.
[4] אוניברסיטת פיראת, הפקולטה לרפואה וטרינרית, מחלקה למיילדות וגינקולוגיה, אלאזיג, טורקייה.

תקופת המעבר בפרות הינה בעלת משמעות רבה. במסגרת מחקר זה הוערכו השפעות אירועי אצירת שליה, דלקת עטין תסמינית ודלקת רחם על רמות מדדים ביוכימיים שונים בפרות סימנטל. הפרות חולקו לחמש קבוצות: פרות עם אצירת שליה (n=17), דלקת עטין (n=25), דלקת רחם (n=21), פרות בריאות לאחר המלטה (n=21) ופרות בריאות בטווח הזמן של 5±15 ימים טרם מועד ההמלטה החזוי (n=20). רמות האנזימים alkaline phosphatase (ALP, 85.18±15.83 U/L), aspartate transaminase (AST, 123.02±19.15 U/L), gamma-glutamyl transferase (GGT, 28.18±2.66 U/L) בפרות עם דלקת רחם היו גבוהות ביחס לפרות הבריאות טרם ההמלטה. בנוסף לכך, GGT (41.83±14.61 U/L), myocardial band of creatine kinase (CK-MB, 155.25±27.85 U/L), הראו את הרמות הגבוהות ביותר בפרות עם אצירת שליה, בעוד שרמות N-acetyl creatine kinase — cysteine (CK-NAC, 540.45±175.67 U/L) וקריאטינין (2.29±0.88 mg/dL) רמות הגבוהות ביותר בפרות עם דלקת רחם. רמות חלבון כללי (6.39±0.38 g/dL) היו הגבוהות ביותר בפרות עם דלקת העטין. מות האוריאה הגבוהות ביותר נמדדו בפרות עם דלקת הרחם (61.40±17.38 mg/dL). ממצאי עבודה זו מראים הבדלים בתמונת המדדים הביוכימיים בפרות עם עצירת שליה, דלקת עטין ודלקת רחם. תמונות המדדים נותחו בעזרת עקומות receiver-operator characteristic (ROC). נמצא כי רמות של 89U/L≥ עבור AST, 24U/L≥ עבור GGT, ו־106U/L≥ עבור CK-MB יכולות לשמש לאבחון ראשוני של דלקת רחם, כמו גם רמות של 21U/L≥ עבור GGT לאבחון עצירת שליה ורמות של 105U/L≥ עבור CK-MB לאבחון ראשוני של דלקת עטין. מחקרים נוספים, לתיקוף ממצאים אלו בקבוצות פרות גדולות יותר, מומלצים בעתיד.

ברטונלה בוביס בבקר בניגריה: זיהוי מולקולרי ואנליזת גורמי סיכון

ג׳ קמני,[1] ג׳ שאר,[2] י׳ נחום־ביאלה,[3] ג׳ בנעט,[3] מ׳ שאנד[4] ו־ש׳ הרוש[3]

[1] המחלקה לפרימטולוגיה, המכון הלאומי למחקר וטרינרית ואסת מחוז פלטו, ניגריה.
[2] החטיבה לפרזיטולוגיה, המכון הלאומי למחקר וטרינרי, מדינת פלטו, ניגריה.
[3] בית הספר לרפואה וטרינרית ע״ש קורט, האוניברסיטה העברית בירושלים, רחובות, ישראל.
[4] בית ספר למדעי גיאוגרפיה וכדור הארץ אוניברסיטת גלזגו, הממלכה המאוחדת.

בקר משמש כמקור חלבון עיקרי בתזונת אנשים בניגריה. שיטת גידול הבקר בניגריה הינה אקסטנסיבית. אמנם עלויות הגידול זולות יחסית, אך השיטה חושפת את הבקר לטפילים חיצוניים וגורמים זיהומיים זואונוטיים המועברים על ידם. ברטונלוזיס הינה מחלה מגיחה בעלת חשיבות וטרינרית והשלכות זואונוטיות. עבודת מחקר זו בחנה 462 דגימות דם לנוכחות DNA של ברטונלה. פרגמנטים של הגנים ציטרט סינטז (*gltA*) ו־RNA פולימרז, תת יחידה בטא (*rpoB*) הוגברו ב־43 (9.3%) ו־6 (1.3%) מהבדיקות, בהתאמה. המקטעים שרוצפו הראו התאמה גבוהה בת 97.6-99.8% לריצפי *ברטונלה בוביס* שהופקדו בבנק הגנים (GenBank). אנליזה פילוגנטית של הרצפים הראתה כי הם הופיעו בקלסטר אחד עם מיני *ברטונלה בוביס* מיונקים שונים ממדינות שונות. היארעות נוכחות DNA של *ברטונלה בוביס* הייתה גבוהה יותר בבקר מעל גיל שנתיים ובדגימות שנאספו מבתי מטבחיים. זהו התיאור הראשון של אבחון *ברטונלה בוביס* בבקר בניגריה. נדרשות עבודות נוספות על מנת לברר האם לממצא זה חשיבות מבחינת בריאות הציבור, במיוחד לנוכח העובדה שקשה לאכוף חוקי פיקוח בשר בניגריה.

תקצירים

דיווח ראשון על הדבקה בטפיל *Pennella balaenopterae* בגופת לוויתן מצוי *(Balaenoptera physalus)* שנסחפה לחופי ישראל.

ס' אורן,[1] נ' עדרי,[1] ד' יסעור לנדאו,[2] ר' קינג,[3] מ' לשקוביץ־מזוז,[2] ס' אבן ברי[1] ו־ל' מוס[1]

[1] המחלקה לפתולוגיה, מכון וטרינרי ע"ש קימרון, ת.ד. 12, בית דגן 50250, ישראל.

[2] החטיבה לפרזיטולוגיה, מכון וטרינרי ע"ש קימרון, ת.ד. 12, בית דגן 50250, ישראל.

[3] רשות הטבע והגנים, ירושלים, ישראל.

זהו דיווח ראשון בדבר זיהוי *Pennella balaenoptera*, טפיל מזודרמלי, המשתייך לתת מחלקת השטרגליים, בגופת יונק ימי בישראל. גופת לווייתן מצוי (*Balaenoptera physalus*) נשטפה לחופי ישראל ונותחה בסמיכות לאירוע דליפת נפט שהתרחש לאורך קו החוף הישראלי. במהלך הבדיקה החיצונית, נראו מספר רב של טפילי *Pennella balaenopterae* בשכבת השומן התת עורית לאורך הגחון וצדי הגוף של הלוויתן. בבדיקות טוקסיקולוגיות נוספות של דגימות ריאה וקנה הנשימה נמצאו רמות גבוהות מהמקובל של טולואן (Toluene), ללא עדות לחריגה מהנורמה של רכיבי נפט אחרים, דבר שעשוי להעיד על שאיפת החומר. מאמר זה מציע לשקול הרעלת טולואן כגורם אטיולוגי אפשרי העלול להשפיע על עמידות המאכסן וכתוצאה מכך הגברת העומס הטפילי.

השפעת תוספת מלטונין על רמות נוגדני IgG בקולוסטרום ועל עקה חימצונית בכבשים מזן אוואסי בהריון מתקדם.

ט"ב טקין ו־ט' אקוס

המחלקה למיילדות וגינקולוגיה, פקולטה לרפואה וטרינרית, אוניברסיטת הרן, סנליפורה, טורקייה.

מטרת המחקר המוצג הייתה לחקור את השפעות תוספת מלטונין בשתל תת עורי על מדדי עקה חימצונית ואיכות הקולוסטרום בכבשי אוואסי בחודש ההריון הרביעי, תוך התייחסות למין הוולדות. המחקר כלל 60 כבשי אוואסי בריאות. תזמון ייחום בוצע במהלך עונת הרבייה בעזרת מתן פרוגסטרון, ובדיקות הריון בוצעו דרך קיר הבטן. כבשים הרות חולקו לשתי קבוצות: מלטונין (n=30) וביקורת (n=30). בחודש הרביעי להריון, כבשים בקבוצת המלטונין טופלו בשתל מלטונין תת עורי באזור האוזן, ולכבשים בקבוצת הביקורת הוזרק מיליליטר אחד של תמיסה פיסיולוגית באותו המיקום. דגימות קולוסטרום ודם מהכבשים ודגימות דם מהוולדות, נלקחו בתוך שעה מההמלטה, לפני שהוולדות ינקו. רמות נוגדני IgG בקולסטרום היו גבוהות יותר בקבוצת המלטונין ביחס לביקורת ($p<0.001$), גבוהות יותר באמהות להן לא היו וולדות ממין נקבה בהשוואה לאלו להן היה וולד נקבה ($p<0.001$) וגבוהות יותר באמהות שהמליטו תאומים בהשוואה לאמהות שהמליטו וולד יחיד ($p<0.001$). רמות IgG בקולוסטרום היו גבוהות יותר ברחלות שהמליטו וולד יחיד, בין אם קיבלו מלטונין ובין אם לאו, ביחס לרחלות ללא וולד ממין נקבה ($p<0.001$). רמות total antioxidant capacity (TAC) בסרום מכבשים בקבוצת המלטונין היו גבוהות בהשוואה לקבוצת הביקורת ($p<0.001$). רמות total oxidant capacity (TOC) ו־oxidative stress index (OSI) היו גבוהות בקבוצת הביקורת ביחס לקבוצת הטיפול במלטונין. ($p<0.001$). רמות TAC בסרום בוולדות לאמהות מקבוצת המלטונין היו גבוהות יותר מאלו אצל וולדות לאמהות מקבוצת הביקורת ($p<0.001$), וגבוהות בוולדות יחידים ביחס לוולדות שנולדו כחלק מתאומים ($p<0.001$). המלטונין רמות TOC ו־OSI בסרום וולדות שנולדו לאמהות מקבוצת הביקורת היו גבוהות ביחס לאלו של וולדות לאמהות בקבוצת הביקורת ($p<0.001$), וגבוהות בוולדות יחידים ביחס לתאומים ($p<0.001$). לסיכום, מתן מלטונין טרם המלטה לכבשים בעדרים בהם מבוצעת רבייה, עשוי לשפר את התנגודת לעקה חמצונית ואת איכות הקולוסטרום ולסייע בכך לשיפור חיסוניות הוולדות.

קוראים יקרים,

בראה שהעולם שרוי במבוכה. בנוסף לכל האירועים ברחבי העולם המשפיעים על מיליוני בני אדם, רעידת אדמה חזקה במיוחד פגעה ב־6 בפברואר 2023 בארצות סמוכות לנו, תורכיה וסוריה.

אני מקיים קשר תדיר עם רופאים ווטרינרים ומדענים מטורקייה התורמים לעיתוננו "רפואה ווטרינרית" בכך שהם מפרסמים מאמרים באיכות גבוהה המתעדים את הממצאים והמחקר הקשורים לרפואה הווטרינרית בטורקייה, היות ומטרת עיתוננו לכסות את אגן הים התיכון.

כעורך "רפואה ווטרינרית", כתבתי לאחדים משולחי המאמרים מטורקייה והבעתי את השתתפותנו ותנחומינו לנפגעי האסון שפגע בעם בתורכי.

שלושה מכתבים ממחברים תורכים מופיעים במדור "מכתבים למערכת".

בשם הסתדרות הרופאים הווטרינרים בישראל, אנו שולחים את השתתפותנו ותמיכתנו בעם הטורקי.

ב ב ר כ ה,

ד"ר טוביה וינר

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תוכן עניינים

תמונת השער: דוכיפת (*Upupa epops*)

צבע גופה חום צהבהב, כנפיה וזנבה מפוספסים בשחור ולבן. רגליה קצרות וכנפיה גדולות יחסית. מקורה דק וארוך. על ראשה מתנשאת ציצית של נוצות ארוכות המזדקרות בזמן מעופה. אין הבדל חיצוני בין הזכר והנקבה. הדוכיפת מקננת בחורים ובחללים שונים על פי רוב בגובה פני הקרקע. להגנת הקן מפרישה הנקבה הפרשה מצחינה שריחה מזכיר ריח של בשר רקוב המרחיק טורפים ואולי אף טפילים. הנוזל מופרש מבלוטה שבבסיס הזנב (Uropygial gland). יכולת ההפרשה מתחילת ההטלה ועד לפריחת הגוזלים מהקן.

הדוכיפת שכיחה באירופה, באסיה ובאפריקה. בארץ היא מצויה בעיקר במישור החוף, אך בשנים האחרונות התפשטה בשאר אזורי הארץ בעקבות ריבוי השדות המעובדים והמדשאות. היא ניזונה מחרקים, זחלים ותולעים אותם היא שולפת מהאדמה בעזרת מקורה הארוך והמחודד. הדוכיפת משמיעה קול כמו "הודהוד" ובלטינית "הופופה" (upupa). במקרא מוזכרת הדוכיפת כעוף טמא [ויקרא י״א]. הגמרא מייחסת לה את הבאת תולעת השמיר לסיתות אבני המקדש, ובקוראן הדוכיפת מופיעה בסיפור שלמה המלך ומלכת שבא [סורא 27]. בימי הביניים יוחסו לדוכיפת סגולות מיסטיות. במסגרת חגיגות שנות השישים למדינה, הובילה החברה להגנת הטבע מיזם לבחירת הציפור הלאומית. המתחרות היו נשר מקראי, בז אדום, תנשמת, צופית, פשוש, בולבול, דוכיפת, חוחית ושלדג לבן חזה. הדוכיפת נבחרה ברוב של 36% מכלל 155,000 המשתתפים בהצבעה. הדוכיפת, הציפור הלאומית, נבחרה לשער הגיליון הזה של כתב העת, לכבוד חגיגות השנה ה־75 להקמת מדינת ישראל, ב־14 במאי 1948.

התמונות המופיעות על הכריכה, נמסרו באדיבותו של **מר משה טחנאי**.

רפואה וטרינרית

בטאון הסתדרות הרופאים הוטרינרים בישראל

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